RESEARCH ARTICLE

Antidiabetic effect of matured fruits of *Diospyros peregrina* in alloxan-induced diabetic rats

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The methanol extract of matured fruits of *Diospyros peregrina* was evaluated for its antidiabetic activity in alloxan-induced diabetic rats. It was also intended to establish correlation with reduction of oxidative state associated with diabetes. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (150 mg/kg body weight). Methanol extract was administered orally at doses of 150 and 300 mg/kg body weight for 14 consecutive days in diabetic rats. Fasting blood glucose level, serum lipid profiles, liver glycogen level, pancreatic thiobarbituric acid reactive substances (TBARS) as well initial and final changes in body weight were evaluated. Experimental findings showed significant antidiabetic potential of extract in terms of reduction of fasting blood glucose level in diabetic rats. Observed data was found statistically significant in reduction of serum lipid and pancreatic TBARS levels whilst significant improvement was observed in liver glycogen level and body weight profiles in extract-treated diabetic rats. The effect of the extract particularly at 300 mg/kg was comparable to that of standard drug glibenclamide (1 mg/kg body weight).

Key words: Alloxan, antihyperglycemic, diabetes, *Diospyros peregrina*, glibenclamide

INTRODUCTION

Diabetes mellitus is a disease in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated by hormone insulin resulting in elevation of fasting and postprandial blood glucose levels.[1] The major chronic complications associated with diabetes include retinopathy, neuropathy, nephropathy, and atherosclerotic coronary artery disease and peripheral atherosclerotic vascular disease.[2] According to recent estimation, the global population is approaching the midst of a diabetes pandemic. By the year 2010 the total number of people worldwide with diabetes is predicted to reach 239 million.[3] Besides hyperglycaemia, several other factors like hyperlipidemia and enhanced oxidative stress play a major role in diabetic pathogenesis. Despite the great strides that have been made in the understanding and management of this disease, the graph of diabetes-related mortality is rising unabated. Although a number of synthetic drugs are available in the market diabetes and its related complications still remain uncontrolled. On the other hand, traditional medicinal plants have been used successfully since ancient times by physicians and laymen to treat diabetes and its related complications, presenting a stirring prospect for the expansion of an alternative way of treatment of diabetes.[4,5] Herbal drugs are prescribed widely, even when their biologically active compounds are unknown, because of their effectiveness, lesser side-effects and relatively low cost.[6,7]

Diospyros peregrina Gurke (Ebenaceae) is a small middle-sized tree, glabrous except the younger parts with numerous spreading branches, forming an impenetrable shady head, which grows luxuriantly in the plains of coastal West Bengal. Ripe fruits are edible with ethnomedicinal significance as tonic and aphrodisiac.[8] Unripe fruits are astringent, acrid, bitter and oleaginous.[9] Unripe fruits are used for the treatment of diarrhoea, dysentery, cholera, ulcer of mouth and in wounds.[10] The fruits contain triterpenes, alkanes, flavonoids and tannins.[11-14] The stem barks of the plant have been reported for their hypoglycaemic activity.[15] The maceration of matured fruits is successfully employed in coastal West Bengal for the treatment of diabetes. The present investigation is directed to the exploration of the antidiabetic activity of the methanol extract of matured fruits of *Diospyros peregrina*. An attempt was also made to find out antioxidant potential of the aforementioned plant with an aim to establish a correlation with the reduction of oxidative state associated with diabetes.

MATERIALS AND METHODS

Plant Material

Matured unripe fruits of *Diospyros peregrina* (Family: Ebenaceae) were collected in the month of June 2006 from the villages of coastal South 24 Parganas, West Bengal, India. The plant was authenticated by Taxonomists of the Botanical Survey of India, Shibpur, Howrah, India.

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A voucher specimen JU/PT/Pcog/01/06 was deposited in the herbarium of the department 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. Matured 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* (yield 8.75% w/w). The extract was stored in a desiccator for use in subsequent experiments.

Phytochemical Analysis

Preliminary phytochemical screening[16] of methanol extract of fruits revealed the presence of tannins, flavonoids, triterpenoids and sugars.

Animals

Healthy adult Wistar strain albino rats of both sexes between two to three months of age and weighing 180-240 g were screened for the study. Animals were allowed to be acquainted for a period of 15 days in our laboratory environment prior to the experiment. Rats were housed in standard polypropylene cages (three animals per cage), maintained under standard laboratory conditions (*i.e.* 12:12 hour light and dark cycle; at an ambient temperature of 25 ± 5°C; 35-60% of relative humidity); the animals were fed with standard rat pellet diet (Hindustan Lever Ltd. Mumbai, India) and water *ad libitum*. The principles of Laboratory Animals' care[17] were followed and instructions given by our institutional animal ethical committee were followed throughout the experiment. All studies were carried out using six rats in each group.

Chemicals

Alloxan monohydrate, a most widely used chemical diabetogen was procured from Loba chemie, Mumbai, India and other reagents used in the experiment were of analytical grade. Chemically alloxan is 2, 4, 5, 6 tetra oxo hexahydro pyrimidine. Glibenclamide, a standard antidiabetic agent was purchased from Aventis Pharma. Ltd., Goa, India.

Antihyperglycaemic Studies

Induction of diabetes

Hyperglycaemia was induced in overnight fasted adult Wistar strain albino rats weighing 180-240 g by a single intraperitoneal injection of freshly prepared alloxan monohydrate in normal saline (150 mg/kg body weight) in a volume 1 ml/kg body weight.[18] Hyperglycaemia was confirmed by the elevated glucose level in plasma, determined at 48 h after injection.[19] The rats found hyperglycaemic were screened for the antihyperglycaemic study.

Experimental Design

Animals were divided into four groups of six rats each. Test groups were administered methanol extract at doses of 150 and 300 mg/kg body weight respectively by oral route. Standard and control animals were treated with standard drug glibenclamide at an oral dose of 1 mg/kg body weight and distilled water respectively. All doses were started 48 h after alloxan injection. Fasting blood glucose levels were estimated on Hour 0, 1, 2, 4, 6 (short-term study after a single administration of doses) and then on Day 0, 1, 2, 4, 7, 14 (long-term study) with the help of single-touch glucometer (Ascensia Entrust, Bayer Health Care, USA). Serum lipid profiles, liver glycogen profile[20] and pancreatic thiobarbituric acid reactive substances[21] were measured after the animals were sacrificed after 12 days by decapitation. Initial and final changes in body weight were also measured.[22]

Statistical Analysis

Data were statistically calculated by utilizing one-way ANOVA and expressed as mean ± S.E.M. followed by Dunnett's *t*-test using computerized GraphPad InStat version 3.05, Graph pad software, U.S.A.

RESULTS

The effect of methanol extract of matured fruits of *Diospyros peregrina* on alloxan-induced animals is indicated in Table 1

Table 1: Effect of methanol extract of matured fruits of *Diospyros peregrina* on fasting plasma glucose level of alloxan-induced diabetic rats after single dose (short-term study)

Group	Initial	1 h	2 h	4 h	6 h
Normal control	78.83 ± 3.28	77.67 ± 2.46	77.83 ± 2.71	78.17 ± 2.32	78.67 ± 2.94
Diabetic control	283.67 ± 9.89*	287.33 ± 11.01*	290.83 ± 12.51*	292.5 ± 10.54*	295.33 ± 9.51*
Diabetic + DPME (150 mg/kg)	289.17 ± 11.98*	279.67 ± 13.05	267.33 ± 12.92	258.33 ± 11.21^c	252.67 ± 10.15^b
Diabetic + DPME (300 mg/kg)	287.17 ± 9.63*	272.17 ± 8.97	256.33 ± 9.34	239.83 ± 8.53^b	233.33 ± 7.24^b
Diabetic + Glibenclamide (1 mg/kg)	286.33 ± 10.84*	263.67 ± 9.31	246.17 ± 8.91^c	230.33 ± 9.17^b	223.83 ± 8.08^b

Values are given as mean ± S.E.M. ($n = 6$); *$P < 0.001$ compared with normal control group; $^cP < 0.05$ compared with diabetic control group; $^bP < 0.01$ compared with diabetic control group

Table 2: Effect of methanol extract of matured fruits of *Diospyros peregrina* on fasting plasma glucose level of alloxan-induced diabetic rats in long-term study

Group	Initial	1st day	2nd day	4th day	7th day	14th day
Normal control	78.83 ± 3.28	77.67 ± 2.23	78.17 ± 3.15	79.16 ± 3.02	78.67 ± 2.33	78.5 ± 2.80
Diabetic control	283.67 ± 9.89*	297.17 ± 8.63*	301.33 ± 8.57*	307.67 ± 9.23*	316.17 ± 8.63*	24.5 ± 9.61*
Diabetic + DPME (150 mg/kg)	289.17 ± 11.98*	247.67 ± 11.21[b]	225.83 ± 9.15[b]	209.17 ± 9.08[b]	179.33 ± 8.80[b]	119.33 ± 7.21[a]
Diabetic + DPME (300 mg/kg)	287.17 ± 9.63*	230.83 ± 6.38[b]	209.17 ± 9.18[b]	192.33 ± 7.05[b]	151.67 ± 5.60[b]	104.83 ± 7.47[a]
Diabetic + Glibenclamide (1 mg/kg)	286.33 ± 10.84*	217.67 ± 7.68[b]	197.83 ± 7.77[b]	189.5 ± 8.73[b]	146.67 ± 6.48[b]	97.33 ± 5.41[a]

Values are given as mean ± S.E.M. (n = 6); *P < 0.001 compared with normal control group; [b]P < 0.01 compared with diabetic control group; [a]P < 0.001 compared with diabetic control group

Table 3: Effect of methanol extract of matured fruits of *Diospyros peregrina* on serum lipids, liver glycogen and pancreatic TBARS levels of alloxan-induced diabetic rats

Group	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Liver glycogen (mg/g)	Pancreatic TBARS (µmol/g)
Normal control	76.83 ± 3.34	78.83 ± 3.12	14.17 ± 1.08	2.68 ± 0.50
Diabetic control	111.17 ± 4.64*	112.67 ± 5.91*	7.45 ± 0.69*	5.18 ± 0.73*
Diabetic + DPME (150 mg/kg)	93.33 ± 5.11[c]	90.33 ± 7.03[c]	11.38 ± 1.01[c]	3.15 ± 0.38[c]
Diabetic + DPME (300 mg/kg)	88.33 ± 4.34[b]	87.67 ± 5.42[c]	13.35 ± 1.21[b]	2.58 ± 0.29[b]
Diabetic + Glibenclamide (1mg/kg)	89.67 ± 3.62[b]	85.5 ± 4.43[b]	14.02 ± 1.19[b]	2.97 ± 0.39[c]

Values are given as mean ± S.E.M. (n = 6); *P < 0.01 compared with normal control group; [c]P < 0.05 compared with diabetic control group; [b]P < 0.01 compared with diabetic control group

(short-term study) and Table 2 (long-term study). The results in Table 1 showed that after a single dose of extract on alloxan diabetic rats, there was a significant reduction (P < 0.01, at 6 h) of fasting blood glucose level within the period of study (6 h) as compared with diabetic control group. The antidiabetic effect was found comparable to that of standard drug glibenclamide (P < 0.01, at 6 h). On chronic administration [Table 2], significant difference was observed between experimental and diabetic control rats in lowering fasting blood glucose level. At a dose of 150 mg/kg body weight, the extract significantly lowered blood glucose level and showed maximum reduction of 58.73% (P < 0.001) on Day 14. The extract, at 300 mg/kg body weight produced maximum reduction of 63.75% (P < 0.001) on Day 14 whereas inhibition of 66.01% (P < 0.001) was found for glibenclamide on Day 14 as a peak. The effect of the extract on serum lipids (*i.e.* triglycerides and cholesterol), liver glycogen levels and pancreatic TBARS level in diabetic rats is indicated in Table 3. Significant increase (P < 0.01) in cholesterol and triglycerides' levels was observed in diabetic rats when compared with normal control groups. Treatment with methanol extract at graded doses significantly lowered the levels of cholesterol and triglyceride when compared with diabetic control group and results are comparable to that of standard drug glibenclamide, particularly at the dose of 300 mg/kg body weight. A significant decrease (P < 0.01) in the level of liver glycogen was observed in diabetic rats when compared with normal control groups. Oral administration of extract at the selected doses significantly increased liver glycogen level to its normal level. The significantly increased (P < 0.01) level of pancreatic TBARS is an index of augmented oxidative state in diabetic rats as compared with normal control was also found to be reverted to near normal status in extract-treated groups. It this case the extract at the dose of 300 mg/kg body weight was found to be more effective than that of standard drug glibenclamide. The changes in initial and final body weight are listed in Table 4. Observed data indicates significant improvement of body weight profile (P < 0.05, at 300 mg/kg body weight) in extract-treated diabetic rats with respect to diabetic control group.

Table 4: Effect of methanol extract of matured fruits of *Diospyros peregrina* on body weight profile of alloxan-induced diabetic rats

Group	Initial body weight (Gram)	Final body weight (Gram)
Normal control	206.67 ± 5.11	213.33 ± 4.01
Diabetic control	204.17 ± 5.69	174.17 ± 6.64
Diabetic + DPME (150 mg/kg)	205.83 ± 8.89	196.67 ± 9.08
Diabetic + DPME (300 mg/kg)	208.33 ± 6.15	201.67 ± 6.67[c]
Diabetic + Glibenclamide (1 mg/kg)	207.5 ± 6.29	204.17 ± 5.97[c]

Values are given as mean ± S.E.M. (n = 6); [c]P < 0.05 compared with diabetic control group

DISCUSSION

Alloxan, a beta cytotoxin, destroys β cells of islet of Langerhans of pancreas resulting in a decrease in endogenous

insulin secretion and paves the ways for the decreased utilization of glucose by the tissue.[23] It results in elevation of blood glucose level. Expression of elevated fasting blood glucose level confirmed induction of diabetes in alloxan-induced experimental rats. The experiment focused on exploring the competence of methanol extract of matured fruits of *Diospyros peregrina* for the correction of diabetes to substantiate folklore claim. The differences between the initial and final fasting blood glucose levels of different groups in both short-term and long-term studies exposed a significant elevation in blood glucose level in diabetic controls as compared with that of normal, extract-treated and glibenclamide-treated animals. Maintenance of blood glucose level with extract-treated rats vindicates the effectiveness of the extract in experimental diabetic animals.

The extract exhibited a significant control of serum lipid profiles in diabetic rats. Diabetes is associated with hyperlipidemia.[24] It is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal conditions. Destruction of β cells leads to depletion of plasma insulin, which results in hyperlipidemia. The significant control of plasma lipid levels suggests that the extract may produce its action by improving insulin secretion.

Excessive hepatic glycogenolysis and gluconeogenesis associated with decreased utilization of glucose by tissue is the fundamental mechanism underlying hyperglycaemia in diabetic state.[25] Aberration of liver glycogen synthesis or glycogenolysis in diabetes may be due to lack of or resistance to insulin, which is essential to activate glycogen synthase system. The significant increase of liver glycogen level in extract-treated diabetic groups may be due to reactivation of the glycogen synthase system by improving insulin secretion. Diabetes is associated with weight loss.[26] The reversal of weight loss in extract-treated diabetic group indicates that the restorative effect of the extract may be by the reversal of gluconeogenesis and glycogenolysis.

Experimental results also reflect that the extract is capable of reducing the oxidative state associated with diabetes. The reduction of thiobarbituric acid levels in tissues in the extract-treated diabetic group ensures the antioxidant potential of the extract. Alloxan produces diabetes by liberating oxygen-free radicals, which cause lipid peroxide-mediated pancreatic injury.[27] The extract may scavenge free radicals and facilitate reconstruction of pancreatic cells to release more insulin and ultimately produces an antidiabetic effect.

In conclusion, the results of this investigation revealed that methanol extracts of matured fruits of *Diospyros peregrina* possesses significant antidiabetic activity in alloxan-induced diabetic rats in a dose-dependent manner. Experimental results also showed that the extract is capable of alleviating the augmented oxidative state associated with diabetes. Preliminary phytochemical screening indicated the presence of flavonoids in the extract. Flavonoids isolated from different sources are reported to have antioxidant activity and antihyperglycaemic activity[28,29] so the lead compound may be flavonoid. Now research is continued to isolate lead compound from this extract.

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