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Antidiabetic, hypolipidemic and histopathological analysis of *Dillenia indica* (L.) leaves extract on alloxan induced diabetic rats

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ABSTRACT

Objective: To investigate antidiabetic, hypolipidemic histopathological analysis of *Dillenia indica (D. indica)* methanolic leaves (DIME) extract in alloxan induced diabetic rat by administering oral doses (250 and 500 mg/kg body weight). **Methods:** Blood glucose levels were measured using blood glucose test strips with elegance glucometer on weekly intervals till the end of study (*i.e.* 3 weeks). Other parameters *e.g.* liver profile, renal profile and total lipid levels were determined in normal and alloxan induced diabetic rats after oral administration of the extract for 21 days. Histopathological changes in diabetic rat organs (pancreas, liver and kidney) were also observed after extract treatment. **Results:** Daily oral administration DIME (250 and 500 mg/kg body weight) and glibenclamide (10 mg/kg) showed beneficial effects on blood glucose level (P<0.001) as well as improving kidney, liver functions and hyperlipidaemia due to diabetes. The extract treatment also showed to enhanced serum insulin level and body weight of diabetic rats as compared to diabetic control group. Furthermore, the extract has a favorable effect on the histopathological changes of the pancreas, liver and kidney in alloxan induced diabetes. **Conclusions:** *D. indica* possess antidiabetic property as well improve body weight, liver profile, renal profile and total lipid levels. DIME has also favorable effect to inhibit the histopathological changes of the pancreas and kidney in alloxan induced diabetes.

1. Introduction

Diabetes is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The total number of people with diabetes is projected to increase from 171 million in 2000 to 366 million in 2030[1].

The pathogenesis of diabetes mellitus is managed by insulin and oral administration of hypoglycemic drugs such as sulfonylureas and biguanides[2]. Unfortunately, apart from having a number of side effects, none of the oral synthetic hypoglycemic agents has been successful in diabetes management and controlling long-term microvascular and macrovascular complications[2-4]. The toxicity of oral antidiabetic agents differs widely in clinical manifestations, severity, and treatment[5].

Alternative medicines particularly herbal medicines are available for the treatment of diabetes. Common advantages of herbal medicines are effectiveness, safety, affordability and acceptability[6]. Medicinal plants and their products have been used in the Indian traditional system of medicine and have shown experimental or clinical anti-diabetic activity[7,8]. Furthermore, World Health Organization has also recommended the evaluation of traditional plant treatments for diabetes[9].

Dillenia indica (D. indica) plant belongs to family Dilleniaceae, commonly called *Dillenia*. The fruit shows laxative properties and is used for relieving abdominal pain. The bark and leaves have astringent effect[12]. The juice of *D. indica* leaves; bark and fruits are mixed and given orally for the treatment of cancer and diarrhea[13]. Fruits and leaves extracts of *D. indica* are reported to have antioxidant activity[14]. CNS depressant activities[15] and anti-inflammatory activity[16] in mice and antimicrobial activity[17] were found from the alcoholic extract of the leaves of *D. indica*. Traditionally, the plant is also used for

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treatment of diabetes[18]. So, considering the traditional use this plant in diabetes treatment, this study was carried out in order to investigate the antidiabetic, hypolipidemic and histopathological analysis of *D. indica* methanolic leaves extract in alloxan induced diabetic rat.

2. Materials and methods

2.1. Plant material

D. indica leaves were collected from the campus of Kurukshetra University, Kurukshetra, India during month of October, 2009 and were identified by Dr. HB 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).

2.2. Extract preparation

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

2.3. Chemicals

Alloxan was purchased from Loba chemie Pvt. Ltd. Mumbai, India. Total cholesterol (TC), serum high-density lipoprotein (HDL), serum Creatinine (SC), serum urea (SU), serum alkaline phosphate (ALP), alanine transaminase (ALT), serum aspartate transaminase (AST) and triglyceride (TG) standard kits were obtained from Erba diagnostics Mannheim Gambh, Germany. Blood glucose level was measured using Elegance glucose meter (CT-X10) of Convergent Technologies, Germany. All reagents used in study were analytical grade.

2.4. Animals

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

2.5. Induction of diabetes

Rats were made diabetic by a single intraperitoneal injection of alloxan monohydrate (Loba Chemie, Bombay; 150 mg/kg i.p.) in sterile saline. Twelve days after Alloxan injection, rats with blood glucose level of >200 mg/dL were separated and used for the study. Blood glucose levels were measured using blood glucose test strips with elegance glucometer (Frankenberg, Germany) at weekly intervals till the end of study (*i.e.* 3 weeks). Blood glucose estimation and body weight measurement were done on 0, 7, 14 and 21 day after administration of extract orally.

2.6. Experimental design

Overnight fasted rats were divided into five groups and for each group six animals and treated orally once a day for 21 days as follows:

Group I. Normal healthy control: given only vehicle (Tween 80, 1% v/v)

Group II. Diabetic control: given only vehicle (Tween 80, 5% v/v)

Group III. Diabetic rats given DIME (250 mg/kg b.w.)

Group IV. Diabetic rats given DIME (500 mg/kg b.w.)

Group V. Diabetic rats given glibenclamide (10 mg/kg b.w.).

2.7. Biochemical parameters

Blood glucose was measured with elegance glucometer (Frankenberg, Germany) at weekly intervals *i.e.* 0, 7, 14 and 21 day after daily administration of extract orally. After blood glucose estimation on day 21, whole blood was collected by cardiac puncture under mild ether anesthesia from rats. Serum cholesterol, triglycerides, creatinine, urea, alkaline phosphatase, HDL and total proteins levels were also evaluated in normal and alloxan induces diabetic rats[19]. Serum alanine transaminase (ALT) and serum aspartate transaminase (AST) were measured by autoanalyser (Erba Chem 7, Mannheim, Germany) using Erba diagnostic kits[20-21]. Serum insulin levels were determined using insulin ELISA kit[22].

2.8. Statistical analysis

All values of 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 various treated groups. Statistical significance was accepted at the $P<0.05$ values.

3. Results

3.1. Antidiabetic activity

Single dose alloxan monohydrate (150 mg/kg) significantly

(P<0.01) increases the blood glucose as shown in Table 1. After the daily oral administration with DIME (250 and 500 mg/kg, p.o.), for 21 days, significant decreased (P<0.01) in the blood glucose levels was observed in the diabetic rats. The reduced insulin level in diabetic rats was also significantly improved by treatment of DIME. At the end of experiment (21st day) blood glucose level was (145.22 ± 2.25) mg/dL and (123.23 ± 2.41) mg/dL of the groups treated with the doses of DIME 250 and 500 mg/kg respectively (Table 1).

3.2. Effect on body weight of rats

In diabetic rats, continuous reduction in body weight was observed as shown in Table 2. Glibenclamide (10 mg/kg) as well as the extracts (DIME 250 and 500 mg/kg) treatment significantly (P<0.05) improved the body weight of diabetic rats.

3.3. Effect on lipid profile

In diabetic rats, there was a significant increase of serum total cholesterol, triglycerides, and significant decrease in HDL cholesterol in compared to that of normal control. 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 3) after 21 days treatment.

3.4. Effect on liver functions

The effect of DIME on liver functions is represented in the Table 3. ALT, AST, ALP and bilirubin levels were significantly elevated in alloxan induced diabetes. The rats treated with DIME (250 and 500 mg/kg) showed significant (P<0.01) reduction in the elevated levels of liver enzymes (transaminase) in a dose dependent manner. Bilirubin level was also decreased diabetic rats after DIME treatment. Total protein level was decreased significantly in diabetic rats and after 21 days DIME increased the level significantly (P<0.01) as shown in Table 4.

3.5. Effect on kidney functions

Kidney function markers like creatinine and urea were elevated in the alloxan induced diabetic rats when compared with the normal rats. DIME reduced both the levels in dose dependent manner (Table 5).

3.6. Histology of liver

Photomicrographs of liver (Figure 1) showed normal hepatic cells with well preserved cytoplasm, nucleus, nucleolus and central vein (A). In case of group II diabetic rats, the normal lobular structure was preserved. The central vein was prominent and prominently congested. Focal areas of hemorrhage were also seen. Fatty change was evident. The portal tracts appeared normal (B). In group IV [diabetic rats + DIME 500 mg/kg], the hepatocytes portal tracts and central veins appear normal (C).

3.7. Histology of pancreas

Histology of pancreas (Figure 2) showed normal acini, and normal cellular in the islets of langerhans in the pancreas of normal control (A). In diabetic animals treated extensive damage to islets of langerhans and reduced dimensions of islets were observed in diabetic rats (B) which were restorated toward normal cellular population size of islets by DIME 500 mg/kg treatment (C).

3.8. Histology of kidney

Histology of kidney (Figure 3) in normal animals showed normal structure (A). In diabetic rats, mild thickening of the basement membrane of the arterioles of glomeruli along with mild change of density of mesangial mesangium were observed. No other significant changes were seen (B). After DIME 500 mg/kg treatment, these changes were improved towards normal condition (C).

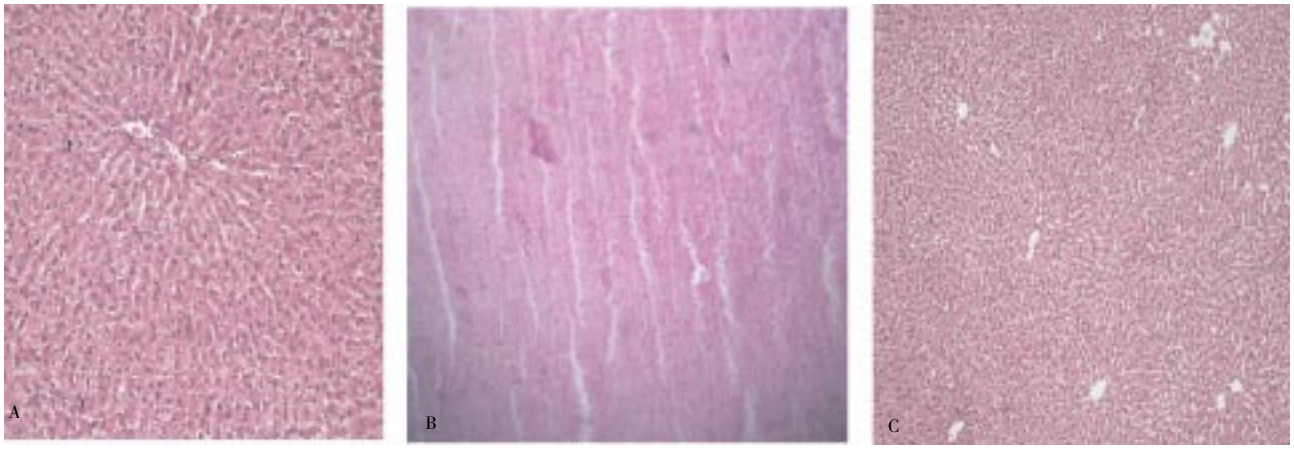

Figure 1. Effect of DIME 500 mg/kg on rat liver (A: Normal rats, B: Diabetic rats, C: A–D + 500 mg/kg).

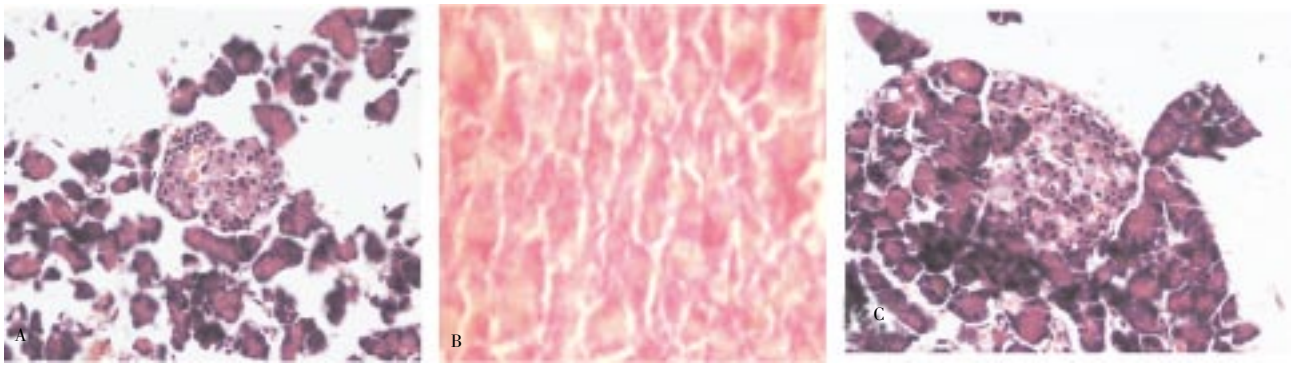

Figure 2. Effect of DIME on rat pancreas (A: Normal rats, B: Diabetic rats, C: A–D + 500 mg/kg).

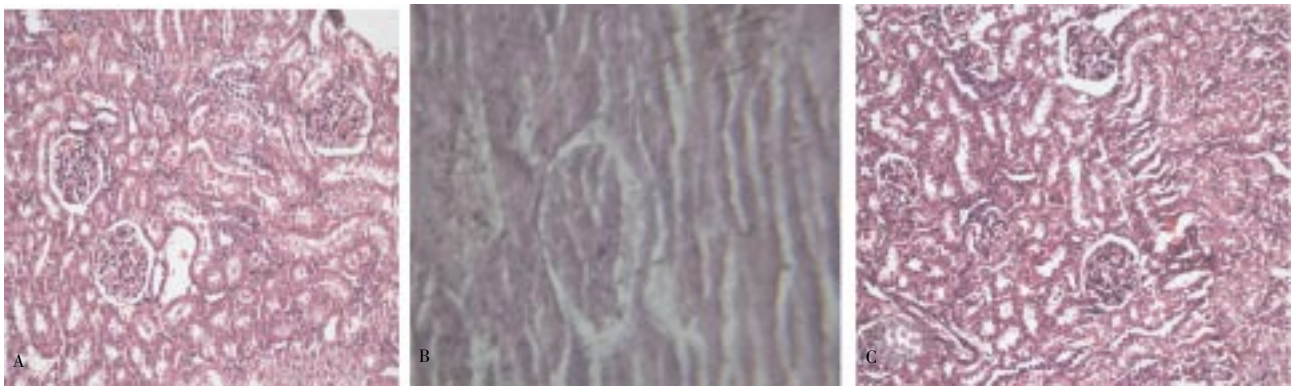

Figure 3. Effect of DIME on rat kidney (A: Normal rats, B: Diabetic rats, C: A–D + 500 mg/kg).

Table 1

Effect of DIME on the blood glucose and insulin levels in alloxan diabetic rat (A–D)(n=6).

Groups/Treatments	Blood glucose level (mg/dL)				Serum insulin(IU/dL)
	Initial day	Day 7	Day 14	Day 21	
I: Normal + Vehicle	115.27 ± 4.50	113.34 ± 3.80	112.70 ± 5.20	113.82 ± 2.40	4.30 ±0.25
II: A–D + vehicle	253.52 ± 2.45	296.54 ± 4.35	325.46 ± 4.27	389.24 ± 4.34	1.20 ±2.50
III: A–D + DIME (250 mg/kg)	252.21 ± 2.24	234.20 ± 2.25*	197.20 ± 2.25*	145.22 ± 2.25*	2.20 ±1.24
IV: A–D + DIME (500 mg/kg)	265.52 ± 2.70	226.43 ± 2.90*	158.58 ± 4.30*	123.23 ± 2.41**	2.90 ±2.48*
V: A–D + Glibenclamide(10 mg/kg. b.w.)	255.24 ± 2.28	201.23 ± 3.52	130.31 ± 2.34**	116.42 ± 2.80**	3.30 ±1.87*

Data represent means ± S.E.M. *P<0.05, **P<0.01, When groups III, IV and V compared with diabetic control i.e. group II, N= Numbers of animals in each group.

Table 2

Effect of DIME on the body weight in diabetic rats (n=6).

Groups/Treatments	Body weight (g)			
	Initial day	Day 7	Day 14	Day 21
I: Normal	215.20 ± 2.35	222.43 ± 4.22	225.41 ± 3.62	228.47 ± 3.17
II: A–D	224.34 ± 2.70	220.21 ± 2.42	212.25 ± 1.52	207.13 ± 2.48
III: A–D + DIME (250 mg/kg)	226.34 ± 2.32	224.42 ± 2.20*	225.44 ± 3.42	227.23 ± 2.35*
IV: A–D + DIME (500 mg/kg)	225.24 ± 2.23	224.35 ± 2.32	225.27 ± 1.35*	226.23 ± 1.43*
V: A–D + Std. (10 mg/kg. b.w.)	222.23 ± 2.71	223.22 ± 2.25*	225.53 ± 2.59*	227.32 ± 2.43*

Data represent means ± S.E.M., *P<0.05, When groups III, IV and V compared with diabetic control *i.e.* group II.

Table 3

Effect of DIME on lipid profile (mg/dL) in alloxan induced diabetic rats.

Groups/Treatments	Total cholesterol	Triglycerides	HDL cholesterol
I: Normal	87.28 ± 3.80	82.42 ± 5.16	37.32 ± 2.90
II: A–D	254.73 ± 7.60	150.52 ± 4.71	28.23 ± 2.20
III: A–D + DIME (250 mg/kg)	125.32 ± 11.20*	117.24 ± 4.51*	34.24 ± 3.50*
IV: A–D + DIME (500 mg/kg)	110.47 ± 4.70*	92.24 ± 6.32**	41.35 ± 2.60*
V: A–D + Std (10 mg/kg. b.w.)	98.72 ± 5.30*	83.47 ± 4.50*	45.28 ± 4.80**

Data represent means ± S.E.M. *P<0.05, **P<0.01.

Table 4
Effect of DIME on liver parameters in normal and diabetic rats.

Groups	Total protein(g/dL)	Bilirubin(mg/dL)	AST(U/L)	ALT(U/L)	ALP(U/L)
I	7.26 ± 2.18	0.45 ± 1.24	43.22 ± 2.34	59.35 ± 3.49	123.35 ± 3.43
II	5.26 ± 1.29	0.94 ± 1.29	102.26 ± 4.87	113.23 ± 3.45	198.26 ± 4.37
III	6.22 ± 1.67*	0.62 ± 0.34*	73.54 ± 3.53	67.26 ± 3.25*	158.55 ± 3.25*
IV	7.12 ± 2.34**	0.49 ± 1.26*	49.23 ± 3.58*	59.68 ± 3.75**	128.35 ± 3.68**
V	7.21 ± 1.25*	0.38 ± 1.83*	45.56 ± 3.54**	58.86 ± 3.58*	125.25 ± 3.25**

Data represent means ± S.E.M. *P<0.05, **P<0.01.

Table 5
Effect of DIME on kidney parameters in normal and diabetic rats (n=6).

Groups/Treatments	Serum urea (mg/dL)	Serum creatinine (mg/dL)
I: Normal	30.25 ± 1.58	0.63 ± 1.34
II: A-D	59.24 ± 1.57	0.97 ± 0.54
III: A-D + DIME (250 mg/kg)	45.24 ± 1.48	0.84 ± 1.08*
IV: A-D + DIME (500 mg/kg)	37.35 ± 0.59*	0.73 ± 0.43**
V: A-D + Std. (10 mg/kg.b.w.)	35.35 ± 0.87*	0.65 ± 0.62**

Data represent means ± S.E.M. *P<0.05, **P<0.01.

4. Discussion

Alloxan acts as diabetogenic by the destruction of β-cells of the islets of langerhans and causes massive reduction in insulin release, thereby inducing hyperglycaemia[23] Insulin deficiency leads to various metabolic alterations in the animals viz increased blood glucose, increased cholesterol, increased levels of alkaline phosphate and transaminases etc[24-25]. Single dose intra-peritoneal (i.p) treatment of rats with alloxan monohydrate (150 mg/kg) significantly (P<0.01) increases the blood glucose as shown in Table 1. DIME and glibenclamide were found to reduce the elevated glucose level significantly in alloxan induced diabetes animals during 21 days treatment. The DIME treatment has also increased the insulin level. So, one possible antidiabetic mechanism of *D. indica* extract may be stimulation of insulin secretion. A significant weight loss was observed in the diabetic group which was improved significantly by the DIME treated groups as urinary glucose and peotein release was restored after treatment.

The most common lipid abnormalities in diabetes are hypertriglyceridemia and hypercholesterolemia[26]. Repeated administration of the DIME for 21 days significantly (P<0.05) decreased hypertriglyceridemia and hypercholesterolemia, the observed hypolipidemic effect may be due to decreased cholesterologenesis and fatty acid synthesis[27-37]. HDL cholesterol level was significantly improved by the extract.

Liver enzymes *e.g.* AST, ALT and ALP level were increased in diabetic rats which is responsible for the liver damage. The elevated serum level of these enzymes was significantly reduced by DIME treatment. The diabetic complications such as increased gluconeogenesis and ketogenesis may be due to elevated enzymes[38]. The restoration of transaminases to their normal levels also treatment also indicates revival of insulin secretion.

DIME also improved renal functions in diabetic rats by reducing serum urea and creatinine. So, DIME normalizes the functions of vital organs of rats. Alloxan has been shown to induce free radical production and cause tissue injury. Histopatholigical studies of tissues of organs (liver, pancreas and kidney were undertaken and it was found that DIME was non-toxic and regenerated the toxic effect of alloxan.

The pancreas is especially susceptible to the action of alloxan induced free radical damage. *D. indica* leaves are rich in polyphenols and have in vitro antioxidant effect[39]. So, antioxidant potential of the plant has protective effect on the organ. Also, various studies have shown that diabetes is associated with increased formation of free radicals and decrease in antioxidant potential.

The result of the present study showed that DIME brings back the blood glucose and body weight to normal in diabetes-induced rats. It also improved kidney, liver function and hyperlipidaemia due to diabetes. After treatment with extract, liver section of diabetic rats hepatocytes, portal tracts and central veins appeared normal. DIME has favorable effect to inhibit the histopathological changes of the pancreas and kidney in alloxan induced diabetes. Antidiabetic action of the plant in diabetic rats may be possible through the insulinomimetic action or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production, or activation of gluconeogenesis in liver and muscle. Although the exact chemical compounds responsible for the hypoglycemic effects of *D. indica* still remain speculative, experimental evidence obtained from this study indicates that *D. indica* possess antidiabetic property, which also is confirmed by histopathological examination.

Conflict of interest statement

We declare that we have no conflict of interest.

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