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Effect of Dianex, A Herbal Formulation on Experimentally Induced Diabetes Mellitus

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Dianex, a polyherbal formulation consisting of the aqueous extracts of *Gymnema sylvestre*, *Eugenia jambolana*, *Momordica charantia Azadirachta indica*, *Cassia auriculata*, *Aegle marmelose*, *Withania somnifera* and *Curcuma longa* was screened for hypoglycemic activity in normal and streptozotocin induced diabetic mice. Dianex was administered in different doses of 100–500 mg/kg/day orally in acute (6 h) and long-term (6 weeks) studies. Blood glucose levels were checked 2–6 h after treatment in acute studies and every 2 weeks in long-term studies. Body weight was recorded on the first and final day of the treatment in the long-term studies with diabetic mice. After 6 weeks, high-density lipoprotein, triglycerides, total cholesterol, alanine transaminase (ALT), aspertate transaminase (AST), urea and creatinine were estimated in serum of the diabetic mice. Glycogen and total protein levels were estimated in the liver. Also, the liver and pancreas was subjected to histological examination. Oral glucose tolerance and in vitro free radical scavenging activity was also studied.

Dianex produced significant ($p < 0.05$) hypoglycemic activity at 250–500 mg/kg doses in both normal and diabetic mice in acute and long-term studies. The body weight of diabetic mice significantly ($p < 0.05$) increased with all tested doses of Dianex. The elevated triglycerides, cholesterol, ALT, AST, urea and creatinine levels in diabetic mice were significantly ($p < 0.05$) reduced at the doses of 250 and 500 mg/kg. The liver glycogen and protein levels were both significantly ($p < 0.05$) increased in diabetic mice at 250 and 500 mg/kg doses. Dianex increased the glucose tolerance significantly ($p < 0.05$) in both normal and diabetic mice at all the doses tested. Histopathological examination showed that the formulation decreased streptozotocin induced injury to the tissues at all the doses tested. It produced significant ($p < 0.05$) free radical scavenging activity against $ABTS^{+}$, DPPH and hydroxyl free radicals at the concentrations ranging between 10–1000 μg/ml.

Thus, in the present study, Dianex produced significant hypoglycemic activity in both normal and diabetic animals. It also reversed other diabetic complications in diabetic mice at 250 and 500 mg/kg doses. In our earlier study, Dianex was well tolerated in laboratory animals at higher doses (upto 10 g/kg in mice, acute toxicity; upto 2.5 g/kg in rats, subacute toxicity studies for 30 days) without exhibiting any toxic manifestation. Hence, Dianex may be useful in the treatment of diabetes mellitus.

Keywords: Dianex; herbal formulation; diabetes mellitus.

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism (Das *et al.*, 1996). The management of diabetes mellitus is considered a global problem and successful treatment is yet to be discovered. The modern drugs like sulfonylureas, biguanides, inhibitors of intestinal α-glucosidases, glitazones, repaglinide and aldose reductase inhibitors control the blood sugar level as long as they are regularly administered but also produce a number of undesirable side effects and suboptimal control of glucose levels (Upadhyay *et al.*, 1996; Reynolds, 1997). Also, an oral hypoglycemic agent equivalent to insulin has yet to be found. These are some reasons why people are still searching for novel treatments. The treatment of diabetes mellitus has been attempted with various indigenous plants and polyherbal formulations (Chaurasia *et al.*, 1994; Mitra *et al.*, 1996; Upadhyay *et al.*, 1996). In the present study, Dianex, a polyherbal formulation, consisting of herbs derived from Ayurveda, has been screened for antidiabetic activity in mice.

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MATERIALS AND METHODS

Animals. Animals used were adult Swiss albino mice (6–8 weeks old) of either sex, weighing 25–30 g (Department of Radiobiology, Kasturba Medical College, Manipal). The animals were housed in polypropylene cages, four per cage, with free access to standard laboratory diet (Lipton Rat Feed, Mumbai, India) and water. The animals were kept at 25 ± 1 °C and 45–55% relative humidity with a 12 h light/dark cycle.

Chemicals and plant material. Sodium deoxycholate, anthrone, thiourea, streptozotocin, 1,1-diphenyl-2-picryl

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hydrazyl (DPPH), 2,2′-azinobis (3-ethyl benzthiazoline-6-sulfonic acid) ($ABTS^+$), ascorbic acid, deoxyribose, thiobarbituric acid and bovine serum albumin were purchased from Sigma, St. Louis (MN), USA. Trichloroacetic acid was purchased from Qualigens, India. All the other chemicals used were of highest analytical grade. The Dianex mixture containing the aqueous extracts of *Gymnema sylvestre* (Asclepiadaceae; leaf, 15 mg), *Eugenia jambolana* (Myrtaceae, seed, 19 mg), *Momordica charantia* (Cucurbitaceae, fruit, 30 mg), *Azadirachta indica* (Meliaceae, leaf, 7.5 mg), *Cassia auriculata* (Caesalpiniaceae, flower, 25 mg), *Aegle marmelose* (Rutaceae, fruit, 12.5 mg), *Withania somnifera* (Solanaceae, root, 20 mg) and *Curcuma longa* (Zingiberaceae, rhizome, 4 mg) was supplied by the Apex Laboratories, Chennai, India. The plant materials were collected from Kancheepuram area, TN, India and identified at Department of Herbals, Apex Laboratories, Chennai, India. Glibenclamide was supplied as a gift sample from Bal Pharma (P) Ltd, India. Dianex mixture or glibenclamide were passed through sieve No. 80 and suspended in 0.5% w/v sodium carboxymethyl cellulose (CMC) for administration.

Hypoglycemic activity in normal mice. Following an overnight fast, mice were divided into five groups (n = 6). Dianex was suspended in 0.5% w/v CMC and administered orally at 100, 250 and 500 mg/kg body weight. For oral administration, a mouse feeding needle was used (metal canula attached to a hypodermic syringe). Another group was administered glibenclamide (5 mg/kg; p.o.). The control group was administered with 0.2 ml of CMC. At time intervals of between 2–6 h after treatment, blood was collected from orbital sinuses for analysis. Blood glucose levels were determined using Accutrend Alpha Glucometer (Roche Diagnostics, Penzberg, Germany).

In the long-term study, the above treatments were administered orally once daily for 6 weeks. Blood glucose levels were determined once in every 2 weeks in over night fasted mice, 2 h after drug administration as previously described.

Induction of diabetes mellitus and hypoglycemic activity in diabetic mice. Diabetes was induced in overnight fasted mice by injecting streptozotocin (150 mg/kg; ip) dissolved in citrate buffer (3 mM; pH 4.5) (Grover *et al.*, 2002). Seven days later, mice with blood glucose levels between 300–400 mg/dL were selected. Hypoglycemic activity was evaluated in over night fasted diabetic mice divided into five groups (n = 6), which were treated as described earlier.

In the long-term study, drugs were administered once daily for six weeks and blood glucose levels were determined, as described previously. Body weight of animals was recorded on the first and final day of treatment.

Oral glucose tolerance test in normal and diabetic mice. After an overnight fast, normal and streptozotocin diabetic mice were divided into four groups (n = 6). Diabetes had been induced as described previously. Normal control and diabetic control groups were given with 0.2 ml of CMC p.o. Other groups were administered 250/500 mg/kg of Dianex p.o. or 5 mg/kg glibenclamide p.o., as before. All the animals then received glucose solution (2 g/kg) p.o. Blood samples for glucose determination were collected just prior to (0 min) and at 30, 60 and 120 min after the glucose administration (Pari *et al.*, 2001).

Biochemical and histopathological studies. At the end of the treatment period, blood was collected from orbital sinuses of diabetic mice and serum was separated off. Lipid profile (high-density lipoprotein, triglycerides and total cholesterol), alanine transaminase (ALT), aspertate transaminase (AST), urea and creatinine levels were estimated in serum using Auto-analyzer (Hitachi 911, Tokyo, Japan). Finally, the animals were sacrificed by cervical dislocation and liver and pancreases were removed. A part of the liver was processed for glycogen estimation (Carrol *et al.*, 1956) and total protein (Lowry *et al.*, 1951). Pieces of liver and pancreas were fixed in Bouin's fixative and processed routinely for histological screening. The slides were stained with haematoxylene and eosin and observed under low power microscope for any pathological changes.

The animal experimental protocol was approved by the Institutional animal ethical committee, Kasturba Medical College, Manipal.

***In vitro* free radical scavenging activity.** The *in vitro* free radical scavenging activity of Dianex was carried out with respect to DPPH, $ABTS^+$ and hydroxyl radicals.

DPPH: To the ethanolic solution of DPPH (0.5 mM) an equal volume of Dianex dissolved in phosphate buffer (pH7.4, 20 mM) was added at various concentrations in a final volume 1.0 ml. An equal volume of phosphate buffer alone was added to control. After 20 min, absorbance was recorded at 517 nm (Sreejayan and Rao, 1996).

ABTS: The reaction mixture contained $ABTS^+$ radical (2 mM ABTS solution, 0.17 mM potassium persulphate), various concentrations of Dianex and buffer (pH7.4, 20 mM) in a total volume of 3.5 ml. After 30 sec, the absorbance was measured at 734 nm (Miller and Evans, 1997).

Hydroxyl radical: Hydroxyl radical scavenging activity was measured by the deoxyribose method (Halliwell *et al.*, 1987). To the reaction mixture, containing deoxyribose (3 mM), ferric chloride (0.1 mM), EDTA (0.1 mM), ascorbic acid (0.1 mM), hydrogen peroxide (2 mM) in phosphate buffer (pH7.4, 20 mM), various concentrations of Dianex was added to give a final volume of 3 ml. After incubation for 30 min at ambient temperature, tri-chloroacetic acid (0.5 ml, 5% w/v) and thiobarbituric acid (0.5 ml, 1% w/v) was added. The reaction mixture was kept in a boiling water bath for 30 min, cooled and absorbance was measured at 532 nm.

The difference in the absorbance between test and control in relation to the blank was taken and % scavenging of free radicals was calculated.

Analysis of results. Student's t-test was employed to analyze the results. Differences below the probability level 0.05 was considered statistically significant.

RESULTS

The results of acute and long-term hypoglycemic activity in normal mice are shown in Tables 1 and 2

Table 1. Blood glucose reduction of Dianex in normal mice (acute study)

Dose (mg/kg)	Absolute blood glucose level (mg/dL)	Reduction in blood glucose level (mg/dL) (Percentage reduction in blood glucose levels)		
		2 h	4 h	6 h
Control	176.1 ± 7.51	171.1 ± 5.05	173.1 ± 5.25	175.0 ± 4.12
(0.2 ml CMC)		(2.57 ± 1.21)	(1.72 ± 2.11)	(0.61 ± 1.82)
Dx-100	177.5 ± 9.24	166.8 ± 4.46	161.5 ± 3.81	136.4 ± 4.21
		(5.51 ± 2.41)	(9.56 ± 2.15)*	(22.81 ± 3.15)*
Dx-250	151.4 ± 9.61	138.0 ± 5.74	104.1 ± 6.21	103.2 ± 5.24
		(8.43 ± 2.21)*	(30.18 ± 3.21)*	(31.01 ± 2.19)*
Dx-500	139.2 ± 8.91	98.1 ± 1.55	88.1 ± 2.22	86.0 ± 3.11
		(29.21 ± 5.41)*	(36.01 ± 3.11)*	(38.21 ± 2.95)*
GLBN-5	101.1 ± 5.78	64.1 ± 2.92	56.2 ± 3.11	55.5 ± 2.51
		(36.42 ± 7.81)*	(44.92 ± 6.22)*	(45.26 ± 5.18)*

All values are expressed as Mean ± SE, $n = 6$; * significant compared to control ($p < 0.05$); CMC = Carboxymethyl cellulose; Dx = Dianex; GLBN = Glibenclamide.

Table 2. Blood glucose reduction of Dianex in normal mice (long-term study)

Dose (mg/kg)	Absolute blood glucose level (mg/dL)	Reduction in blood glucose level (mg/dL) (Percentage reduction in blood glucose levels)		
		2 Week	4 Week	6 Week
Control	138.8 ± 8.61	136.3 ± 7.26	136.0 ± 4.08	134.5 ± 5.56
(0.2 ml CMC)		(0.98 ± 1.01)	(0.98 ± 3.31)	(2.21 ± 2.14)
Dx-100	173.1 ± 6.32	160.4 ± 0.56	164.0 ± 1.49	162.1 ± 2.21
		(7.21 ± 3.52)	(5.81 ± 2.82)	(6.32 ± 2.81)
Dx-250	167.5 ± 7.01	149.0 ± 2.52	152.1 ± 3.11	153.2 ± 2.92
		(10.21 ± 3.11)*	(8.92 ± 2.52)	(9.12 ± 1.51)*
Dx-500	186.1 ± 8.65	132.1 ± 2.31	137.0 ± 2.81	125.0 ± 3.12
		(29.01 ± 4.21)*	(26.12 ± 3.51)*	(32.11 ± 4.24)*
GLBN-5	151.2 ± 5.11	95.1 ± 2.31	101.2 ± 2.01	98.0 ± 2.81
		(37.01 ± 4.21)*	(34.41 ± 3.28)*	(34.01 ± 2.92)*

All values are expressed as Mean ± SE, $n = 6$; * significant compared to control ($p < 0.05$); CMC = Carboxymethyl cellulose; Dx = Dianex; GLBN = Glibenclamide.

Table 3. Blood glucose reduction of Dianex in diabetic mice (acute study)

Dose (mg/g)	Absolute blood glucose level (mg/dL)	Reduction in blood glucose level (mg/dL) (Percentage reduction in blood glucose levels)		
		2 h	4 h	6 h
DC	324.5 ± 12.13	317.9 ± 6.31	315.1 ± 5.28	313.1 ± 4.8
(0.2 ml CMC)		(1.65 ± 1.15)	(2.12 ± 1.51)	(3.01 ± 2.51)
Dx-100	345.1 ± 13.85	315.1 ± 4.81	304.1 ± 5.51	269.2 ± 3.91
		(8.73 ± 2.33)*	(11.01 ± 2.21)*	(21.21 ± 5.28)*
Dx-250	331.5 ± 8.65	295.1 ± 6.21	250.5 ± 8.15	235.1 ± 6.16
		(12.11 ± 3.11)*	(25.11 ± 4.55)*	(29.12 ± 3.33)*
Dx-500	328.1 ± 8.67	242.3 ± 9.51	223.1 ± 10.15	211.1 ± 8.91
		(25.91 ± 5.56)*	(33.01 ± 4.51)*	(35.11 ± 5.15)*
GLBN-5	345.5 ± 12.13	240.0 ± 5.21	223.1 ± 2.15	219.0 ± 3.01
		(31.11 ± 2.01)*	(34.92 ± 4.12)*	(35.91 ± 3.12)*

All values are expressed as Mean ± SE, $n = 6$; * significant compared to DC (Diabetic control) ($p < 0.05$); CMC = Carboxymethyl cellulose; Dx = Dianex; GLBN = Glibenclamide.

respectively. Dianex produced significant ($p < 0.05$) reduction in blood glucose levels at 250 and 500 mg/kg doses in both acute and long-term studies. The results of acute and long-term hypoglycemic activity in diabetic mice are shown in Table 3 and 4 respectively. Streptozotocin produced moderate to severe hyperglycemia in mice (300–600 mg/dL). The animals with 300–400 mg/dL blood glucose levels were used for hypoglycemic study. Dianex produced significant ($p < 0.05$) hypoglycemic activity in diabetic mice at all doses tested in both acute and long-term studies, which was comparable to glibenclamide.

The body weight of untreated diabetic mice was markedly less (18.03 ± 4.27%) than that of the untreated normal mice at the end of 6 weeks. The administration of Dianex for 6 weeks significantly ($p < 0.05$)

Table 4. Blood glucose reduction of Dianex in diabetic mice (long-term study)

Dose (mg/kg)	Absolute blood glucose level (mg/dL)	Reduction in blood glucose level (mg/dL) (Percentage reduction in blood glucose levels)		
		2 Week	4 Week	6 Week
DC (0.2 ml CMC)	324.5 ± 12.1	321.2 ± 6.56 (0.91 ± 0.98)	316.5 ± 5.55 (2.22 ± 1.12)	320.4 ± 4.58 (0.89 ± 1.12)
Dx-100	345.2 ± 13.8	312.0 ± 7.15 (9.81 ± 1.91)*	309.1 ± 6.53 (10.91 ± 3.52)*	314.0 ± 4.82 (9.01 ± 3.21)*
Dx-250	331.1 ± 18.4	296.0 ± 1.98 (11.10 ± 2.28)*	285.0 ± 3.11 (14.59 ± 3.58)*	290.1 ± 3.87 (12.01 ± 3.98)*
Dx-500	328.2 ± 15.0	241.0 ± 3.01 (26.01 ± 3.91)*	238.5 ± 2.91 (27.11 ± 4.01)*	236.0 ± 1.98 (27.91 ± 3.25)*
GLBN-5	375.5 ± 12.1	275.5 ± 11.11 (27.01 ± 4.28)*	268.0 ± 9.18 (28.01 ± 5.01)*	260.0 ± 8.92 (30.33 ± 4.98)*

All values are expressed as Mean ± SE, $n = 6$; * significant compared to DC (Diabetic control) ($p < 0.05$); CMC = Carboxymethyl cellulose; Dx = Dianex GLBN = Glibenclamide.

Table 5. Oral glucose tolerance test in normal and diabetic mice

Dose (mg/kg)	Absolute blood glucose level (mg/dL) (Percentage change in blood glucose level of 0 min)			
	0 min	30 min	60 min	120 min
NC (0.2 ml CMC)	130.0 ± 5.01	234.5 ± 4.04 (+82.04 ± 2.81)	210.0 ± 3.12 (+63.59 ± 3.41)	141.1 ± 5.28 (+9.55 ± 0.79)
Dx 250 + N	141.0 ± 6.81	209.0 ± 12.78 (+42.60 ± 4.09)*	177.5 ± 3.83 (+25.84 ± 3.33)*	145.0 ± 4.93 (+3.55 ± 1.77)*
Dx 500 + N	151.0 ± 4.21	163.0 ± 6.93 (+7.85 ± 1.71)*	155.0 ± 5.25 (+2.61 ± 0.71)*	145.5 ± 5.12 (−4.00 ± 0.88)*
GLBN 5 + N	155.0 ± 3.24	168.4 ± 7.18 (+7.04 ± 4.69)*	161.0 ± 2.12 (+3.90 ± 0.83)*	151.0 ± 8.28 (−2.49 ± 2.86)*
DC (0.2 ml CMC)	321.0 ± 11.01	401.5 ± 5.28 (+25.10 ± 2.68)	440.5 ± 4.52 (+37.29 ± 3.23)	410.0 ± 12.0 (+27.86 ± 0.87)
Dx 250 + D	355.0 ± 5.21	402.4 ± 6.12 (+13.24 ± 0.14)#	391.0 ± 8.51 (+10.11 ± 0.83)#	382.0 ± 5.0 (+7.61 ± 0.11)#
Dx 500 + D	360.0 ± 6.52	381.0 ± 4.21 (+3.76 ± 1.24)#	372.0 ± 5.51 (+0.28 ± 0.31)#	359.2 ± 6.01 (−3.23 ± 0.10)#
GLBN 5 + D	365.0 ± 8.12	371.4 ± 8.58 (+1.63 ± 0.12)#	361.0 ± 5.28 (−1.05 ± 0.77)#	351.0 ± 7.12 (−3.82 ± 0.23)#

All values are expressed as Mean ± SE, $n = 6$; * significant compared to NC (Normal control) ($p < 0.05$); # significant compared to DC (Diabetic control) ($p < 0.05$); CMC = Carboxymethyl cellulose; Dx = Dianex; N = Normal mice; GLBN = Glibenclamide; D = Diabetic mice.

increased the body weight of diabetic mice compared to untreated diabetic mice (6.36 ± 2.03%, 8.85 ± 0.55% and 17.56 ± 0.96% for 100, 250 and 500 mg/kg Dianex respectively).

Table 5 shows the blood glucose levels of normal control, diabetic control, Dianex and glibenclamide treated mice in response to an oral glucose tolerance test. Dianex treated mice (both normal and diabetic) showed significant ($p < 0.05$) decrease in blood glucose levels, compared to the respective controls, indicating increased glucose tolerance.

The results of biochemical studies are presented in Table 6. The glycogen and total protein levels in the liver of diabetic mice were significantly ($p < 0.05$) lowered compared to normal mice. Dianex treatment significantly ($p < 0.05$) increased liver glycogen and protein levels in diabetic mice at 250 and 500 mg/kg doses. The lipid profile (total cholesterol, triglycerides and HDL-cholesterol) was significantly ($p < 0.05$) increased in diabetic control mice compared to normal mice. Dianex treatment significantly ($p < 0.05$) reduced the elevated lipid profile in diabetic mice at 100, 250 and 500 mg/kg doses (Table 6). The hepatic enzyme levels (ALT and AST) were significantly ($p < 0.05$) increased in untreated diabetic mice in comparison with normal mice. The elevated levels of ALT and AST were significantly ($p < 0.05$) decreased upon administration of Dianex at 250 and 500 mg/kg doses (Table 6). The serum urea and creatinine levels were significantly ($p < 0.05$) elevated in diabetic mice compared to normal mice. Dianex treatment at 250 and 500 mg/kg doses significantly ($p < 0.05$) lowered these changes (Table 6). The histopathological studies of pancreas and liver from diabetic mice showed inflammation, necrosis and degeneration. These conditions were considerably reversed by Dianex treatment indicating that Dianex administration produces improved repair of the tissues after streptozotocin induced injury.

The results of in vitro free radical scavenging activity of Dianex are shown in Table 7. Dianex showed significant ($p < 0.05$) DPPH, $ABTS^+$ and hydroxyl radical scavenging activity between 10–1000 μg/ml concentrations.

Table 6. Lipid profile, ALT, AST, urea, creatinine, liver glycogen and liver protein levels in diabetic mice treated with different doses of Dianex

Dose (mg/kg)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	ALT (IU/L)	AST (IU/L)	Urea (mg/dL)	Creatinine (mg/dL)	Liver glycogen (mg/g)	Liver protein (mg/g)
Normal (0.2 ml CMC)	101.1 ± 8.11	65.6 ± 7.12	41.1 ± 2.55	90.4 ± 5.11	51.5 ± 8.31	42.6 ± 0.91	0.41 ± 0.02	3.71 ± 0.43	36.9 ± 4.96
DC (0.2 ml CMC)	311.1 ± 5.41#	181.5 ± 7.41#	101.8 ± 4.11#	165.2 ± 8.12#	91.1 ± 8.31#	52.6 ± 2.56#	0.53 ± 0.02#	1.49 ± 0.15#	11.5 ± 1.88#
Dx-100	221.5 ± 12.11*	122.4 ± 6.22*	49.5 ± 5.12*	145.5 ± 6.11	86.1 ± 5.25	48.1 ± 1.61	0.51 ± 0.01	2.47 ± 0.19*	16.1 ± 2.12
Dx-250	161.6 ± 8.41*	101.1 ± 5.71*	51.1 ± 6.42*	141.7 ± 5.21*	71.1 ± 9.51	45.5 ± 2.11*	0.46 ± 0.02*	2.71 ± 0.20*	24.3 ± 3.42*
Dx-500	124.1 ± 5.25*	82.5 ± 6.61*	52.1 ± 7.24*	117.9 ± 6.42*	62.2 ± 7.12*	44.1 ± 1.81*	0.43 ± 0.02*	3.29 ± 0.19*	30.3 ± 3.79*
GLBN-5	125.8 ± 8.41*	81.2 ± 5.55*	54.1 ± 5.11*	111.1 ± 6.51*	61.1 ± 6.41*	46.5 ± 0.75*	0.48 ± 0.01*	2.93 ± 0.33*	25.4 ± 4.51*

All values are expressed as Mean ± SE, $n = 6$; # significant compared to normal ($p < 0.05$); * significant compared to DC (Diabetic control) ($p < 0.05$); CMC = Carboxymethyl cellulose; Dx = Dianex; GLBN = Glibenclamide; TC = Total cholesterol; TG = Triglycerides; HDL-C = High density lipoprotein-cholesterol; ALT = Alanine transaminase; AST = Aspertate transaminse.

Table 7. *In vitro* free radical scavenging activity of Dianex (%)

Concentration (μg/ml)	$ABTS^+$	DPPH	OH
Control (No drug)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
10	24.43 ± 2.13	2.11 ± 0.15	28.52 ± 1.12
25	28.36 ± 0.92	5.68 ± 0.95	24.48 ± 2.53
50	65.46 ± 1.96	9.54 ± 1.12	21.56 ± 2.19
100	74.19 ± 1.01	14.92 ± 1.81	11.61 ± 1.10
200	81.98 ± 0.08	17.85 ± 2.49	–
400	96.87 ± 0.13	22.21 ± 1.21	–
500	95.49 ± 0.39	24.59 ± 3.69	–
1000	–	33.95 ± 2.52	–

All values are expressed as Mean ± SE, $n = 3$; All values are significant compared to control ($p < 0.05$); DPPH = 1,1-diphenyl-2-picryl hydrazyl; $ABTS^+$ = 2,2′-azinobis (3-ethyl benzthiazoline-6-sulfonic acid); OH = Hydroxyl radical.

DISCUSSION

The present study was undertaken with the aim of evaluating the antidiabetic activity of Dianex, a polyherbal formulation. Dianex showed significant ($p < 0.05$) hypoglycemic activity in both normal and diabetic mice upon single and long-term administration. The observed hypoglycemic activity of Dianex may be due to the presence of various herbs whose antidiabetic activity is already well documented (Das *et al.*, 1996; Upadhyay *et al.*, 1996; Andallu *et al.*, 2000; Nischino *et al.*, 2000). Plants like *Gymnema sylvestre*, *Eugenia jambolana* (Achrekar *et al.*, 1991), *Azadirachta indica*, *Cassia auriculata* and *Momordica charantia* (Pari *et al.*, 2001) present in Dianex have been reported to potentiate plasma insulin effect by increasing the pancreatic secretion of insulin from β-cells. *Azadirachta indica* was found to increase the peripheral uptake of glucose (Bajaj and Srinivasan, 1999). The possible mechanism by which Dianex brings about its hypoglycemic action may be due to stimulation of the pancreatic beta cell function and extrapancreatic action, by increasing the peripheral utilization of glucose.

The body weight of untreated diabetic mice was markedly less than that of untreated normal mice (18.03 ± 4.27%) at the end of 6 weeks. The administration of Dianex for 6 weeks significantly ($p < 0.05$) increased the body weight of diabetic mice compared to untreated diabetic mice. Similar results were obtained with *Celosia argentea* (not contained in Dianex). The ability of the plant to protect the body weight loss of diabetic animals could be due to its antidiabetic activity (Vetrichelvan *et al.*, 2002) and a similar mechanism of action might be attributed to Dianex.

In oral glucose tolerance test, Dianex treated mice (both normal and diabetic) showed significant ($p < 0.05$) decrease in blood glucose levels, indicating increased glucose tolerance. *Momordica charantia*, *Cassia auriculata*, *Azadirachta indica* and *Aegle marmelose* have been reported to increase the glucose tolerance in laboratory animals (Pari *et al.*, 2001).

The glycogen and total protein levels in the liver of diabetic mice were significantly ($p < 0.05$) lowered compared to normal mice. Dianex treatment significantly increased liver glycogen and protein levels in diabetic

mice (Table 6). In the diabetic condition, the levels of glycogen phosphorylase, an important enzyme of glycolytic pathway is increased, and hence liver glycogen content is decreased. Insulin stimulates glycogen synthesis and inhibits glycogenolysis. Insulin deficiency in diabetes causes excessive catabolism of protein, which is utilized for gluconeogenesis (Vasanthakumari and Shyamaladevi, 1998). Hence, increased insulin release and increased peripheral uptake of glucose after Dianex treatment might be responsible for elevated glycogen and total protein content of the liver. Further more, a polyherbal formulation containing *Momordica charantia* and *Gymnema sylvestre* has shown increased hepatic glycogen levels in diabetic rats (Annapurna *et al.*, 2001).

The increased lipid profile in diabetic mice was significantly ($p < 0.05$) reduced by Dianex treatment (Table 6). Some of the plants like *Momordica charantia*, *Azadirachta indica*, *Withania somnifera* and *Aegle marmelose* present in Dianex are reported to produce hypolipidemic action (Bopanna *et al.*, 1997; Andallu *et al.*, 2000; Annapurna *et al.*, 2001). A possible mechanism for antihyperlipidemic action of *Azadirachta indica*, a component of Dianex, was attributed to the inhibition of endogenous synthesis of cholesterol and enhancement of its excretion through intestinal tract (Bopanna *et al.*, 1997).

The increased AST and ALT levels in diabetic mice (Table 6) reflected the increased hepatic damage in diabetic mice. This was further confirmed by the histopathological studies. The elevated levels of AST and ALT in diabetic mice were significantly decreased upon administration of Dianex. Many of the plants in Dianex, e.g. *Curcuma longa* and *Azadirachta indica* are reported to be hepatoprotective (Chattopadhyaya *et al.*, 1992; Song *et al.*, 2001). Hence improvement of liver function and subsequent increase in the uptake of glucose and its utilization may be the possible mechanisms for this observation (Vetrichelvan *et al.*, 2002).

The serum urea and creatinine levels were significantly ($p < 0.05$) elevated in diabetic mice of control group. Dianex treatment significantly ($p < 0.05$) lowered these changes (Table 6). The observed nephroprotective effect of Dianex may be due to the antihyperglycemic activity, which increases the uptake and utilization of glucose by the tissues. Similar results were observed with a polyherbal formulation consisting of *Eugenia jambolana*, *Momordica charantia* and *Gymnema sylvestre* (Dubey *et al.*, 1994). Generally, renal hypertrophy is significantly high in the diabetic state (Grover *et al.*, 2001). In an earlier study, *Momordica charantia* and *Eugenia jambolana* significantly prevented the renal hypertrophy in diabetes (Grover *et al.*, 2001). *Aegle marmelose* has also been reported to reduce the blood urea levels in diabetic animals (Ponnachan *et al.*, 1993).

The histopathological studies of pancreas and liver from diabetic control and Dianex treated diabetic mice were carried out. Tissues from diabetic animals showed inflammation, necrosis and degeneration. These conditions were considerably reversed by Dianex treatment indicating that Dianex administration produces improved repair of the tissues after streptozotocin induced injury. These observations are in accordance with the earlier report where *Aegle marmelose*, a component of Dianex, was found to repair or reduce the damage to the tissues (Das *et al.*, 1996).

Dianex showed significant ($p < 0.05$) *in vitro* DPPH, $ABTS^+$ and hydroxyl radical scavenging activity between 10–1000 µg/ml concentrations (Table 7). Free radicals are generated under various pathological conditions including hyperglycemia and hence diabetes is generally associated with increased oxidative stress (Cai and Kang, 2001; Kumari and Augusti, 2002). The present results demonstrate the free radical scavenging and hence, antioxidant activity of Dianex, which could decrease the damage caused by oxidative stress involved in the pathogenesis of diabetes. The antioxidant property of *Momordica charantia*, *Curcuma longa*, *Withania somnifera* and *Azadirachta indica* has been reported (Scartezzini and Speroni, 2000).

To conclude, Dianex produced significant hypoglycemic activity in experimental animals besides reversing other diabetic complications. In another study, Dianex was well tolerated in laboratory animals at higher doses (upto 10 g/kg in mice, acute toxicity; upto 2.5 g/kg in rats, subacute toxicity studies for 30 days) without exhibiting any toxic manifestation (Mutalik *et al.*, 2003). Hence, Dianex may be effectively used in the treatment of diabetes mellitus. Studies on diabetic patients are under progress to confirm its effectiveness in human volunteers.

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