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Hypoglycemic activity of *diospyros peregrina* fruits in diabetic rats

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SUMMARY

Diospyros peregrina Gurke. (Ebenaceae) is a small middle sized tree grows luxuriantly in the plains of costal West Bengal, India. The objective of the study was to explore the antidiabetic activity of methanol extract of matured fruits of *Diospyros peregrina* to substantiate the folklore claim of traditional practitioners. It was also aimed to establish correlation with reduction of oxidative state associated with diabetes. Methanol extract of matured fruits of *Diospyros peregrina* was administered orally at doses of 150 and 300 mg/kg body weight for 12 consecutive days to normal and streptozotocin induced diabetic rats. Fasting blood glucose level was estimated in both normal and diabetic rats while serum lipid profiles, liver glycogen level and pancreatic thiobarbituric acid reactive substances (TBARS) were evaluated for diabetic rats. Initial and final changes in body weight were also recorded. Oral glucose tolerance test was performed during the course of study. Experimental findings showed significant antidiabetic potential of extract in term of reduction of fasting blood glucose level of both normal and diabetic rats. It was found that extract at the dose of 300 mg/kg body weight is more effective and percentage reduction (55.64) of elevated blood glucose level is comparable to that of standard drug glibenclamide (60.60) at a dose of 10 mg/kg body weight. Observed data found statistically significant in reduction of serum lipid and pancreatic TBARS levels whilst improvement was observed in liver glycogen level and body weight profiles in extract treated diabetic rats.

Key words: *Diospyros peregrina*; Ebenaceae; Diabetes; Streptozotocin; Antihyperglycemic

INTRODUCTION

The term diabetes mellitus encompasses a heterogeneous group of disorders of carbohydrate, fat and protein metabolism characterized by insulin hyposecretion or insensitivity resulting elevation of blood glucose levels (Balkau *et al.*, 2000) and a greatly increased risk of heart disease, stroke, kidney disease, retinopathy and loss of nerve function (Kuyvenhoven *et al.*, 1999). In spite of the great strides that have been made to understand the management of diabetes, the disease and its related complications are increasingly unabated. Phytochemicals obtained from traditional medicinal plants are presenting a stirring prospect for the expansion of an alternative way of treatment (Bailey *et al.*, 1989; Rahman *et al.*, 1989). Moreover, plant drugs are frequently considered to be less toxic with fewer side effects (Momin, 1987). A

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number of medicinal plants namely *Gymnema sylvestre*, *Momordica charantia, Trigonella foenum graecum, Panax ginseng*, *Allium sativum*, *Allium cepa, Aloe barbadensis*, *Silybum marianum*, *Ginkgo bialoba* (Kaczmar, 1998) have been reported to possess significant antidiabetic activity. Thus the aim of present investigation lies on scientific exploration of antidiabetic efficacy of a traditional plant based on its folklore claim to lengthen the queue of antidiabetic herbs.

Diospyros peregrina Gurke. (Ebenaceae) is a small middle sized tree, glabrous except younger parts with numerous spready branches, forming an impenetrable shady head grows luxuriantly in the plains of costal West Bengal. Ripe fruits are edible with ethnomedicinal significance as tonic and aphrodisiace (Kirtikar *et al.,* 1975). Unripe fruits are astringent, acrid, bitter and oleaginous (Anjaria *et al.,* 2002). Unripe fruits are used for the treatment of diarrhoea, dysentery, cholera, ulcer of mouth and in wounds (Asolkar *et al.,* 1992). The fruits contain triterpenes, alkanes, flavonoids and tannins (Misra *et al.,* 1971; Chauhan *et al.,* 1982; Chopra *et al.,* 1992; Jain *et al.,* 1994, 1997). The stem barks of the plant have been reported for its hypoglycemic activity (Ghani, 1998). The maceration of matured fruits is successfully employed in costal West Bengal for the treatment of diabetes. The present investigation is directed to the exploration of antidiabetic activity of methanol extract of matured fruits of *Diospyros peregrina*. An attempt was also made to find out antioxidant potential of aforementioned plant with an aim to establish correlation with the reduction of oxidative state associated with diabetes.

MATERIALS AND METHODS

Plant material

Matured fruits of *Diospyros peregrina* (Family: Ebenaceae) were collected in the month of June and July, 2006 from the villages of South 24 Parganas, West-Bengal, India. The plant was authenticated by the taxonomist of Central National Herbarium, Botanical Survey of India, Shibpur, Howrah, India. A voucher specimen of number entitled SCM-JU-09 was deposited at our departmental herbarium for future reference.

Preparation of methanol extract

Methanol extract of fruits was prepared in accordance to the method of National Institute of Health and Family Welfare, New Delhi, India. Matured fruits of *Diospyros peregrina* were dried in an incubator for two days at 40 ºC, crushed in a mechanical grinder to fine powder of mesh 40. 500 g of powder was then extracted with 2.5 L of 90% methanol in a soxhlet apparatus until the powder became exhausted totally. Resulting extract was filtered by course sieve filter paper. The filtrate was dried under reduced pressure with the help of rotary vacuum evaporator and finally lyophilized to give an extract sample with a yield of 8.75% w/w. The extract was stored in a dessicator for use in subsequent experiments.

Animals

Healthy adult Wister strain albino rats of both sex between 2 - 3 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 order; at an ambient temperature of 25 ± 5 ºC; 35 - 60% of relative humidity); the animals were fed with standard rat pellet diet (Hindustan Liver Ltd. Mumbai, India) and water *ad libitum*. The principles of Laboratory Animals care (PHS, 1986) 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

Streptozotocin used for the induction of diabetes

was procured from Sisco Research Laboratory Pvt. Ltd., India and other reagents used in the experiment were of analytical grade. Glibenclamide (Daonil™, Hoechst, India) tablets used as standard antidiabetic agent were purchased from local medical store, Jadavpur, India.

Oral glucose tolerance test (OGTT)

Eighteen rats were divided into three groups for oral glucose tolerance test. The OGTT was performed on overnight (18 h) fasted normal Wister strain albino rats. Control group received distilled water. Test groups were treated with methanol extract at doses of 150 and 300 mg/kg body weight respectively oral route. Glucose (2 g/kg body weight) was fed orally 30 min prior to the administration of extracts (Bonner-Weir, 1988). Blood glucose level was measured at 0, 1 and 2 h with the help of single touch glucometer (Ascensia Entrust, Bayer Health Care, USA).

Normoglycemic study

Eighteen animals were divided into three groups of six animals each subjected for normoglycemic study. Test groups were treated with methanol extract at 150 and 300 mg/kg, respectively by oral route once in a day for twelve days whilst control group received only distilled water. After subjecting an over night fast on day 1, 5, 9 and 12 blood samples were withdrawn from tail vein of each animal for fasting blood glucose estimation. Blood glucose level was estimated by the one touch glucometer.

Antihyperglycemic studies

Induction of diabetes

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 (dissolved in 0.1 M ice-cold citrate buffer, pH 4.5, immediately before use) in a volume 1 ml/kg body weight (Siddque *et al.,* 1987). 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 Antihyperglycemic study.

Experimental design

Animals were divided into four groups of six rats in each group. 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 10 mg/kg body weight and distilled water respectively. All doses were started forty eight hours after streptozotocin injection. Fasting blood glucose levels were estimated on overnight fasted rats on day 1, 5, 9 and 12. Serum lipid profiles, liver glycogen profile (Caroll *et al.,* 1956) and pancreatic thiobarbituric acid reactive substances (Hiroshi *et al.,* 1979) were measured after the animals were sacrificed after 12 days by decapitation. Initial and final changes in body weight were also measured (Shirwaikar *et al.,* 2004).

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, USA.

RESULTS

In OGTT, the extract, at 2nd h, showed significant reduction in plasma glucose level indicated in Table 1. Expression of elevated fasting blood glucose level confirmed induction of diabetes in streptozotocin induced experimental rats. The effect of methanol extract of matured fruits of *Diospyros peregrina* on streptozotocin induced animal was presented in Table 2. The difference between experimental and diabetic control rats in lowering fasting blood glucose level was found to be statistically significant ($P < 0.01$) from 5th day onward and very

Table 1. Effect of methanol extract of matured fruits of *Diospyros peregrina* on oral glucose tolerance test (OGTT)

Group	Dose (Oral)	Fasting blood glucose level (mg/dl)		
		0 h	1st h	2nd h
Control	-	73.83 ± 2.23	96.67 ± 2.32	95.33 ± 2.7
Extract treated	150 mg/kg	71.67 ± 1.71	93.67 ± 2.94	76.17 ± 2.57**
Extract treated	300 mg/kg	69.33 ± 1.28	90.33 ± 2.58	72.83 ± 1.62**

Values are expressed as mean ± S.E.M. (n = 6). $^{**}P < 0.01$, when compared with normal control.

Table 2. Effect of methanol extract of matured fruits of *Diospyros peregrina* on fasting plasma glucose level in normal and streptozotocin induced diabetic rats

Group	Dose (mg/kg)	Fasting blood glucose level (mg/dl)			
		1st day	5th day	9th day	12th day
Normal Control	-	70.67 ± 2.11	69.67 ± 3.43	71.83 ± 2.04	71.33 ± 1.98
Normal + Extract treated	150 mg/kg	74.17 ± 2.47	71.83 ± 1.57 (4.88)	69.5 ± 1.96 (6.29)	67.17 ± 1.58 (9.44)
Norma l + Extract treated	300 mg/kg	75.17 ± 2.30	70.50 ± 1.84 (6.21)	68.33 ± 2.04 (9.10)	65.50 ± 1.54 (12.86)
Diabetic control	-	253.83 ± 5.23	265.33 ± 3.21	272.50 ± 4.60	275.83 ± 5.01
Diabetic + Extract treated	150 mg/kg	258.83 ± 4.35	194.67 ± 5.77** (24.79)	156.67 ± 2.92** (39.47)	128.33 ± 3.24** (50.42)
Diabetic + Extract treated	300 mg/kg	267.17 ± 5.68	179.33 ± 5.99** (32.88)	146.33 ± 4.82** (45.23)	118.50 ± 4.15** (55.64)
Diabetic + Glibenclamide	10 mg/kg	275.83 ± 5.19	171.33 ± 4.40*** (37.88)	130.67 ± 3.95*** (52.63)	108.67 ± 3.21*** (60.60)

Values are expressed as mean ± S.E.M. (n = 6). $^{**}P < 0.01$, $^{***}P < 0.001$ when compared with diabetic control.

much comparable to that of standard drug glibenclamide. At a dose of 150 mg/kg body weight, the extract significantly lowered blood glucose level and showed maximum percentage reduction of 50.42 on 12th day. The extract, at 300 mg/kg oral dose, maximum percent reduction was found at a value of 55.64 on 12th day whereas percentage inhibition of 60.60 was found for glibenclamide on 12th day as a peak. It was found that methanol extract is also capable to lower blood glucose level in normal rats. Percentage reduction of fasting blood glucose levels was found 9.44 and 12.86 for normal rats at the doses of 150 and 300 mg/kg body weight respectively on 12th day as a peak. Significance differences were found in serum lipids (*i.e.* triglycerides and cholesterol) liver glycogen levels and pancreatic thiobarbituric acid reactive substances (TBARS) level indicated in Table 3. The changes in initial and final body weight were enlisted in Table 4. Observed data

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

Group	Dose (Oral)	Cholesterol (mg/dl)	Triglycerides (mg/dl)	Liver glycogen (mg/g)	TBARS (ìmol/g)
Diabetic control	-	105.83 ± 3.00	99.00 ± 5.58	6.25 ± 0.64	4.85 ± 0.57
Diabetic + Extract treated	150 mg/kg	66.17 ± 2.04**	74.83 ± 3.13**	10.87 ± 0.84*	3.13 ± 0.40*
Diabetic + Extract treated	300 mg/kg	61.5 ± 2.55**	64.83 ± 1.66**	14.27 ± 1.22**	2.02 ± 0.19**
Diabetic + Glibenclamide	10 mg/kg	57.5 ± 2.39**	53.17 ± 2.83**	15.05 ± 1.21**	2.16 ± 0.18**

Values are expressed as mean ± S.E.M. (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ when compared with diabetic control.

Table 4. Effect of methanol extract of matured fruits of *Diospyros peregrina* on changes in body weight in normal and streptozotocin induced diabetic rats

Group	Dose (Oral)	Initial body weight (Gram)	Final body weight (Gram)
I (Normal control)	-	199.17 ± 7.68	205.83 ± 5.23**
II (Diabetic control)	-	198.33 ± 6.15	169.17 ± 4.55
III (Diabetic + Extract)	150 mg/kg	206.67 ± 6.28	195.83 ± 5.83*
IV (Diabetic + Extract)	300 mg/kg	205.33 ± 7.68	198.33 ± 8.23**
V (Diabetic + Glibenclamide)	1 mg/kg	207.5 ± 7.5	201.67 ± 6.41**

Values are expressed as mean ± S.E.M. (n = 6). $^{*}P < 0.05$, $^{**}P < 0.01$ when compared with diabetic control rats.

indicated that, the significant improvement of body weight profile for extract treated diabetic rats with respect to diabetic control group.

DISCUSSION

Streptozotocin, an N-nitroso derivative of glucosamine (Leslie *et al.,* 1994) is a potent toxin for β cell of islet of Langerhans of pancreas and causes hyperglycemia in rats (Palmer *et al.,* 1998). The experiment focused to explore the competence of methanol extract of matured fruits of *Diospyros peregrina* for the correction of diabetes to substantiate folklore claim. In OGTT, hypoglycemic effect was observed at 2 h after administration of extract. It reflects the efficiency of extract to control elevated blood glucose levels. The differences between initial and final fasting blood glucose levels of different groups exposed a significant elevation in blood glucose level in diabetic control as compared with that of normal, extract treated and glibenclamide treated animals at the end of 12th day. Maintenance of blood glucose level in both normal and diabetic rats with extract treatment vindicates the effectiveness of extract. It also vindicates the significant control of serum lipid profiles in the extract treated diabetic rats and results are comparable with that of standard drug. Diabetes is associated with hyperlipidemia (Chase *et al.,* 1976). It is well known that insulin activates enzyme lipoprotein lipase, which hydrolyzes triglyceride under normal condition. Destruction of β cells leads depletion of plasma insulin, which results 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 decrease utilization of glucose by tissue is the fundamental mechanism underlying hyperglycemia in diabetic state (Swanston Flatt *et al.,* 1990). 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 glycogen synthase system by improving insulin secretion. Diabetes is associated with weight loss (Huang *et al.,* 2000). The reversal of weight loss in extract treated diabetic group indicates the restorative effect of extract may be by the reversal of gluconeogenesis and glycogenolysis.

Experimental results also reflect that the extract is capable to reduce oxidative state associated with diabetes. The reduction of thiobarbituric acid levels in tissues in extract treated diabetic group ensures the antioxidant potential of extract. Streptozotocin produces diabetes by liberating oxygen free radicals, which cause lipid peroxide mediated pancreatic injury (Halliwell *et al.,* 1985). The extract may scavenge free radicals and facilitate reconstruction of pancreatic cells to release more insulin. Preliminary phytochemical screening indicated that the presence of flavonoids in the extract. Flavonoids isolated from different sources are reported to have antioxidant activity and antihyperglycemic activity (Olmedilla,

1999; Miura *et al.,* 2001) so the lead compound may be flavonoid. Now our intention is guided to isolate bioactive flavonoid from extract and to substantiate its effectiveness against diabetes.

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