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RESEARCH ARTICLE

Antidiabetic and antioxidant activity of the methanol extract of *Diospyros peregrina* fruit on Type I diabetic rats

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Abstract

Chronic diabetes complications are mainly associated with augmented oxidative stress. Thus the present study evaluated the hypoglycemic, as well the antioxidant effect, of the methanol extract of *Diospyros peregrina* Gurke. (Ebenaceae) fruits on experimental diabetic rats. Oral administration of methanol extract at 150 and 300 mg/kg body weight per day to diabetic rats was found to have profound hypoglycemic activity in term of reduction of fasting blood glucose level. The diabetic rats showed lower activities of superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) content in hepatic and renal tissues as compared with normal rats. The activities of SOD, CAT, and GSH were found to be increased in extract-treated diabetic rats in selected tissues. The increased levels of lipid peroxidation (thiobarbituric acid reactive substances and hydroperoxides) in diabetic rats were also found to be reverted back to near-normal status in extract-treated groups. It was found that the extract is more effective at the dose of 300 mg/kg body weight and this effect is almost comparable to that of standard glibenclamide.

Keywords: *Antidiabetic; antioxidant;* Diospyros peregrina*; oxidative stress*

Introduction

Diabetes mellitus is a disease in which homeostasis of carbohydrate, protein, and lipid metabolism is improperly regulated by hormone insulin resulting elevation of fasting and postprandial blood glucose levels (Tiwari & Rao, 2002). The major chronic complications associated with diabetes include retinopathy, neuropathy, nephropathy, atherosclerotic coronary artery disease, and peripheral atherosclerotic vascular disease (Kaczmar, 1998). Hyperglycemia alone does not cause diabetic complications. It is rather the detrimental effect of glucose toxicity due to chronic hyperglycemia mediated and complicated through augmented oxidative stress (Menon et al., 2004). Hyperglycemia increases the production of reactive oxygen species (ROS) inside the aortic endothelial cells. ROS-induced activation of protein kinase-C isoforms, increased formation of glucose-derived advanced glycation end products, increased glucose flux through aldose reductase pathways, and activation of cytokines are some of the known biochemical mechanisms of hyperglycemia-induced tissue and cell damage (Koya & King, 1998; Brownlee, 1995). The mammalian cells are equipped with enzymatic as well as non-enzymatic antioxidant defenses which minimize ROS-mediated cellular damage (Haliwell & Gutteridge, 1994). Recent studies showed that the majority of plasma antioxidants are depleted in diabetes patients (Valabhji et al., 2001). Thus, antioxidant therapy in diabetes may be helpful in relieving symptoms and complications observed in diabetes patients. Hence, attention has been focused on hypoglycemic properties coupled with antioxidant properties to combat diabetes and its related complications. Currently, traditional medicinal plants present a stirring prospect of expansion in diabetes treatment (Bailey & Day, 1989; Rahman & Zaman, 1989). As plants often contain a substantial amount of antioxidants, so herbal hypoglycemic properties coupled with

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antioxidant properties may serve as a wonderful antidiabetic agent (Larson, 1988).

Diospyros peregrina Gurke. (Ebenaceae) is a small medium-sized tree, glabrous except younger parts with numerous spreading branches, forming an impenetrable shady head, which grows luxuriantly in the plains of costal West Bengal. Ripe fruits are edible with ethnomedicinal significance as a tonic and aphrodisiac (Kirtikar & Basu, 1975). Unripe fruits are astringent, acrid, bitter and oleaginous (Anjaria et al., 2002). Unripe fruits are used for the treatment of diarrhea, dysentery, cholera, mouth ulcer, and in wounds (Asolkar et al., 1992). The fruits contain triterpenes, alkanes, flavonoids, and tannins (Chopra et al., 1992; Jain & Yadav, 1994, 1997; Chauhan et al., 1982; Misra et al., 1971). The stem bark of the plant has been reported to possess hypoglycemic activity (Ghani, 1998). Though there is no scientific literature to support the antidiabetic effect of *Diospyros peregrina* fruit, the villagers of costal West Bengal continue to use the maceration of dried unripe fruits in the management of diabetes. The present investigation was directed to ascertain the efficacy of this plant in management of diabetes as well as the oxidative stress associated with it.

Materials and methods

Collection of plant material

Mature unripe fruits of *Diospyros peregrina* were collected in June 2006 from the villages of costal South 24 Parganas, West-Bengal, India. The plant was authenticated by H.J. Chowdhury, Joint Director, Botanical Survey of India, Shibpur, Howrah, India. A voucher specimen JU/PT/Pcog/01/06 was deposited at our laboratory for future reference.

Preparation of methanol extract

Methanol extract of fruits was prepared in accordance with the method of the National Institute of Health and Family Welfare (NIHFW), New Delhi, India. Mature unripe fruits of *Diospyros peregrina* were dried in an incubator for two days at 40°C, crushed in a mechanical grinder to fine powder of mesh 40. The powder (500 g) was then extracted with 2.5 L of 90% methanol in a Soxhlet apparatus at 65°C, until the powder became exhausted totally. The resulting extract was filtered, concentrated, and dried *in vacuo* at 40-45°C and 0. 8 MPa in a Buchi evaporator, R-114. The dried extract (8.75% w/w) was stored in a desiccator.

Estimation of total phenolics and flavonoids

The total concentrations of phenolics in methanol extract of *Diospyros peregrina* fruits were determined according to the method of Singleton et al. (1999). Total soluble flavonoid content was determined according to the method of Hsu (2006) with little modification.

Antidiabetic studies

Animals

Healthy adult Wister strain albino rats of both sexes between two and three months of age and weighing 180-240 g were used for the study. The animals were allowed to acclimatize for a period of two weeks in our laboratory environment prior to the study. The rats were housed in polypropylene cages (three animals per cage), maintained under standard laboratory conditions (i.e. 12:12 h light and dark sequence; at an ambient temperature of 25° ± 2°C, 35-60% humidity). The animals were fed with standard rat pellet diet (Hindustan Liver, Mumbai, India) and water *ad libitum*. The *Principles of Laboratory Animal Care* (PHS, 1986) were followed and instructions given by our institutional animal ethical committee were followed throughout the experiment.

Chemicals

Streptozotocin, 5, 5-dithio bis-2-nitro benzoic acid (DTNB), and reduced glutathione (GSH) were procured from SISCO Research Lab (Mumbai, India). Thiobarbituric acid, nitroblue tetrazolium (NBT), and nicotinamide adenine dinucleotide (NAD) were purchased from Loba Chemie (Mumbai, India). All chemicals and reagents used were of analytical grade.

Induction of diabetes

Experimental hyperglycemia was induced in overnight fasted adult Wister strain albino rats weighing 180-240 g by a single intraperitoneal injection of 65 mg/kg streptozotocin in a volume 1 mL/kg body weight (bw) (Siddque et al., 1987). Because of the instability of streptozotocin in aqueous media, the solution was made in citrate buffer (pH 4.5) immediately before injection (Karunanayake et al., 1974). Hyperglycemia was confirmed by the elevated glucose level in plasma, determined at 48 h after injection (Mandal et al., 1997). The rats found hyperglycemic were screened for the antidiabetic study.

Experimental design

Animals were divided into five groups of six rats each.

Group I: Normal rats administered distilled water daily for 12 days.

Group II: Diabetic control rats administered distilled water daily for 12 days.

Group III: Diabetic rats administered methanol extract (150 mg/kg, orally) daily for 12 days.

Group IV: Diabetic rats administered methanol extract (300 mg/kg, orally) daily for 12 days.

Group V: Diabetic rats administered standard drug glibenclamide (1 mg/kg, orally) daily for 12 days.

All doses were started 48 h after streptozotocin injection. Fasting blood glucose levels were estimated at hour 0, 2, 6, 12 (short-term study) and then on day 1, 6, 12 with the help of a single-touch glucometer (Ascensia Entrust, Bayer Health Care Milpitas, California, USA).

After 12 days of treatment all the rats were anesthetized and sacrificed by cervical dislocation; livers and kidneys were excised and washed thoroughly to clear off blood. The tissues were immediately transferred to ice-cold saline and homogenized in 0.1N Tris-HCl buffer (pH 7.4). These tissue homogenates were used for the estimation of thiobarbituric acid reactive substances (TBARS) (Fraga et al., 1988), hydroperoxides (HP) (Fraga et al., 1988), reduced glutathione (GSH) (Ellman, 1959), superoxide dismutase (SOD) (Kakkar et al., 1984), and catalase (CAT) (Sinha, 1972).

Statistical analysis

Data were statistically calculated by utilizing one-way ANOVA and expressed as mean ± SEM followed by Dunnett's *t*-test using computerized GraphPad InStat version 3.05, GraphPad software. InStat version 3.05, GraphPad software, Avenida de la Playa La Jolla, California, USA

Results

The level of total polyphenolic compounds was found to be 42.94 mg of pyrocatechol equivalent per gram of dry weight of methanol extract of *Diospyros peregrina* fruit. Total flavonoids content was found to be 17.96 mg of quercetin equivalent per gram of methanol extract.

The diabetic rats showed a significant elevation of blood glucose level. The extract was found to be an effective hypoglycemic in terms of reduction of blood glucose level. Even a single oral administration of extract at the doses of 150 and 300 mg/kg to diabetic rats was found to reduce blood glucose level steadily within a period of 12 h (Table 1). Daily administration of extract to diabetic rats maintained fasting blood glucose level to near-normal status in a dose-dependent manner within 12 days (Table 2). The antioxidant effect of the extract on tissue antioxidant markers was studied (Table 3). The diabetic rats showed a significant increase in TBARS and HP in hepatic and renal tissues. Oral administration of extract reduced these to normal level. There was a significant reduction in GSH in diabetic rats. Extract administration to diabetic rats significantly increased liver and kidney GSH to near normal value. The decreased levels of SOD and CAT in diabetic rats were found to be reverted back to near normal status after treatment of fruit extract. The readings obtained from the treated groups were comparable to that of the standard drug glibenclamide.

Table 1. Effect of methanol extract of *Diospyros peregrina* on blood glucose level after single dose treatment.

		Blood glucose (mg/dL)			
Group (n = 6)	Treatment	0 h	2 h	6 h	12 h
I	Normal control (Distilled water)	70.67 ± 2.11	71 83 ± 3.23	70.83 ± 3.12	69.83 ± 2.29
II	Diabetic control (Distilled water)	253.83 ± 5.23[a]	256.17 ± 8.59[a]	256.67 ± 8.29[a]	259.83 ± 8.85[a]
III	Diabetic + DPME (150 mg/kg)	258.83 ± 4.35	223.5 ± 6.2[b]	213.67 ± 7.61[b]	210.67 ± 8.01[b]
IV	Diabetic + DPME (300 mg/kg)	267.17 ± 5.68	216.17 ± 5.4[b]	209.17 ± 5.74[b]	206.83 ± 4.73[b]
V	Diabetic + Glibenclamide (1 mg/kg)	275.83 ± 5.19	202.33 ± 8.76[b]	193.5 ± 5.69[b]	188.67 ± 3.9[b]

Values are expressed as mean ± SE.
[a]$p < 0.001$ compared with normal control group.
[b]$p < 0.01$ compared with diabetic control group.

Table 2. Effect of methanol extract of *Diospyros peregrina* on fasting blood glucose level.

		Blood glucose (mg/dL)			
Group (n = 6)	Treatment	0 day	1 day	6 day	12 day
I	Normal control (Distilled water)	70.67 ± 2.11	69.33 ± 2.03	72.17 ± 2.17	71.33 ± 1.98
II	Diabetic control (Distilled water)	253.83 ± 5.23[a]	263.5 ± 8.12[a]	272.17 ± 7.69[a]	275.83 ± 5.01[a]
III	Diabetic + DPME (150 mg/ kg)	258.83 ± 4.35	209.17 ± 6.89[b]	188.83 ± 5.14[b]	128.33 ± 3.24[b]
IV	Diabetic + DPME (300 mg/ kg)	267.17 ± 5.68	203.17 ± 3.54[b]	174.5 ± 6.19[b]	118.5 ± 4.15[b]
V	Diabetic + Glibenclamide (1 mg/kg)	275.83 ± 5.19	187.83 ± 3.02[b]	166.17 ± 8.32[b]	108.67 ± 3.21[b]

Values are expressed as mean ± SE.
[a]$p < 0.001$ compared with normal control group.
[b]$p < 0.01$ compared with diabetic control group.

Table 3. Effect of methanol extract of *Diospyros peregrina* on tissue TBARS, HP, GSH, SOD, and CAT in diabetic rats.

Parameters	Normal control (Distilled water)	Diabetic control (Distilled water)	Diabetic + DPME (150 mg/ kg)	Diabetic + DPME (300 mg/ kg)	Diabetic + Gliben clamide (1 mg/kg)
TBARS- Liver *	0.87 ± 0.14	1.55 ± 0.12[a]	1.22 ± 0.08	0.93 ± 0.11[b]	1.01 ± 0.05[b]
TBARS – Kidney*	1.44 ± 0.06	2.12 ± 0.15[a]	1.71 ± 0.13[c]	1.55 ± 0.07	1.47 ± 0.06[b]
HP – Liver*	64.35 ± 5.59	91.58 ± 2.59[a]	76.13 ± 4.33	68.67 ± 5.79[b]	70.08 ± 4.57[c]
HP – Kidney*	51.67 ± 3.7	80.75 ± 5.59[a]	65.78 ± 4.69	61.93 ± 2.52[c]	62.48 ± 3.6[c]
GSH – Liver**	43.53 ± 3.16	21.13 ± 1.52[a]	33.9 ± 3.88[c]	41.02 ± 2.7[b]	40.83 ± 2.18[b]
GSH – Kidney**	20.12 ± 1.92	4.12 ± 0.89[a]	12.02 ± 1.08[b]	17.83 ± 1.5[b]	18.18 ± 2.08[b]
SOD – Liver[#]	6.78 ± 0.23	3.22 ± 0.3[a]	5.57 ± 0.63[b]	6.22 ± 0.28[b]	6.48 ± 0.32[b]
SOD – Kidney[#]	14.25 ± 1.14	6.77 ± 0.88[a]	10.15 ± 0.77[c]	12.33 ± 1.02[b]	13.23 ± 0.58[b]
CAT – Liver[##]	82.42 ± 3.44	46.28 ± 4.02[a]	64.93 ± 5.85[c]	73.08 ± 5.83[b]	71.72 ± 3.49[b]
CAT – Kidney[##]	34.12 ± 2.34	19.2 ± 1.35[a]	29.58 ± 1.73[b]	32.02 ± 1.81[b]	32.85 ± 1.19[b]

* mmoles/100 g wet tissue.

** mg/100 g wet tissue.

[#] U/mg of protein, one unit is defines as the enzyme concentration require to inhibit the OD at 560 nm of chromogen produced 50% in 1 min.

[##] µmoles of H_2O_2 consumed/min/mg protein.

Values are expressed as mean ± SE (n = 6).

[a] $p < 0.01$ compared with normal control group.

[b] $p < 0.01$, [c] $p < 0.05$ compared with diabetic control group.

Discussion

Streptozotocin-induced hyperglycemia is a useful experimental model to study the activity of hypoglycemic agents (Junod et al., 1969). In this experiment, significant elevation of blood glucose level was observed in streptozotocin-induced diabetic rats as compared with normal control. A significant decrease in blood glucose level was observed in extract-treated diabetic rats. In this context, methanol extract of matured unripe fruits of *Diospyros peregrina* possesses profound hypoglycemic effect in experimental diabetic rats.

Streptozotocin is a potent β-cell toxin. It produces reactive oxygen species (ROS) in the body, namely superoxide radicals, hydroxyl radicals, and hydrogen peroxide, participating as a major role in causation of diabetes. Hence, antioxidants may have an important role in the alleviation of diabetes (Baynes, 1991). The concentration of ROS is modulated by scavenging enzymes like GSH, SOD, and CAT, which are known as the markers of tissue antioxidants.

Lipid peroxidation is one of the characteristic features of chronic diabetes (Maxwell et al., 1997). An increase in hepatic and renal TBARS is an index of enhanced lipid peroxidation in diabetes (Kamalakkanan & Prince, 2004). Increased TBARS in diabetic rats suggests augmented oxidative stress that could be due to enhance production or decrease destruction of ROS (Griesmacher et al., 1995). Oral administration of extract to diabetic rats significantly lower this enhanced TBARS level in both the tissues.

Hydroperoxide molecules participate in the destruction of enzymatic protein and cell membranes (Wang et al., 1996). Increased HP levels in liver and kidney were observed in diabetic rats. It may be due to decreased activity of antioxidant enzymes resulting in uncontrolled generation of ROS. Oral administration of methanol extract significantly lowered hepatic and renal HP in diabetic rats and it indicates that the extract is capable of alleviating lipid peroxidation.

Increased lipid peroxidation in diabetes can be due to enhanced oxidative stress in the cells as a result of depletion of the antioxidant scavenger system. GSH is a major endogenous antioxidant which counteracts free radical-mediated damage. Depletion of liver and kidney GSH levels represents enhanced oxidative stress (Anuradha & Selvam, 1993).

SOD is an antioxidant enzyme which reduces superoxide radicals to water and molecular oxygen (McCord et al., 1976) whilst CAT reduces hydrogen peroxide (Gutteridge, 1995). Diminished activity of these antioxidant enzymes results in elevation of ROS and ROS-mediated cell destruction. Reduced activities of SOD and CAT in liver and kidney were observed in diabetic rats and these were reverted to near-normal status on extract treatment.

The present investigation showed that the methanol extract of unripe mature fruits of *Diospyros peregrina* possesses considerable hypoglycemic activity. The extract also exhibited a profound antioxidant effect in diabetic rats. Scientists claimed that, "the treatment of diabetes with antioxidant therapy is like applying water to a burning house and is certainly helpful in limiting the conflagration" (Tiwari & Rao, 2002). Hence, the synergistic combination of hypoglycemic activity and antioxidant activity may prove to be very effective in the management of diabetes and the associated oxidative stress. The quantitative estimation of total polyphenolics and flavonoids confirmed that the methanol extract of the fruits of *Diospyros peregrina* contains substantial quantity of

polyphenolics and flavonoids which are the known antioxidant from plant sources (Georgetti et al., 2003). So, the activity may be due to the presence of substantial quantities of polyphenolics and flavonoids in the extract.

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***Declaration of interest*:** The authors alone are responsible for the content and writing of the paper.

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