Antidiabetic activity of *Diospyros malabarica* Kostel bark: a preliminary investigation for possible mode of action

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SUMMARY

The defatted methanol extract of bark of *Diospyros malabarica* (DM) at doses of 200 and 400 mg/kg, p.o. showed significant hypoglycemic activity on normal rats. The extract also exerted significant antihyperglycemic effect in alloxan-induced hyperglycemia and resulted in increase in plasma protein content and decrease in alkaline phosphatase, cholesterol and triglyceride levels when compared with those in the diabetic control group. However there were no significant changes in body and kidney weights of the DM extract-treated animals, compared to those of the untreated diabetic rats as a control. However, the DM extract showed a potential antioxidant activity by increasing catalase activity and reducing lipid peroxidation in liver. The results demonstrate antidiabetic activity of the defatted methanol extract of DM bark.

Key words: *Diospyros malabarica*; Hypoglycemia; Hyperglycemia; Biochemical parameters

INTRODUCTION

Diabetes is a metabolic disorder that affects metabolism of carbohydrate, protein and fat. It has now become a global burden (Raghunathan and Raghunathan, 1992). The number of diabetic patients was nearly 135 million in 1995 and is likely to be 300 million in 2025 (Amos *et al.*, 1997; King *et al.*, 1998). But there are little drugs developed as of yet, which can counteract the problem efficiently particularly the type II diabetes. Although the second and third generation sulfonylureas are widely used as an oral hypoglycemic agent, their long-term use produces many side effects, such as severe hypoglycemia, skin rashes, thrombocytopenia etc (Tripathy, 2004). Thus it is necessary to look for new compounds to manage and solve this problem.

Diospyros malabarica (DM) Kostel (Ebenaceae) is a medium size evergreen plant, found throughout India. It is popular as Gab or Tinduk. Common names are Riber Ebony (English), Tinduka (Sanskrit), Pei Shih (Chinese) and Abnusehindi (Arabic). The plant has oblong, deep green leaves (10 - 28 × 3 - 9 cm). Its bark is smooth, thick and blackish in color (Kirtikar *et al.*, 1975). Several phytochemicals have been isolated from leaf and bark, which include β-sitosterol, betulin, betulinic acid, oleanolic acid, lupeol and gallic acid (Gupta *et al.*, 1964; Misra *et al.*, 1971).

The plant is traditionally used for the treatments

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of dysentery, intermittent fevers, and menstrual problems (Kirtikar *et al.,* 1975; Chopra *et al.,* 1994) in India. The plant also possesses antifertility activity (Choudhary *et al.,* 1990). The bark portion of stem is reported to have hepatoprotective effect and antioxidant property (Mondal *et al.,* 2005, 2006). Hypoglycemic activity of the bark was primarily evaluated by Dhar *et al.* (1968). A detailed literature survey afforded no more information on antidiabetic activity of the plant. Thus the present study was to investigate the hypoglycemic and antihyperglycemic activities of DM bark, using an animal model.

MATERIALS AND METHODS

Plant material

The bark of DM was collected in February 2004 from Bangaon, West Bengal, India and authenticated by the Botanical Survey of India, Howrah, India. A voucher specimen (PG - 211) was kept in our laboratory for further reference.

Extraction

The bark was dried under shade and powdered in a mechanical grinder. The powdered material was first defatted with petroleum ether (60 - 80 °C) [SRL, Mumbai, India] followed by extraction with methanol [SRL, Mumbai, India] using soxhlet apparatus. This methanol extract of *Diospyros malabarica* (MEDM) was concentrated in *vacuo* and kept in a vacuum desiccator for four weeks for complete removal of solvent. The yield was 9.1% w/w with respect to dried powder.

Preliminary qualitative analysis of the methanol extract showed the presence of alkaloid, tannin, C-glycoside, saponin, reducing sugar and triterpenes (Mondal *et al.,* 2005). MEDM was used for the present study.

Animals

Swiss albino female mice (18 - 22 g) and Swiss albino male rats (140 - 180 g) were housed at room temperature (25 ± 1 °C) with free access to food

[Hindustan Lever, Kolkata, India] and water. Ethical clearance was obtained from the University Ethical Committee to carry out the work.

i) Acute toxicity study

MEDM in the dose range of 200 - 2,000 mg/kg was administered orally to different groups of mice comprising of ten mice in each group. Mortality was observed after 72 h (Litchfield and Wilcoxon, 1949; Mondal *et al.,* 2005).

ii) Hypoglycemic effect of extract on normal rats

Animals were divided into four groups consisting of six animals in each group. Group I received the vehicle (0.025% Carboxy methyl cellulose [Sigma, USA] in water, 3 ml/kg) for seven days while group II and III received MEDM orally, once a day, at doses of 200 and 400 mg/kg respectively. Group IV was treated similarly with metformin [gift sample from Deys' Medical, Kolkata, India] at a dose of 150 mg/kg. After 3 h of the last dose 1.0 ml of blood (from inner canthus of eye) was collected in microcentrifuge tubes containing 50 µl of anticoagulant (10% Sodium citrate [S. D. Fine-Chem Ltd, Boisar, India]). The samples were centrifuged to obtain plasma. Plasma was analyzed for glucose by GOD-POD method (Trinder, 1969) by using diagnostic kit [Qualigens Fine Chemicals, Mumbai, India].

iii) Effect of extract on hyperglycemic rats

Six animals were taken to form Group I (Normal control) which received vehicle through out the study. Diabetes was induced to other rats (day 0) by intraperitoneal injection of alloxan [Loba Chemie, Mumbai, India] at a dose of 150 mg/kg. The animals were kept for seven days to stabilize the diabetic condition. Animals showing plasma sugar level 200 - 350 mg/dl on day 7 were considered as diabetic. These diabetic rats were divided into three groups consisting of six animals in each group. One group (Group II) served as diabetic control; another group (Group III) received MEDM orally, once a day, at the dose of 400 mg/kg for

eight days (from 7th to 14th day) and the last group (Group IV) was treated similarly with metformin (150 mg/kg). Body weights were measured on 0th, 7th and 14th day. 3 h after the last dose, blood was collected as before. Hemoglobin content was measured. A portion of blood was centrifuged to obtain plasma. The plasma was analyzed for the content of sugar, total protein, alkaline phosphatase, cholesterol and triglycerides using commercially available kits (E merck, Germany). Immediately after blood collection, the animals were sacrificed under light ether anesthetic condition. Both the kidneys were collected and weighed. Liver was also collected and analyzed for catalase activity and extent of lipid peroxidation.

Determination of catalase activity

300 mg liver tissue was homogenized in 3.0 ml M/150 phosphate buffer in ice and centrifuged [Remi Centrifuge-CPR-30, Remi Instruments Ltd, Mumbai, India] at 30,000 g for 1hour at 4 °C. The supernatant was taken and catalase activity was measured according to the method of Luck (1963).

Determination of lipid peroxidation

900 mg of liver tissue was collected from each experimental rat, washed in normal saline and soaked in filter paper. The tissues were then homogenized in 3.0 ml M/150 Phosphate buffer (pH 7.0) and centrifuged at 30,000 g at 4 °C for 1 h. The supernatant was further centrifuged at 10,000 g for 10 min at 4 °C. The supernatant was collected and estimated for lipid peroxidation following the

method of Ohkawa *et al.* (1979).

Statistical analysis

The data are represented as mean ± S.E.M. Student's *t*-test was used for statistical analysis and $P < 0.05$ was considered significant.

RESULTS

Both the extract and metformin showed significant ($P < 0.05$) hypoglycemic effect on normal rats. MEDM at doses of 200 and 400 mg/kg and metformin (150 mg/kg) showed 5.82, 14.30 and 24.04% reduction in plasma sugar level respectively compared with the control group (Table 1). The extract also exhibited antihyperglycemic effect on alloxan-induced diabetic rats. MEDM (400 mg/kg) showed 29.89% reduction in plasma sugar level ($P < 0.001$) while metformin caused 31.63% reduction ($P < 0.001$) when compared with that of the diabetic control group (Table 2).

There were significant ($P < 0.001$) decrease in plasma protein content and increase in alkaline phosphatase, cholesterol and triglycerides in the diabetic control group compared to those of the normal group. MEDM and metformin treated groups afforded inhibition towards the changes in the diabetic state. Total protein content in plasma was found to be 6.57 and 7.00 g/dl respectively when treated with MEDM and metoformin while that in the diabetic control was 4.82 g/dl (Table 3). Levels of alkaline phosphatase, cholesterol and triglycerides were decreased by 6.64, 10.45, 14.18% respectively in extract treated animals and 19.92,

Table 1. Hypoglycemic effect of extract on normal rats

Groups	Plasma sugar level (mg/dl)	
	0 day	7th Day
Control 3.0 ml/kg	85.24 ± 5.28	82.41 ± 4.82
MEDM 200 mg/kg	84.62 ± 3.83	77.61 ± 5.33* (5.82)
MEDM 400 mg/kg	88.39 ± 6.03	70.62 ± 5.80** (14.30)
Metformin 150 mg/kg	82.31 ± 3.77	62.60 ± 4.62*** (24.04)

Values are mean ± S.E.M. (n = 6). *$P < 0.02$; **$P < 0.01$; ***$P < 0.001$ vs. control group. Values in the parentheses are percentage reduction of plasma sugar level compared with the control group.

Table 2. Effect of extract on plasma sugar levels in hyperglycemic rats

Group	Plasma sugar level (mg/dl)	
	7th Day	14th Day
Normal control 3.0 ml/kg	84.96 ± 3.80	86.12 ± 4.33
Diabetic control 3.0 ml/kg	246.08 ± 13.26[*]	263.70 ± 13.62[*]
MEDM 400 mg/kg	252.39 ± 12.85[*]	184.88 ± 8.25[#] (29.89)
Metformin 150 mg/kg	253.67 ± 9.44[*]	180.30 ± 10.10[#] (31.63)

Values are mean ± S.E.M. (n = 6). [*]$P < 0.001$ vs. normal control group; [#]$P < 0.001$ vs. diabetic control group. Values in the parentheses are percentage reduction of sugar level compared with the diabetic control group.

Table 3. Effect of extract on some biochemical parameters of hyperglycemic rats

Groups	Biochemical parameters measured on 14th day			
	Total protein (g/dl)	Alkaline phosphatase (U/L)	Cholesterol (mg/dl)	Triglycerides (mg/dl)
Normal control 3.0 ml/kg	7.08 ± 0.34	169.10 ± 16.59	150.21 ± 9.82	98.98 ± 6.76
Diabetic control 3.0 ml/kg	4.82 ± 0.54[*]	238.50 ± 21.87[*]	238.60 ±14.33[*]	170.50 ± 11.42[*]
MEDM 400 mg/kg	6.57 ± 0.48[°°°]	222.67 ± 12.22[°] (6.64)	213.67 ± 12.61[°°] (10.45)	146.33 ± 8.37[°°] (14.18)
Metformin 150 mg/kg	7.00 ± 0.39[°°°]	191.00 ± 14.57[°°] (19.92)	203.44 ± 14.49[°°°] (14.74)	127.50 ± 12.31[°°] (25.22)

Values are mean ± S.E.M. (n = 6). [*]$P < 0.001$ vs. normal control group; [°]$P < 0.05$, [°°]$P < 0.01$, [°°°]$P < 0.001$ vs. diabetic control group. Values in the parentheses are percentage reduction compared with the diabetic control group.

Table 4. Effect of MEDM on body weight and kidney weight of hyperglycemic rats

Groups	Body weight (g)			Kidney weight on 14th day	
	0 day	7th day	14th day	Kidney weight (g)	2 kidney wt/bw (mg/g)
Normal control	158.90 ± 8.11	160.23 ± 9.08	164.98 ± 11.16	0.498 ± 0.009	6.04 ± 0.52
Diabetic control	160.00 ± 6.29	142.86 ± 10.26	146.43 ± 10.94[*]	0.696 ± 0.006	9.51 ± 0.46[*]
MEDM 400 mg/kg	154.33 ± 8.90	136.67 ± 10.64	152.73 ± 11.13	0.592 ± 0.012	7.75 ± 0.65
Metformin 150 mg/kg	162.50 ± 9.26	149.39 ± 8.88	150.74 ± 10.68	0.590 ± 0.008	7.82 ± 0.40

Values are mean ± S.E.M. (n = 6). [*]$P < 0.05$ vs. normal control group on 14th day.

Table 5. Effect of MEDM on Catalase activity and lipid peroxidative state of liver of hyperglycemic rats

Groups	Catalase activity (Units/mg protein)	Lipid peroxidation (µmol MDA/g Liver)
Normal control	90.37 ± 6.45	40.55 ± 6.65
Diabetic control	24.74 ± 3.28[#]	120.82 ± 9.98[#]
MEDM 400 mg/kg	71.53 ± 5.67[*]	58.43 ± 5.92[*]
Metformin 150 mg/kg	105.99 ± 9.97[*]	50.94 ± 7.17[*]

Values are mean ± S.E.M. (n = 6). [#]$P < 0.001$ vs. normal control; [*]$P < 0.001$ vs. diabetic control.

14.74, 25.22% respectively in metformin treated animals compared with the untreated ones (Table 3). However, both the extract and the standard drug failed to show any significant effect on body weight and kidney weight (Table 4).

Table 5 indicates that the diabetic control group showed significant increase in lipid peroxidation with significant reduction in catalase activity. The treatment with MEDM and metformin restored the above parameters towards the normal levels.

DISCUSSION

The present study revealed that the extract possesses blood-sugar lowering effect on normal rats as well as alloxan-induced diabetic rats. Thus MEDM possesses both hypoglycemic and antihyperglycemic activity in animal model.

Alloxan is selectively taken up by the rat islets and hepatocytes. The liver has a very high concentration of free radical scavenging enzymes such as catalase, superoxide dismutase and glutathione peroxidase which are, by comparison, low in islet cells. Alloxan gets converted to dialuric acid by two electron reduction. However, dialuric acid is unstable and is oxidized back to alloxan producing oxidative free radicals and hydrogen peroxide which, through Fenton reaction, generates highly toxic hydroxyl radical. All these radicals damage the pancreatic β cells by inducing membrane lipid peroxidation and extensive DNA strand breakage (Chattopadhyay *et al.*, 1997). As alloxan induces diabetes causing β cell damage, the hyperglycemia is characterized by insulin deficiency (Halliwell and Gutteridge, 1985). In addition to increase in blood sugar level, insulin deficiency leads to various other metabolic aberrations *viz*. increase in cholesterol and alkaline phophatase, decrease in plasma protein content (Shanmugasundaram *et al.*, 1983; Vasu *et al.*, 2003; Dhanbal *et al.*, 2004). It is reported that subjects with insulin deficiency fail to activate the lipoprotein lipase enzyme and suffer from hypertriglyceridemia (Taskimen, 1987). Reports are available indicating increased level of serum triglycerides and cholesterol in diabetic rats (Nikhila, 1973; Kadnur and Goyal, 2005). The extract and metformin treated groups not only showed reduction in blood sugar level (Table 2) but also increased plasma protein content and decreased alkaline phosphatase, cholesterol and triglyceride levels. These findings imply that there is increased secretion of insulin in the treated groups. So the hypoglycemic activity may be due to insulinotropic effect of MEDM. This notion however could be confirmed if insulin level was estimated.

In uncontrolled and poorly controlled diabetes there is increased glycosylation of a number of proteins including hemoglobin (Sheela and Augusti, 1992; Prince and Menon, 2003). So total hemoglobin level decreases in alloxan induced group. Administration of MEDM caused increase in total hemoglobin level (Table 3) indicating improved utilization of blood sugar, which in turn ascertains the antidiabetic potential of MEDM.

It is well known that the diabetic subjects lose body weight gradually (Grover *et al.*, 2003; Vishwakarma *et al.*, 2003) which is once again evident from the present study. It was observed that the extract prevented the loss of body weight as in diabetic control group. However, such protection was not significant (Table 4). One explanation may be that, to obtain maximum effect, therapy with plant products be continued for longer duration like 1 - 2 months (Yadav *et al.*, 2002; Grover *et al.*, 2003). Unlike sulfonylureas and biguanides the onset of action is slow and it cannot be used for management of emergency situations.

It is also known that diabetes is associated with enlargement of kidneys. Renal enlargement is probably dependent on duration as well as degree of hyperglycemia although contradictory theory is there (Rasch 1979; Grover *et al.*, 2003). Whatever may be the reason, treatment with MEDM did not show appreciable change in kidney weight. It may be due to short term treatment protocol (Yadav *et al.*, 2002; Grover *et al.*, 2003).

It is known that oxidative stress can lead to diabetes (Alder, 1984; Vander *et al.*, 1990; Lata and Ahuja, 2003). In rats, alloxan is selectively taken up by the Islets of Langerhans and hepatocytes. Thus they suffer high oxidative stress due to the free radicals generated from alloxan (Kershbaum *et al*, 1968). These radicals reduce the level of antioxidants i.e. reduced glutathione, catalase etc and increase liver lipid peroxidation rendering the subject to exacerbate the symptoms of diabetes (Vander *et al.*, 1990; Krishnakumar *et al.*, 1999). Table 5 shows that

there was significant reduction in catalase activity and increased level of lipid peroxidation in diabetic control which were reversed in the treated groups. This indicates that the extract possesses antioxidant property.

The *in vivo* and *in vitro* antioxidant effect of MEDM has already been reported (Mondal *et al.,* 2005, 2006).

This is established that many plants show antidiabetic activity due to their antioxidant potential (Raven *et al.,* 1995; Cunningham, 1998; Garg and Bansal, 2000; Mc Cune *et al.,* 2002; Mazumder *et al.,* 2005). So the antidiabetic activity of MEDM may be due to its antioxidant property.

Thus from all the results it can be concluded that antidiabetic activity of MEDM may be due to insulinotropic action and antioxidant property.

Phytochemical analysis of MEDM showed the presence of tannins and triterpenes. Some drugs containing these phytochemicals are known to have antidiabetic activity (Iwu, 1980, 1983; Rahman and Zaman, 1989). Thus it can be assumed that tannins or triterpenes present in MEDM is responsible for the antidiabetic activity. However further chemical and pharmacological investigations are needed to find out the active phytochemical and the exact mechanism of action.

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