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Article

Antidiabetic and antihyperlipidemic effects of *Dillenia indica* (L.) leaves extract

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The present study was carried out to evaluate antidiabetic and antihyperlipidemic effects of *Dillenia indica* methanolic leaves extracts in streptozotocin induced diabetic Wistar rats by administering graded oral doses (250 and 500 mg/kg body weight) for 21 days. The extract showed significant antidiabetic activity ($p<0.001$). Furthermore, the decreased body weight of rats was significantly improved after extract treatments. Daily oral treatment with the extract for 21 days to diabetic rats, also resulted in significant reduction in serum cholesterol, triglycerides and serum transaminase levels but HDL-cholesterol level was found to be improved ($p<0.001$) as compared to the diabetic control group. The extract treatment also showed to enhance serum insulin level in diabetic rats as compared to the diabetic control group. In conclusion, *D. indica* leaf extract might be useful for diabetes mellitus management and other abnormalities associated with this metabolic disorder.

Uniterms: *Dillenia indica*/antidiabetic effects/experimental study. *Dillenia indica*/antihyperlipidemic effects/experimental study. Antidiabetics/experimental study. Serum transaminase. Serum cholesterol. HDL-cholesterol level.

Realizou-se o presente estudo para avaliar os efeitos antidiabético e anti-hiperlipidêmico de extratos metanólicos de folhas de *Dillenia indica* em ratos wistar com diabetes induzido por estreptozotocina por meio da administração de doses orais (250 e 500 mg/kg de peso corporal) por 21 dias. O extrato mostrou atividade antidiabética significativa ($p<0,001$). Além disso, a diminuição do peso corporal dos ratos foi significativamente melhorada após o tratamento com os extratos. O tratamento com doses orais do extrato por 21 dias aos ratos diabéticos também resultou em redução significativa do colesterol, triglicerídios e níveis de transaminase séricos, mas o nível de HDL-colesterol foi melhorado ($p<0,001$), quando comparado ao grupo controle diabético. O tratamento com extrato também mostrou aumento do nível sérico de insulina em ratos diabéticos comparativamente ao grupo controle diabético. Em conclusão, o extrato de folha de *D. indica* poderia ser útil para o controle do diabetes mellitus e de outras anormalidades associadas a essa disfunção metabólica.

Unitermos: *Dillenia indica*/efeitos antidiabéticos/estudo experimental. *Dillenia indica*/efeito anti-hiperlipidêmico/estudo experimental. Antidiabéticos/estudo experimental. Transaminase sérica. Colesterol sérico. Nível de HDL-colesterol.

INTRODUCTION

Diabetes mellitus is a metabolic disorder affecting carbohydrate, fat and protein metabolism that affects nearly 25% of the population afflicting 150 million people, a figure set to rise to 300 million by 2025 (Vats *et al.*, 2000; Vetrichelvan *et al.*, 2002). The disease causes numerous complications such as retinopathy, neuropathy, and peripheral vascular insufficiencies (Chehade, Mooradian, 2000). Hyperglycemia can be handled initially with oral synthetic agents and insulin therapy. However, these synthetic agents produce some serious side effects and are relatively expensive for developing countries. Therefore, searching for effective, low cost hypoglycemic agents with fewer side effects is important (Rubin *et al.*, 1994). Since ancient times, diabetes has been treated orally with several medicinal plants or their extracts based on folklore medicine. The World Health Organization has also recom-

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mended the evaluation of traditional plant treatments for diabetes (Day, 1998).

Dillenia indica Linn. (Family: Dilleniaceae) is an evergreen tree, 30–80 ft. in height, which bears large and hard fruit 3-5 in. in diameter and grows in moist and evergreen forests of India. The ripe fruits are widely used in the flavoring of curries and preparation of jam and jelly. The acidic juice is sweetened with sugar and used as a cooling drink (The Wealth of India, 1992). The fruit is said to possess tonic laxative properties and is used for relieving abdominal pain. The bark and leaves are astringent (Kirtikar, Basu, 2003). The mixed juices of leaves, bark and fruits are given orally for the treatment of cancer and diarrhea (Sharma *et al.*, 2001). Traditionally, the plant is also used for treatment of diabetes (Sood *et al.*, 2005). The literature survey revealed that there is no experimental evidence of the plant's antidiabetic effect. Therefore, the present work was undertaken to explore the antidiabetic and antihyperlipidemic potentials of the leaves extract of the plant.

MATERIAL AND METHODS

Plant material

D. indica leaves were collected from the campus of Kurukshetra University, Kurukshetra, India during the month of October, 2009 and were identified by Dr. H.B. Singh, scientist F& Head, Raw Material Herbarium & Museum, NISCAIR, and New Delhi, India. A voucher specimen of the plant is preserved in the herbarium (NISCAIR/RHMD/Consult/-2009-10/1381/182/1).

Extract preparation

The leaves were dried under shade and powdered to coarse particles. The powdered plant material was defatted with petroleum ether (60-80°C) in a Soxhlet extraction apparatus at 60°C and further the same amount of plant material extracted with methanol. The extract was dried at 45^{0}C in a rotary evaporator to produce a semisolid mass and stored in airtight containers in a refrigerator below 10 ^{0}C.

Chemicals

Streptozotocin was purchased from Sigma-Aldrich, India. Total cholesterol, High density lipoprotein (HDL)-cholesterol and triglyceride (TC), serum transaminase were assayed by an autoanalyser (Erba Chem 7, Mannheim, Germany) using standard kits from Erba diagnostics Mannheim Gambh, Germany, and Blood glucose level was measured using an Elegance glucose meter (CT-X10) by Convergent Technologies, Germany. All reagents used in the study were analytical grade.

Animals

Wistar rats of both sexes (15 male and 15 female rats), weighing about 150-250 g were used in the study. Animals were maintained under standard environmental conditions i.e. ambient temperature of 22 ± 2 ^{0}C and at 45–55% relative humidity for 12 h, each of dark and light cycle, and fed with a standard pellet rat diet obtained from Ashirwad Industries, Chandigarh, India and water was supplied *ad libitum*. All the studies were conducted in accordance with the Animal Ethics Committee of the University.

Antidiabetic studies

Induction of diabetes

After one week of acclimatization, the rats were subjected to a 12-h fast. Diabetes was induced with a single injection of streptozotocin (STZ) 60 mg/kg body weight i.p. The STZ was freshly dissolved in citrate buffer (0.01 M, pH 4.5). The blood glucose level was checked before and 72 h after streptozotocin injection to confirm the development of diabetes. The diabetic animals were stabilized for five days and the experiment was started on the next day (day 0). Only those animals which showed hyperglycemia (blood glucose levels >250 mg/dL) were used in the experiment.

Experimental design

Overnight fasted rats were divided into five groups and six animals were taken for each group. The extract was dissolved in Tween 80, 1% v/v in saline and given orally. Group I (Normal healthy control) received only vehicle (Tween 80, 1% v/v in saline). Group II served as the diabetic control, Group III and IV received *D. indica* methanolic leaves extract (DIME) 250 and 500 mg/kg.b.w respectively once a day for 21 days and Group V received (Glibenclamide 10 mg/kg.b.w.) once a day for 21 days and served as the standard.

Biochemical parameters

Blood glucose was measured with an Elegance glucometer (Frankenberg, Germany) at weekly intervals i.e. 0, 7, 14 and 21 days after daily administration of extract orally. On the 21st day all the animals were sacrificed and evaluated for the biochemical status of serum alanine

transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) by autoanalyser using Erba diagnostic kits (Brandely, Maynard, 1972; Wilkinson *et al.*, 1969). Serum insulin levels were determined using insulin ELISA kits (Kratzsch *et al.*, 1990).

Antihyperlipidemic effect

Serum total cholesterol and triglycerides were determined by the method of Rifai *et al.*, 1999. HDL-cholesterol was also evaluated in normal and streptozotocin-induced diabetic rats by autoanalyser using Erba diagnostic kits (Burstein *et al.,* 1970).

Statistical analysis

Results are presented as mean ± standard error of mean (S.E.M.) The statistical analysis involving two groups was evaluated by means of Student's t-test whereas One way analysis of variance (ANOVA) followed by Dunnet's multiple comparison post-test was used for statistical comparison between control and the various treated groups. Statistical significance was accepted at the $p < 0.05$ values.

RESULTS

Effect on blood glucose level and serum insulin

Table I shows the results of blood glucose values in STZ-induced diabetic rats, after the daily treatment with the methanolic extract of *D. indica* (DIME 250 and 500 mg/kg, p.o.), for 21 days. A significant decrease ($p<0.001$) in blood glucose levels were observed in the groups treated with both doses of DIME of 48.64 and 55.86%, respectively, as compared to the same group before treatment. At the end of the experiment (21st day) blood glucose level was 139.43 ± 2.28 and 124.32 ± 1.75 mg/dL of the groups treated with the doses of DIME 250 and 500 mg/kg, respectively. Serum insulin level was also significantly improved by treatment with DIME in the diabetic rats.

Effect on body weight

Body weight was slightly increased in the normal control rats (group I) compared to initial body weight whereas in the diabetic control rats (group II) there was a significant decrease in body weight. Glibenclamide (10 mg/kg) as well as the extracts (DIME 250 and 500 mg/kg) treatment significantly ($p < 0.05$) prevented this reduction in body weight. Thus, based on weekly observation of the extract-treated diabetic rats, there were significant weight gains on day 21 compared to day 0 as shown in Table II.

Effect on lipid profile and serum transaminase

In diabetic rats, there was a significant increase in total cholesterol and triglycerides, as well as a significant decrease in HDL cholesterol in serum compared to that of normal controls. The standard drugs as well as DIME (250 and 500 mg/kg) plant extracts used in the experimental study significantly decreased ($p < 0.05$) the levels of cholesterol and triglycerides whereas HDL cholesterol level was improved (Table III) after 21 days of treatment. Also, at the end of study, the serum transaminase, such as AST, ALT and ALP activity, was significantly elevated in the diabetic control groups. After supplementation with DIME (250 and 500 mg/kg) and glibenclamide (10 mg/kg), the serum transaminase level was restored to normal levels (Table III).

TABLE I - Effect of *Dillenia indica* leaves extract on blood glucose and insulin levels in diabetic rats

Groups/Treatments	Blood glucose level (mg/dL)				Serum Insulin (IU/dL)
	Initial day	Day 7	Day 14	Day 21	
I: Vehicle	115.27 ± 4.5	113.34 ± 3.8	112.7 ± 5.2	113.82 ± 2.4	4.3 ± 0.25
II: STZ	258.41 ± 2.32	294.47 ± 5.47	348.7 ± 5.32	402 ± 3.47	1.2 ± 0.23
III: STZ+ DIME (250 mg/kg)	271.45 ± 5.17	265.4 ± 3.27*	178.3 ± 3.17*	139.43 ± 2.28*	2.3 ± 1.25*
IV: STZ+ DIME (500 mg/kg)	281.62 ± 3.4	215.27 ± 3.7*	155.24 ± 2.7**	124.32 ± 1.75**	2.9 ± 1.22/, *
V: Glibenclamide (10 mg/kg. b.w.)	274.27 ± 3.52	210.72 ± 4.25**	125.41 ± 3.45*	118.53 ± 3.5**	3.2 ± 1.23*

Data represent means ± S.E.M. of six animals in each group. *$p<0.05$, **$p<0.001$, when groups III and VI are compared with diabetic control, i.e. group II. SZT: streptozotocin (60 mg/kg body weight); DIME: *D. indica*

TABLE II - Effect of DIME on body weight in diabetic rats

Groups	Body weight (g)			
	Initial day	Day 7	Day 14	Day 21
I: Vehicle	215.2 ± 2.35	222.43 ± 4.22	225.41 ± 3.62	228.47 ± 3.17
II: STZ	225.23 ± 3.4	219.42 ± 3.85	211.35 ± 2.72	208.25 ± 4.32
III: STZ+ DIME (250 mg/kg)	228.27 ± 2.41	223.25 ± 3.4*	224.72 ± 2.37*	227.34 ± 3.42*
IV: STZ+ DIME (500 mg/kg)	225.52 ± 1.28	223.27 ± 3.21	225.72 ± 2.42*	227.33 ± 2.21*
V: Glibenclamide (10 mg/kg.b.w.)	225.34 ± 2.71	227.34 ± 2.36*	228.78 ± 2.35*	231.25 ± 1.83*

Data represent means ± S.E.M of six animals in each group. *p<0.05, when groups III and VI are compared with diabetic control (Group II). SZT: streptozotocin (60 mg/kg body weight); DIME: *D. indica*

TABLE III - Effect of DIME on lipid profile and serum enzymes in diabetic rats

Groups	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL cholesterol (mg/dL)	AST (U/L)	ALT (U/L)	ALP (U/L)
I	87.28 ± 3.8	82.42 ± 5.16	37.32 ± 2.9	56.27 ± 3.15	62.34 ± 3.27	123.34 ± 3.25
II	254.73 ± 7.6	150.52 ± 4.71	28.23 ± 2.2	145.34 ± 4.32	135.43 ± 3.13	240.37 ± 4.47
III	125.32 ± 11.2*	117.24 ± 4.51*	34.24 ± 3.5*	94.32 ± 2.83**	88.45 ± 4.24*	179.52 ± 2.83*
IV	110.47 ± 4.7*	92.24 ± 6.32*	41.35 ± 2.6*	73.25 ± 3.35*	76.54 ± 3.25**	153.44 ± 3.23*
V	98.72 ± 5.3**	83.47 ± 4.5*	45.28 ± 4.8**	65.53 ± 4.25**	71.25 ± 2.45*	131.24 ± 2.43*

Data represent means ± S.E.M. of six animals in each group. *p<0.05, **p <0.001, when groups III and VI are compared with diabetic control (Group II). SZT: streptozotocin (60 mg/kg body weight); DIME: *D. indica;* HDL: high-density lipoprotein; AST: aspartate aminotransferase: ALT: alanine aminotransferase: Alp: alkaline phosphatase

DISCUSSION

Streptozotocin possess diabetogenic properties mediated by pancreatic beta cell destruction, the cells that normally regulate blood glucose levels by producing the hormone insulin, and this compound has been widely used to induce diabetes in experimental animals (Junod, 1969). A 21-day treatment by DIME and glibenclamide in the STZ-induced diabetic rats significantly reduced the elevated blood sugar levels. This shows that the extract may not be able to produce the effect by one dose but by continuous treatment it acts effectively. It is well established that glibenclamide (a long lasting sulfonylurea) acts mainly by stimulating insulin secretion. The DIME treatment also increased the insulin level. Thus, one possible antidiabetic mechanism of *D. indica* extract may be stimulation of insulin secretion.

STZ also induces oxidative stress or relative overload of oxidants i.e. reactive oxygen species (Wright, 1999). *D. indica* leaves are rich in polyphenols and have an *in vitro* antioxidant effect (Arbianti, 2007). Various studies have shown that diabetes is associated with increased formation of free radicals and a decrease in antioxidant potential.

STZ-induced diabetes is characterized by severe loss in body weight and this reduction is due to loss or degeneration of structural proteins, as the structural proteins are a known major contributor to body weight (Chen, Ianuzzo, 1982). A significant weight loss was observed in the diabetic group which was improved significantly by the DIME in treated groups.

The most commonly observed lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia (Shepherd, 2005; Shirwaikar, 2006) and contribute to coronary artery disease (Arvind *et al.*, 2005). In fact, lipid abnormalities accompanying with atherosclerosis is the major cause of cardiovascular disease in diabetes. Therefore, the ideal treatment of diabetes, in addition to glycemic control, should have a favorable effect on lipid profiles. Marked increases in total cholesterol, triglyceride levels and decrease in HDL cholesterol have been observed in diabetic control rats. In the present study, the total cholesterol and triglycerides increased in the diabetic control group and were reduced after the 21 days of treatment with DIME whereas the HDL cholesterol level was significantly increased. This suggests that the extract may inhibit the pathway of cholesterol synthesis (Rang, Dale, 1999).

Elevated transaminase such as AST, ALT and ALP was observed in diabetic rats and indicates hepatic damage. The elevated transaminase activities were significantly reduced by the DIME treatment. Diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated transaminase activity (Ghosh, Suryawansi, 2001). These results suggest that DIME may also act as a hepatoprotective agent.

The present study demonstrated that DIME could be useful in the management of diabetes associated with abnormalities in lipid profiles and transaminase. The study needs to be validated in human volunteers prior to proposed usage of DIME in other human volunteers.

CONCLUSION

Based on these results, it may be concluded that the antidiabetic activity of DIME may sensitize the insulin receptor or stimulate the secretion of insulin from beta cells of Islets of Langerhans in pancreas of STZ-induced diabetic rats, where this was supported by improved *in vitro* antioxidant status. The extract also improved other biochemical parameters associated with diabetes. Therefore, the present study clearly reveals the importance of leaves of *D. indica* as an economical antidiabetic agent. The plant bears potential for further research to isolate the antidiabetic principle.

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