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Research Article

ANTIDIABETIC ACTIVITY OF DIBOLIN (A POLYHERBAL FORMULATION) IN STREPTOZOTOCIN-NICOTINAMIDE INDUCED TYPE 2 DIABETIC RATS

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ABSTRACT

Objective: The objective of the study was aimed to investigate the antidiabetic activity of Dibolin (a polyherbal formulation) in streptozotocin-nicotinamide induced type 2 diabetic rats.

Methods: Oral glucose tolerance test (OGTT) was performed to evaluate Dibolin effect on elevated glucose level. The type 2 diabetes was induced by overnight fasted rats by a single intraperitoneal (i.p.) injection of 65 mg/kg streptozotocin, 15 min. after the i.p. administration of 110 mg/kg nicotinamide. The diabetic rats were treated with Dibolin (500 and1000 mg/kg, p.o.) or glibenclamide (5 mg/kg, p.o) for four week. Various parameters were studied included fasting blood sugar level, serum insulin levels, glycated hemoglobin (HbA_{1C}), serum lipid levels, AST, ALT, serum creatinine, urea, uric acid.

Results: Treatment with Dibolin significantly reduced blood sugar levels in OGTT. Diabetic rats showed a significant increase in the levels of glycated hemoglobin, serum lipids, AST, ALT, serum creatinine, urea and uric acid, whereas there was a significant decrease in Hb, serum insulin and HDL-C levels as compared to normal control rats. The administration of Dibolin or glibenclamide significantly decreased the levels of glycated hemoglobin, TG, TC, LDL-C, AST, ALT, serum creatinine, urea and uric acid, whereas there was a significant increase in the level of Hb, serum insulin and HDL-C as compared to diabetic control rats.

Conclusion: These results concluded that Dibolin caused antidiabetic and antihyperlipidemic activities which are responsible for its use in traditional medicine.

Keywords: Streptozotocin, Dibolin, glycated hemoglobin, serum lipids.

INTRODUCTION

Type 2 diabetes mellitus is one of the world's most common chronic metabolic disorders of multiple etiologies and it is projected to increase to 438 million in 2030[1]. Diabetes mellitus is characterized by increase blood glucose level resulting from defects in insulin secretion, insulin action, or both [2]. The early phenomenon of type 2 diabetes mellitus is insulin insensitivity, which not only has negative metabolic consequences, but also contributes subsequent pancreas β-cell exhaustion, resulting in the onset of clinical hyperglycemia [3].

Globally, it is most common serious and largest endocrine disorders and considered to be one of the five leading causes of death in the world [4]. It has been reported that in both type 1 and type 2 diabetes, there is a significant increase in cardiovascular diseases and two to three fold morbidity and mortality rate compared to non-diabetic person [5]. These facts show that proposing an immediate strategy for diabetes prevention and treatment is a global subject. For a long time, diabetics have been treated with several medicinal plants or extracts based on folklore medicines [6]. Synthetic hypoglycemic agents can produce serious side effects and they are also expensive. Thus, the management of diabetes without any side effects is still a challenge. Dibolin (Ayur Herbal Pvt. Ltd, Vadodara) is a polyherbal formulation contains herbal extract with known antidiabetic action (Table 1) [7-17].

There is a lack of scientific evidence regarding the effect of Dibolin (PHF) in experimental diabetes. Therefore, the aim of study was designed to investigate the effect of Dibolin, a polyherbal formulation in type 2 diabetes.

MATERIALS AND METHODS

Chemicals

Streptozotocin and nicotinamide were purchased from Himedia (Mumbai, India). Kits used in the study were procured from standard company. All other chemical used were of analytical grade.

Table 1: Composition of Dibolin capsule

Ingredients	Botanical name	Part used	Weight (mg)
Shatavari	*Asparagus racemosa*	Tuberous root	50
Amalaki	*Emblica officinalis*	Fruits	70
Vijaysaar	*Pterocarpus marsupium*	Stem/bark	20
Amruta	*Tinospora cordifolia*	Leaves	20
Haritaki	*Terminalia chebula*	Fruits	25
Gokshru	*Tribulus terrestris*	Fruits	25
Saptarangi	*Casearia esulenta*	Root/bark	20
Gudmar	*Gymnema sylvestre*	Leaves	35
Mamejwa	*Enicostemma littorale*	Whole plant	20
Haridra	*Curcuma longa*	Rhizome	25
Punarnava	*Boerhaavia diffusa*	Whole plant	20
Musta	*Cyperus rotundus*	Rhizome	30
Lajjalu	*Mimosa pudica*	Whole plant	10
Methi	*Trigonela foenum graecum*	Seeds	10
Dakshini thor	*Opuntia dillenii*	Fruits	50
Bilvapatra,tulsi,amlaki juice, haldi and neem			q.s.

Experimental animals

Albino wistar rats (200-250 g) either sex, procured from the institutional animal house facility were used for all the experiments. Animals were provided with standard pellet diet and water *adlibitum* and they were maintained under standardized conditions (12-h light/dark cycle, 25 ± 2 °C & humidity 45-55 %). The rats were

left for 48 h for acclimatization prior to the commencement of the experiment. The study was approved by Institutional Animal Ethics Committee (IAEC) and carried out in accordance with CPCSEA (Committee for the Purpose of Control and Supervision of Experiment on Animal) guidelines.

Acute toxicity studies

Acute oral toxicity was determined using albino Wistar rats of either sex weighing 200-250g. OECD guideline no. 423 was followed for toxicity studies [18]. Animals were administered orally with a single dose of Dibolin and were observed for their mortality during 14 days.

Oral glucose tolerance test (OGTT)

Oral glucose tolerance test was performed in overnight fasted normal rats [19]. Rats were divided into four groups of six of each. Groups I received drinking water, group II received glibenclamide (5 mg/kg p.o.), group III and IV Dibolin (500 and 1000 mg/kg, p.o.), respectively.

Glucose (2g/kg, p.o.) was fed 30 min prior to the administration of above mention treatments. Blood glucose levels were measured by collecting the blood samples from the tail vein. Blood samples were obtained by repeated needle puncture to the same tail tip vein. Blood glucose level was determined by Accu-check glucometer at 0, 30, 60, 90 and 120 min after the treatment.

Induction of type 2 diabetes

Type 2 diabetes was induced in overnight fasted adult albino wistar rats weighing 200-250g by a single intraperitoneal (i.p.) injection of 65 mg/kg streptozotocin (dissolved in citrate buffer, pH 4.5), 15 min after the i.p. administration of 110 mg/kg of nicotinamide (dissolved in normal saline)[20]. Hyperglycemia was confirmed by elevated blood glucose levels at 72 h and then on day 7 after injection and only animals with fasting blood glucose level greater than 200 mg/dl were selected for antidiabetic study.

Experimental design

The rats were divided into five groups each consisting of six animals. Group I: Normal control rats (drinking water); Group II: Diabetic control rats (0.5% sodium carboxy methyl cellulose); group III: diabetic rats (Dibolin 500 mg/kg); group IV: diabetic rats (Dibolin 1000 mg/kg); group V: diabetic rats (Glibenclamide 5 mg/kg). The vehicle, Dibolin and glibenclamide were administered orally to the respective group of animals for 28 days. The fasting blood sugar levels were estimated on 1, 7, 14, 21 and 28 days periodically. Urine volume and urine glucose levels were estimated. At the end of the experiments, blood samples were collected from the retro orbital plexus of rats under light ether anesthesia, using glass capillaries and stored in with or without disodium ethylene diamine tetra-acetate for estimation of biochemical parameter. After, allowing the blood to clot for serum separation for 15 minutes, it was centrifuged at 5000 rpm for 20 minutes for separation of serum. Then the serum was stored at -20°C until further estimation.

Blood glucose, glycated hemoglobin (HbA1C), Hemoglobin (Hb) were estimated using whole blood. The total cholesterol (TC), triglycerides (TG), low density lipoprotein (LDL-C), high density lipoprotein (HDL), serum creatinine, urea, uric acid, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated from serum using standard diagnostic kit (SPAN Diagnostics and Crest Biosystems, India). The serum insulin was determined by radioimmunoassay method [21].

Statistical analysis

All the data are expressed as mean ± SEM (n=6). The statistical significance between more than two groups was tested using one-way ANOVA followed by the Bonferroni multiple comparisons post test using a computer-based fitting program (Prism, Graph pad 5). Differences were considered to be statistically significant when $p < 0.05$

RESULTS

Acute oral toxicity

The oral administration of Dibolin in rats up to the dose 5000mg/kg did not show any sign of toxicity and no mortality for 14 days. It was shown that Dibolin was safe up to oral dose of 5000 mg/kg of body weight. The experimental protocol was carried out using $1/10^{th}$ (500 mg/kg) and $1/5^{th}$ (1000 mg/kg) dose based on toxicity study.

Effect of Dibolin on oral glucose tolerance test (OGTT)

Glucose challenge to normal rats increased blood glucose levels with maximum level at 30 min and slight reduction in blood glucose was observed at 60 min onwards. The treatment with Dibolin and Glibenclamide improved glucose tolerance significantly ($p<0.05$) at 30 min to 120 min compared to normal control animals (Table 2).

Table 2: Effect of Dibolin on oral glucose tolerance test in non-diabetic rats

Group (n=6)	Treatment	Blood glucose (mg/dl)				
		0 min	30 min	60 min	90 min	120 min
I	Normal control	92 ± 3.05	126.7 ± 4.77	114.5 ± 4.90	110.2 ± 4.08	108.8 ± 4.15
II	Glibenclamide (5 mg/kg)	90.3 ± 4.14	105 ± 4.45*	98 ± 4.04*	93.50 ± 3.59*	93 ± 3.52*
III	Dibolin (500 mg/kg)	87.1 ± 2.63	103. 3 ± 4.94*	93.6 ± 2.95*	91.8 ± 4.60*	90 ± 4.16*
IV	Dibolin (1000 mg/kg)	85.8 ± 2.76	97.5 ± 5.20*	89.1 ± 4.88*	86.8 ± 3.62*	83.1 ± 4.57*

Values are expressed in mean ± SEM (n=6), *p<0.05 represents significant change as compared to normal control.

Effect of Dibolin on fating blood glucose level in STZ-nicotinamide induced type 2 diabetes The effect of Dibolin on fasting blood glucose level of diabetic rats is shown in Table 3. Diabetic rats showed a significant increase in the fasting blood glucose levels as compared to normal control rats. A significant dose dependent decrease in blood glucose level was observed in diabetic rats after treatment with Dibolin (500 mg/kg and 1000 mg/kg) for 28 days.

Table 3: Effect of Dibolin on fasting blood glucose levels in STZ-nicotinamide induced type 2 diabetes

Group (n=6)	Treatment	Fasting Blood glucose (mg/dl)				
		Day 1	Day 7	Day 14	Day 21	Day 28
I	Normal control	102.5 ± 5.72	93.5 ± 2.77	99.17 ± 2.73	93.8 ± 2.40	89.6 ± 4.30
II	Diabetic control	343.5 ± 15.56	298.3 ± 13.15###	282. ± 8.62###	261.0 ± 7.53###	256.5 ± 10.77###
III	Dibolin (500 mg/kg)	312.5 ± 8.63	301.7 ± 7.36**	221.3 ± 10.15***	185.0 ± 5.02***	162.3 ± 4.56***
IV	Dibolin (1000 mg/kg)	314.7 ± 8.89	267.2 ± 5.94***	203.2 ± 6.52***	167.3 ± 3.68***	145.7 ± 6.62***
V	Glibenclamide (5 mg/kg)	320.8 ± 5.83	261.5 ± 5.73***	198.5 ± 9.82***	155.7 ± 3.65***	133.0 ± 5.85***

Values are expressed as Mean + S.E.M (n=6). Where, ###p<0.001 represents significant to normal control; ** p<0.01, *** p<0.001 represents significant to Diabetic control.

Effect of Dibolin on body weight, urine volume and urine sugar
The body weight of the diabetic rats showed a significant decrease ($p<0.01$) after the administration of STZ-nicotinamide compared to normal control rats. The treatment with Dibolin at the dose of 500 and 1000mg/kg significantly increased the body weight as compared to diabetic control. Administration of Dibolin or glibenclamide in diabetic rats showed a significant reduction in urine volume as compared to diabetic control rats.

An effect of Dibolin or glibenclamide on urine glucose is shown in Table 4

Table 4: Effect of Dibolin on Body weight, urine volume and urine sugar in STZ-nicotinamide induced type 2 diabetes

Group (n=6)	Treatment	Initial body weight (g)	Final body weight (g)	Urine Volume (ml)	Urine glucose
I	Normal control	225.0 ± 4.28	245.0 ± 4.28	20.83 ± 1.24	Nil
II	Diabetic control	231.7 ± 10.14	183.3 ± 8.82$^{\#\#}$	71.67 ± 2.78$^{\#\#\#}$	+++
III	Dibolin (500 mg/kg)	235.0 ± 7.63	200.0 ± 11.55*	39.33 ± 2.40***	+
IV	Dibolin (1000 mg/kg)	221.7 ± 9.09	211.7 ± 11.38**	31.00 ± 2.59***	Nil
V	Glibenclamide (5 mg/kg)	235.0 ± 11.76	196.7 ± 9.88**	27.50 ± 1.84***	Nil

Values are expressed as Mean + S.E.M (n=6). Where, $^{\#\#}p<0.01$, $^{\#\#\#}$ $p<0.001$ represents significant to normal control; * $p<0.05$, ** $p<0.01$, *** $p<0.001$ represents significant to Diabetic control

(+) - Trace elements of sugar and (+++) - more than 2% of sugar

Effect of Dibolin on Hb, Glycated hemoglobin and serum insulin in STZ-nicotinamide induced type 2 diabetes

The present data indicated that there was a significant elevation in the level of glycated hemoglobin (HbA_{1C}) and reduction in the level of hemoglobin (Hb), serum insulin in diabetic rats as compared to normal control rats. The treatment with Dibolin (500 and 1000 mg/kg) or glibenclamide (5 mg/kg) showed a significant reduction in level of glycated hemoglobin and a significant increase in the levels of Hb and serum insulin at dose dependant manner (Table 5).

Table 5: Effect of Dibolin on Hb, Glycated hemoglobin and serum insulin in STZ-nicotinamide induced type 2 diabetes

Group (n=6)	Treatment	Hb (g/dl)	Glycated hemoglobin (HbA_{1C} %)	Serum insulin (µU/ml)
I	Normal control	13.92 ± 0.40	5.35 ± 0.40	144.7 ± 4.77
II	Diabetic control	8.48 ± 0.46$^{\#\#\#}$	11.0 ± 0.80$^{\#\#\#}$	84 ± 3.02$^{\#\#\#}$
III	Dibolin (500 mg/kg)	10.68 ± 0.49*	7.7 ± 0.29***	111.2 ± 4.19**
IV	Dibolin(1000 mg/kg)	12.80 ± 0.41***	6.5 ± 0.22***	127.2 ± 3.46***
V	Glibenclamide (5 mg/kg)	11.95 ± 0.44***	5.46 ± 0.27***	131.2 ± 5.45***

Values are expressed as Mean + S.E.M (n=6). Where, $^{\#\#\#}p<0.001$ represents significant to normal control; * $p<0.05$, ** $p<0.01$,

*** $p<0.001$ represents significant to Diabetic control.

Effect of Dibolin on serum lipid levels in STZ-nicotinamide induced type 2 diabetes:

Diabetic rats showed a significant increase in the levels of TG, TC and LDL-C and a significant reduction in level of HDL-C as compared to normal control rats. Treatment with Dibolin (500 and 1000 mg/kg) or glibenclamide (5 mg/kg) showed a significant reduction in level of TG, TC, LDL-C and a significant increase in HDL-C level in diabetic rats when compared to diabetic control rats (Table 6).

Table 6: Effect of Dibolin on serum lipid levels in STZ-nicotinamide induced type 2 diabetes

Group (n=6)	Treatment	TG (mg/dl)	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)
I	Normal control	63.6 ± 3.67	78.6 ± 3.15	29.3 ± 2.41	49.6 ± 2.45
II	Diabetic control	118.5 ± 4.37$^{\#\#\#}$	147.3 ± 6.45$^{\#\#\#}$	59.3 ± 2.64$^{\#\#\#}$	25.0 ± 2.64$^{\#\#\#}$
III	Dibolin (500 mg/kg)	86.1 ± 3.44***	107.0 ± 6.28***	43.8 ± 2.61**	38.5 ± 4.4*
IV	Dibolin (1000 mg/kg)	74.17 ± 4.47***	97.1 ± 6.24***	38.6 ± 1.62***	42.3 ± 3.41**
V	Glibenclamide (5 mg/kg)	73.0 ± 3.17***	85.0 ± 4.35***	37.0 ± 4.10***	46.6 ± 2.37***

Values are expressed as Mean + S.E.M (n=6). Where, $^{\#\#\#}$ $p<0.001$ represents significant to normal control;

* $p<0.05$, ** $p<0.01$, *** $p<0.001$ represents significant to Diabetic control.

Effect of Dibolin on serum creatinine, urea, and uric acid, AST and ALT in STZ-nicotinamide induced type 2 diabetes There was a significant increase in the levels of serum creatinine, urea, uric acid, AST and ALT in diabetic control rats as compared to normal control rats. The administration of Dibolin (500 and 1000 mg/kg) or glibenclamide (5 mg/kg) showed a significant decrease in the levels of serum creatinine, urea, uric acid, AST and ALT as compared to diabetic control rats (Table 7).

Table 7: Effect of Dibolin on serum creatinine, urea, and uric acid, AST and ALT in STZ-nicotinamide induced type 2 diabetes

Group (n=6)	Treatment	Serum creatinine (mg/dl)	Urea (mg/dl)	Uric acid (mg/dl)	AST (IU/L)	ALT (IU/L)
I	Normal control	0.70 ± 0.02	34.00 ± 1.65	2.91 ± 0.12	33.8 ± 2.73	37.5 ± 1.96
II	Diabetic control	1.28 ±0.03$^{\#\#\#}$	59.17 ± 2.70$^{\#\#\#}$	9.55 ± 0.47$^{\#\#\#}$	59 ± 2.35$^{\#\#\#}$	69.8 ± 3.65$^{\#\#\#}$
III	Dibolin (500 mg/kg)	0.99 ± 0.11*	49.17 ± 1.80*	7.58 ± 0.32**	45.33 ± 3.33*	58 ± 2.68*
IV	Dibolin (1000 mg/kg)	0.88 ± 0.06**	49.17 ± 1.86**	4.66 ± 0.30***	41 ± 3.75**	51.6 ± 2.44***
V	Glibenclamide (5 mg/kg)	0.77 ± 0.05***	40.33 ± 0.95***	3.66 ± 0.38***	36.7 ± 2.86***	39.17 ± 2.16***

Values are expressed as Mean + S.E.M (n=6). Where, $^{\#\#\#}$ $p<0.001$ represents significant to normal control; * $p<0.05$, ** $p<0.01$,

*** $p<0.001$ represents significant to Diabetic control

DISCUSSION

Streptozotocin-nicotinamide administration caused diabetes, which might be due to the destruction of beta cells of the islet of langerhans of the pancreas [22]. Excessive production and decreased consumption of glucose by the tissue are the fundamental basis of hyperglycemia in diabetes mellitus [23]. When Dibolin was administered to glucose loaded overnight fasted normal rats, hypoglycemia was observed after 30 min. The blood glucose was reduced to its maximum extend at 2 h.

In present study, administration of Dibolin showed a significant reduction in the level of blood glucose and an increase serum insulin level. The antidiabetic effect of Dibolin might be due to increase in the insulin level and this effect can be attributed to the stimulation of beta cells of pancreas. In our study, we observed that increase in the level of HbA1c was due to the persistent hyperglycemia. The level of HbA1c is considered as a tool for the diagnosis and prognosis of diabetes associated with diabetic complication [24].

In diabetes, insulin deficiency leads to decrease in protein synthesis in all tissue and thus the synthesis of hemoglobin is also reduced [25]. In present study, Administration of Dibolin significantly decreased in the level of HbA1c and increased Hb level in diabetic rats. The ability of Dibolin to reduce glycated hemoglobin level in diabetic rats showed it's potentially to prevent the diabetic associated complication.

It was previously reported that decreased in body weight in diabetes might be due to metabolic alteration caused by deficiency of insulin [26]. In our study, we observed that diabetic rats showed a significant reduction in body weight, which was significantly improved in Dibolin or glibenclamide treated rats.

Coronary heart disease and cerebrovascular disease are major consequence of diabetes. The atherogenic condition is more proceeding at a more rapid rate in diabetic than nondiabetic subjects. The elevated in levels of TG, TC and LDL-C and decreased HDL-C levels were reported in diabetic condition [28].

In our study, administration of Dibolin significantly decreased elevated levels of TG, TC and LDL-C and increased level of HDL- C in diabetic rats compared to diabetic control rats. Lipid lowering effect of Dibolin might be helpful in controlling diabetic associated complication.

It was reported that there is liver toxicity in streptozotocin induced diabetic rats [29]. Therefore, elevated levels of AST and ALT may be due to the leakage of these enzymes from liver cytosol into blood, which gives an indication on the hepatotoxicity effect of streptozotocin [30]. The treatment with Dibolin or glibenclamide significantly lowered AST and ALT levels in diabetic rats, which supports its protective effect on liver.

Protein glycation in diabetes might be responsible for muscle wasting and increased release of purine, major source of uric acid, as well as increased the activity of xanthine oxidase [31, 32]. In our study, administration of Dibolin or glibenclamide significantly reduced the serum uric acid, urea and creatinine levels in diabetic rats. These finding supports that Dibolin improved renal function in diabetic condition.

CONCLUSION

From this study, we conclude that Dibolin has a significant antidiabetic effect. The Dibolin also showed improvement in lipid profile, body weight and renal function in diabetic condition. Therefore it might be helpful in preventing diabetic associated complication. Our present investigation supports the traditional use of Dibolin in the treatment of diabetes.

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CONFLICT OF INTEREST

All authors have none to declare

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