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ANTIHYPERGLYCEMIC AND ANTIDYSLIPIDEMIC ACTIVITY OF AQUEOUS EXTRACT OF *DIOSCOREA BULBIFERA* TUBERS

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SUMMARY

Dioscorea bulbifera, the 'air potato', has been used in the Chinese system of medicine to treat diseases of the lungs, kidneys and spleen, and many types of diarrhea. Commonly known as yams, these plants have been traditionally used to lower glycemic index, thus providing a more sustained form of energy and better protection against obesity and diabetes. The present study was carried out to scientifically evaluate the aqueous extract of *Dioscorea bulbifera* tubers (DBEA003) for its antihyperglycemic activity in glucose primed and streptozotocin (STZ) treated Wistar rats and antidyslipidemic potential in high fat diet fed C57BL/6J mice, respectively. The antihyperglycemic effect was evaluated in temporarily established hyperglycemic condition by priming Wistar rats with 1.5 g/kg p.o. glucose and rendering them diabetic by the injection of STZ (45 mg/kg,

intraperitoneally). Dyslipidemic condition was induced in C57BL/6J mice by feeding them high fat diet. DBEA003 at 250, 500 and 1000 mg/kg doses administered for 3 weeks to STZ treated rats and for 4 weeks to high fat diet fed C57BL/6J mice showed significant antihyperglycemic and antidyslipidemic effects. In STZ treated rats with severe diabetes, the 7-week DBEA003 treatment produced significant reduction in blood glucose level and increase in body weight. Serum glucose and lipid levels were reversed towards normal in DBEA003 treated high fat diet fed mice.

INTRODUCTION

Diabetes mellitus is a metabolic syndrome in which homeostasis of the carbohydrate and lipid metabolism is improperly regulated (1). In this metabolic disorder, there is either defective/deficient insulin secretory response (2) for normal function of many cells of the body resulting in persistent hyperglycemia (3), or inadequate utilization of insulin at the level of receptors (4). Under the condition when insulin is inadequate in the body, there are disorders of all kinds of metabolism, commonly with an increase in blood sugar accompanied by glycosuria, polyphagia, polyuria and polydipsia (5,6). Insulin unavailability may be due to degenerative changes in β -cells of

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pancreatic islets, reduced effectiveness of the hormone due to the formation of anti-insulin antibodies or inactive complexes, immune-mediated islet cytotoxicity or inappropriate secretion of hormones by neoplasm in other endocrine organs (7).

In the modern system of medicine, there is hardly any drug available for the complete and safe treatment of diabetes mellitus. Although the different classes of currently available therapeutic agents like sulfonylureas (8,9), biguanides (10), α -glucosidase inhibitors (11) and thiozolidinedione (12) have been used in abundance for the disease management, they fall short of permanent cure of the disease while exerting serious side effects of hepatotoxicity and severe hypoglycemia (13). The search for effective and safer antidiabetic plant drugs is thus of great importance. The World Health Organization (WHO) has also recommended that this area warrants attention (14).

The search is still generally focused on identification and isolation of natural products to be used in the treatment of diabetes mellitus and its secondary complications, which could have beneficial effects in diabetics either by enhancing insulin secretion and/or by improving/mimicking insulin action (13). The current piece of work reports on the study of herbal extracts in diabetes and related markers.

Dioscorea bulbifera is native to Africa and Asia and is commonly known as 'air potato'. The tubers often have a bitter taste, which can be removed by boiling. They can then be prepared in the same way as other yams, potatoes, and sweet potatoes. The air potato is one of the most widely-consumed yam species (15).

Several species, known as yams, are important agricultural crops in tropical regions, grown for their large tubers. Many of these are toxic when fresh, but can be detoxified and eaten, and are particularly important in some parts of Africa, Asia, and Oceania (16).

Dioscorea bulbifera has been widely used in the Chinese system of medicine as a valuable herb in the process of rebuilding and maintaining kidney function. This herb was also found to have a beneficial effect in treating diseases of the lungs and spleen, and many

types of diarrhea, improving digestion and metabolism. In Asia, this herb has been highly recommended for treating diabetes disorder. It has been traditionally used to lower glycemic index, providing a more sustained form of energy and better protection against obesity and diabetes; however, this property has not yet been scientifically proven (17). Some scanty reports are available on *Dioscorea bulbifera* use in diabetes mellitus and other related disorders, but no scientifically validated study has been carried out to justify its potential in experimental diabetes and dyslipidemia. In the present study, we investigated the aqueous extract of *Dioscorea bulbifera*, DBEA003, for its antihyperglycemic effect in glucose primed and streptozotocin (STZ) treated Wistar rats, and antidyslipidemic effect in high fat diet (HFD) fed C57BL/6J mice.

MATERIALS AND METHODS

Experimental animals

Adult male Wistar rats (8 weeks) weighing 180-200 g and C57BL/6J mice weighing 20-25 g bred in Animal House, Indian Institute of Integrative Medicine (IIIM), formerly Regional Research Laboratory (CSIR), Jammu, were used. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC), IIIM (formerly Regional Research Laboratory), CSIR, Jammu. The animals were housed in polycarbonate cages in a room with 12-h day-night cycle, temperature of 22 ± 2 °C and humidity of 45%-64%. Throughout the experimental period, animals were fed a balanced commercial pellet diet (Ashirwad Industries, Chandigarh, India) and water *ad libitum*.

Rearing up of animals and their upkeep in the experimental period conformed to the norms of IAEC and ethical guidelines for investigations of experimental pain in conscious animals (18).

Induction of temporary hyperglycemia

Adult male Wistar rats, 6 animals in each group, were fasted overnight. The animals were divided into normal untreated control, negative control (glucose

primed), test (glucose primed + test extract/fraction) treated and reference (glucose primed + glibenclamide) treated groups. The test and reference drug treatment was performed at 0 h, glucose (1.5 g/kg; 10% sol.) administered to all groups except for normal untreated group at 1.5 h. Blood glucose determination was done at 0 h (prior to any treatment), 0.5 h and 1.5 h (post-glucose administration) (19).

Induction of experimental diabetes

Five groups of 6 animals (Wistar rats) each received a freshly prepared STZ solution (45 mg/kg) in 0.1 M sodium citrate buffer, pH 4.5, injected intraperitoneally in a volume of 1 mL/100 g (20). Normal rats (n=6) received 1 mL citrate buffer as a vehicle. Five days after STZ administration, rats with glycosuria and hyperglycemia (i.e. blood glucose level of 250-350 mg/dL) were used for the experiment. These diabetic animals were divided into 5 groups and one additional group of 6 animals that received no STZ served as normal controls.

Induction of experimental dyslipidemia

Adult C57BL/6J male mice, 6 animals *per* group, were housed at 22-26 °C, 12 h-12 h light/dark cycle, fed with normal feed containing crude protein (22.05%), crude oil (4.55%), crude fiber (3.2%), ash (6.45%) and sand silica (1.25%). As calculated from 9 kcal/g fat, 4 kcal/g protein and 4 kcal/g carbohydrates, 100 g of current normal fat diet (NFD) yields 142 kcal energy, of which only 29% is derived from 4.5 g of oil/fat present in the feed. The high fat diet (HFD) should contain enough fat to denote 60% of kcal energy. Therefore, adding extra fat, 912.5 g of lard, to 100 g NFD, makes the following diet composition *per* 100 g feed: protein 22 g; fat 4.5 g; lard 17 g; and carbohydrate 3.2 g. HFD was prepared by thoroughly mixing the ingredients and stored at 4 °C until used.

Plant material

Dioscorea bulbifera tubers were collected from Akhnoor, Rajouri and Poonch areas of Jammu (J&K) region. These tubers of *Dioscorea bulbifera* were identified and authenticated by the taxonomist from

the IIM, formerly Regional Research Laboratory (CSIR), Jammu. The test part of the plant was shade dried, ground to fine powder and sieved through mesh, and then stored at room temperature in screw top jars until used. The fine powdered plant material was subjected to extraction with distilled water to prepare the aqueous extract DBEA003.

Preparation of aqueous extract of *Dioscorea bulbifera* tubers

The aqueous extract of *Dioscorea bulbifera* tubers was prepared by percolation with water. In brief, 0.5 kg of powdered material was successively percolated at ambient temperature for 4x16-h times with 2 L water, then desolventized at 55±5 °C under diminished pressure to obtain the aqueous extract labeled as DBEA003. The yield of lyophilized aqueous extract was 22.0%-22.4%.

Experimental procedure for glucose tolerance

The rats were divided into six groups of six animals each:

- group 1: normal (untreated)
- group 2: glucose primed control
- group 3: glucose + 0.5 mg/kg p.o. glibenclamide
- group 4: glucose + 250 mg/kg p.o. DBEA003
- group 5: glucose + 500mg/kg p.o. DBEA003
- group 6: glucose + 1000 mg/kg p.o. DBEA003

Glucose 1.5 g/kg; 10% sol. and glibenclamide were prepared in distilled water; freshly prepared stock suspension (10%, w/v) of DBEA003 in gum acacia (0.2 % in distilled water) was used. The test and reference drug treatment was administered at 0 h, glucose (1.5 g/kg; 10% sol.) to all groups except for normal untreated group at 1.5 h. Blood glucose was determined at 0 h (prior to any treatment), 0.5 and 1.5 h (post-glucose administration).

Experimental procedure for antihyperglycemic study in STZ treated rats

The rats were divided into six groups of 10 animals each:

- group 1: normal (untreated)
- group 2: STZ control
- group 3: STZ + 0.5 mg/kg p.o. glibenclamide
- group 4: STZ + 250 mg/kg p.o. DBEA003
- group 5: STZ + 500 mg/kg p.o. DBEA003
- group 6: STZ + 1000 mg/kg p.o. DBEA003

Five groups of 6 animals each received a freshly prepared STZ solution (45 mg/kg) in 0.1 M sodium citrate buffer, pH 4.5, injected intraperitoneally in a volume of 1 mL/100 g (20). Normal rats (n=6) received 1 mL citrate buffer as a vehicle. Five days after STZ administration, rats with glycosuria and hyperglycemia (i.e. blood glucose level of 250-350 mg/dL) were used for the experiment. These diabetic animals were divided into 5 groups and one additional group of 6 animals that received no STZ served as normal controls. The test treatment of 5 groups was done as *per* the group defined above. The treatment period was six weeks starting on day 7. Blood glucose and body weight were measured weekly.

Experimental procedure for antidyslipidemic study in mice

The rats were divided into five groups of 10 animals each:

- group 1: NFD control group: 0.5% CMC, @ 2 mL/kg (wk 5-8) for 28 days
- group 2: HFD control group: 0.5% CMC, @ 2 mL/kg;
- group 3: HFD + 300 mg/kg p.o fenofibrate
- group 4: HFD + 250 mg/kg p.o. DBEA003
- group 5: HFD + 500 mg/kg p.o. DBEA003
- group 6: HFD + 1000 mg/kg p.o. DBEA003

To a cage containing 6 mice, 60 g of NFD/HFD was added daily for 4 weeks. Animals had free access to water. Treatment was administered daily at the same time and was continued for up to 4 weeks.

On day 28, after 4 weeks of initial HFD feeding, 4 mice were sacrificed to measure serum glucose and lipid profile to assess the effect of HFD.

On day 56, all mice were sacrificed by bleeding and serum glucose and lipid profile were measured in these samples to assess the effect of feeding HFD for 8 weeks and to test extract treatment for 4 weeks.

During this period, body weight and daily food consumption were recorded. To assess the anti-dyslipidemic potential of DBEA003, serum glucose, triglycerides, total cholesterol, HDL and LDL were measured on days 0, 28 and 56.

Procedure for blood collection and biochemical testing

Blood was drawn by retro-orbital venepuncture technique using a microcapillary (21). Blood samples were collected into plain vials for serum. The blood was allowed to clot and then centrifuged at 2000 rpm for 10 min to obtain clear serum. Blood glucose in serum plasma was estimated by the glucose oxidase method (22). All lipid profile parameters were determined in serum. Total cholesterol (TC) was estimated by the enzymatic method as described by Allain *et al.* (23). Triglycerides (TGs) were determined by the enzymatic colorimetric method (24-26). High density lipoprotein (HDL) was determined by the phosphotungstate method (23,27-30). Low density lipoprotein (LDL) was calculated by using Friedewald *et al.* 1972 formula (31).

Acute toxicity study

The acute toxicity study of DBEA003 was done according to the Organisation for Economic Co-operation and Development (OECD) guideline No. 423. Briefly, male and female Wistar rats, 3 animals/sex weighing 150-180 g body weight, were administered DBEA003 at a limit dose of 2000 mg/kg p.o., prepared in 1% gum acacia. Upon administration, animals were closely observed individually at least once during the first 30 minutes, 4-hourly during the first 24 hours, with special attention paid during the first 4 hours, and then daily for a total of 14 days.

Table 1. Effect of aqueous extract of *Dioscorea bulbifera* (DBEA003) on blood glucose level in glucose primed rats

Treatment group	Dose (mg/kg p.o.)	Blood glucose (mg/dL) (mean±SE)		
		0 h	30 min [#]	90 min [#]
Normal control	Untreated	79±3.98	85±4.43	83±5.51
Glucose primed	Control vehicle	82±4.32	161±9.87	143±8.78
Glibenclamide	0.5	81±5.13	118±5.7***	77±4.32***
DBEA003	250	79±6.87	152±8.77	140±6.89
	500	84±7.33	139±5.46***	121±6.71***
	1000	81±6.44	129±8.81***	97±5.83***

Number of animals: 10; [#] time post glucose administration; *** p < 0.001 compared to glucose primed control at 30 and 90 min.

Statistical analysis

Data were evaluated by Student's unpaired t-test, one-way analysis of variance (ANOVA) or two-way analysis of variance where appropriate. The level of high statistical significance was set at $P < 0.001$.

RESULTS

Acute treatment

Glucose tolerance in glucose primed model

Table 1 and Figure 1 depict the antihyperglycemic effect of DBEA003 in glucose primed rats at 30 and 90 min post glucose administration. DBEA003 was evaluated at 250, 500 and 1000 mg/kg p.o. dose level. The lowest dose showed no significant reduction in blood sugar, whereas the two higher doses of 500 and 1000 mg/kg p.o. produced a highly significant antihyperglycemic effect ($P < 0.001$) in comparison to the glucose primed controls. This antihyperglycemic effect, however, was less than that of glibenclamide (0.5 mg/kg p.o.) at both time points.

Long-term/subacute treatment

Blood glucose and body weight in STZ treated rats

The antihyperglycemic effect of DBEA003 was also studied in STZ treated Wistar rats. The animals that developed diabetes after 5 days of injection were administered DBEA003 in test doses of 250, 500 and 1000 mg/kg p.o. Six-week treatment with DBEA003

Figure 1. Effect of acute treatment with different doses of DBEA003 on glucose level in glucose primed rats. Data were compared with glucose primed control. The maximum antihyperglycemic effect was shown by glibenclamide (0.5 mg/kg p.o.), followed by 1000 and 500 mg/kg p.o. doses of DBEA003. Thus, antihyperglycemic effect of DBEA003 was not superior to that of glibenclamide; *** $P < 0.001$ compared to glucose primed control at 30 and 90 min.

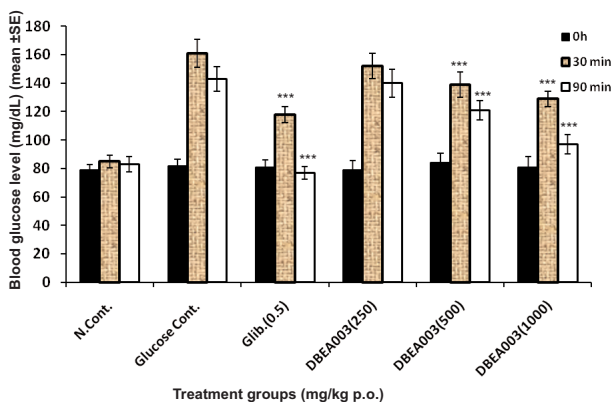
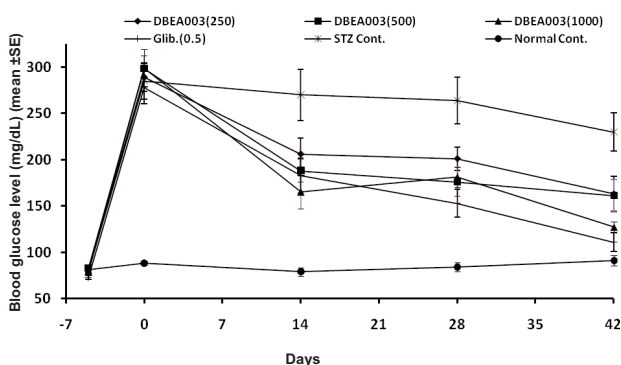


Figure 2. Effect of subacute treatment with different doses of DBEA003 on glucose level in STZ treated rats. Data were compared with STZ treated control. The antihyperglycemic effect of DBEA003 was not superior to that of glibenclamide.



resulted in remarkable glycaemic control with 500 and 1000 mg doses, as recorded on biweekly estimation of blood sugar showing significant decrease ($P<0.001$) as compared with STZ treated controls (Table 2). A significant gain in body weight (data not shown) in the treated rats indicated reversal of diabetes. This showed DBEA003 to have a long lasting effect in the experimental diabetic condition, which may be due to its action at the tissue or receptor level. However, the mechanism of action by which DBEA003 exerted the antidiabetic effect needs to be worked out.

Serum lipid profile

The effect of DBEA003 on blood glucose and serum lipid profile in HFD fed mice is summarized in Table 3 and graphically presented in Figure 3-5. The level of blood glucose normalized in these obese animals, whereas TG, TC, and LDL were reduced and HDL increased significantly ($P<0.001$) in HFD fed mice treated with 500 and 1000 mg kg p.o. doses of DBEA003. Atherogenic index (TC/HDL ratio) was also significantly decreased in DBEA003 treatment groups. The antidyslipidemic effect of DBEA003 was comparable to that of fenofibrate (300 mg/kg p.o.).

Table 2. Effect of *Dioscorea bulbifera* aqueous extract DBEA003 on streptozotocin (STZ) induced diabetes in rats

Treatment group (mg/kg p.o.)	Blood glucose (mg/dL) (mean±SE)				
	Day - 5	Day 0	Day 14	Day 28	Day 42
Normal control	81±3.55	88±2.11	79±4.53	84±4.8	91±5.61
STZ control	80±2.98	285±19.51	270±30.7	264±31.09	230±24.5
Glibenclamide (0.5)	73±2.41	278±17.94	183±18.91***	153±15.13***	111±9.9***
DBEA003 (250)	81±3.21	289±15.04	206±17.57***	201±12.73***	163±18.96***
DBEA003 (500)	83±2.10	298±14.03	188±12.55***	176±15.89***	161±17.67***
DBEA003 (1000)	79±3.77	299±19.76	165±17.69***	181±11.14***	127±5.89***

Data are expressed as mean ± SEM (n=10) and were evaluated by Student's unpaired t-test, one-way analysis of variance (ANOVA). Drugs were given to rats daily for a period of 42 days. Blood glucose and body weight were measured biweekly; *** $P<0.001$ compared to diabetic control.

Table 3. Effect of *Dioscorea bulbifera* aqueous extract DBEA003 on high fat diet induced obesity in C57BL/6J mice

Treatment group	mg/dL (mean±SE)								
	Blood glucose			Triglycerides			Total cholesterol		
	Day 0	Day 28	Day 56	Day 0	Day 28	Day 56	Day 0	Day 28	Day 56
Normal fat diet control	98±12.3	90±6.2	95 ±8.9	99± 6.9	107±9.1	93±9.4	91±10.9	101±13.8	115±8.7
High fat diet control	99±7.0	130±3.7	125±7.3	103±10.2	135±10.0	150±19	89±5.7	120±14.8	135±8.4
Fenofibrate (300)	85±4.8	79±5.0**	82±13.9**	100±10.1	81±16.8**	89±4.9**	90±7.6	85±7.19**	80±12.5**
DBEA003 (250)	95±4.1	110±6.5*	115±10.8 ^{ns}	98±7.3	120± 6.6 ^{ns}	135±7.7 ^{ns}	88±10.6	109±7.4 ^{ns}	125±11.2 ^{ns}
DBEA003 (500)	100±6.3	97±10.6**	98±9.7**	101±8.9	103±13.8**	100±11.1**	81±8.4	99±5.3**	112±7.9**
DBEA003 (1000)	91±4.4	97±11.3**	77±3.7**	93±10.8	90±10.2**	87±11.0**	90.5±7	80±12.8**	91±20.7**

Treatment group	mg/dL (mean±SE)					
	HDL			LDL		
	Day 0	Day 28	Day 56	Day 0	Day 28	Day 56
Normal fat diet control	66±12.3	64±5.3	60±5.4	54±7.3	60±11.6	65±6.0
High fat diet control	68±4.7	54±3.4	49±6.6	57±6.3	87±5.1	89±9.4
Fenofibrate (300)	68±4.7	73±6.5**	67±7.1**	53±5.3	69±4.1**	60±8.9**
DBEA003 (250)	65±2.4	50±5.8 ^{ns}	46±4.3 ^{ns}	50±4.8	80±7.7 ^{ns}	86±9.3 ^{ns}
DBEA003 (500)	75±8.4	65±5.3**	69±7.2**	51±5.6	74±8.7*	76±11.3*
DBEA003 (1000)	66±10.4	80±7.2**	77±12.0**	49±3.1	68±9.1**	70±3.6**

Number of animals: 10; * $P<0.005$ compared to high fat diet control; ** $P<0.001$ compared to high fat diet control; ^{ns} non-significant

Toxicity study

The male and female rats treated with the limit dose of 2000 mg/kg p.o. DBEA003 did not show any drug-induced physical signs of toxicity and no drug related death occurred throughout the study period.

DISCUSSION AND CONCLUSION

In the present study, preliminary evaluation of the antihyperglycemic and antidyslipidemic activity of the aqueous extract of *Dioscorea bulbifera* tubers, DBEA003, was performed. DBEA003 showed a dose dependent antihyperglycemic effect in glucose primed rats at 500 and 1000 mg/kg p.o. doses at both points of measurement at 30 and 90 min post glucose administration. In the STZ treated Wistar rats, the 6-week treatment with DBEA003 prevented blood glucose increase. The two higher doses of DBEA003 significantly ($P < 0.001$) lowered the level of blood glucose, as illustrated in Table 2. Moreover, recovery of body weight records (data not shown) of DBEA003 treated animals additionally pointed to reversal of diabetic condition in the treated animals.

In the present study, the antihyperglycemic effect of DBEA003 was observed in temporary hyperglycemia in glucose primed rats and permanent hyperglycemic condition established in rats by injecting STZ. In the glucose primed rats, in response to the presence of excess glucose in the body, the insulin is secreted from β -cells of the islets of Langerhans. The decrease of blood sugar in the glucose primed controls after treatment with DBEA003 indicated that the test extract either potentiated insulin secretion or acted like insulin in quick glucose metabolism. In severe diabetic condition induced by injecting STZ to Wistar rats, the recovery of high blood sugar and regaining of body weight showed that the test extract might also reach target tissues in the body and act in glucose metabolism. It also showed that DBEA003 possibly exerted a prolonged effect that led to the reversal of diabetic condition.

STZ, a highly cytotoxic agent selective for pancreatic β -cells (32), induces diabetes by damaging the cells and thus reducing the release of insulin. It is reported that treatment of diabetic animals with medicinal plant

Figure 3. Effect of DBEA003 on blood glucose and triglyceride levels in high fat diet induced obesity in C57BL/6J mice.

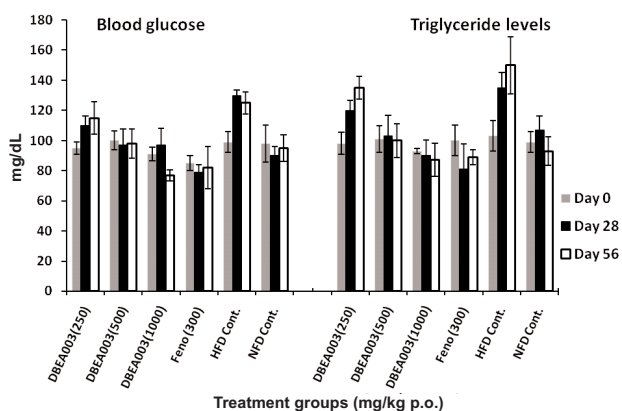


Figure 4. Effect of DBEA003 on total cholesterol and HDL levels in high fat diet induced obesity in C57BL/6J mice.

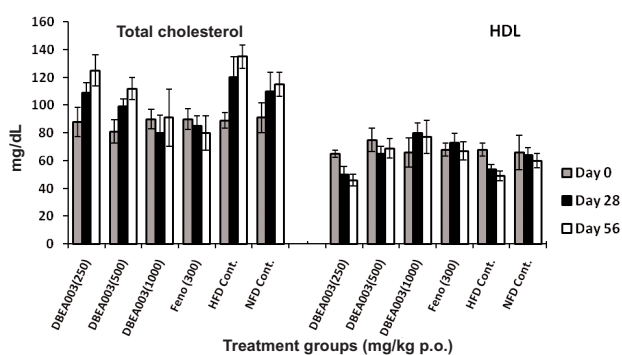
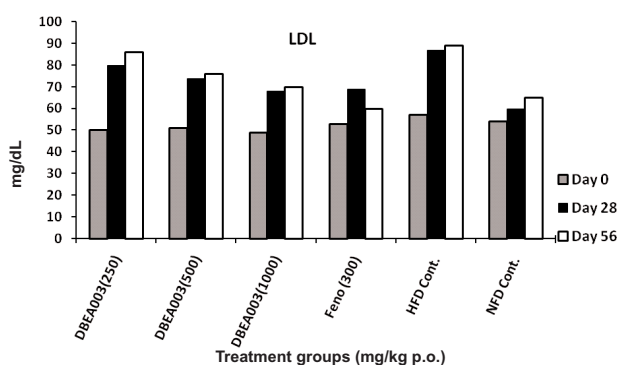


Figure 5. Effect of DBEA003 on LDL levels in high fat diet induced obesity in C57BL/6J mice.



extracts resulted in activation of β -cells and granulation returned to normal, showing an insulinogenic effect (33). The possible mechanism of action of DBEA003 for its antihyperglycemic effect might have been due to the stimulation of insulin secretion from the remnant β -cells and/or regenerated β -cells. A number of other plants have been reported to have antihyperglycemic activity, with a stimulatory effect on insulin release (34,35). Since DBEA003 produced a highly significant antihyperglycemic effect in severe diabetic rats in which most of the β -cells were damaged, it is likely that this extract might have an insulin sensitization mechanism of action at tissue level.

In diabetes mellitus, hyperglycemia is accompanied by dyslipidemia (36,37), i.e. characterized by an increase in TC, LDL, VLDL and TG, and fall in HDL. This altered serum lipid profile was normalized after treatment with the extract. DBEA003 exhibited a dose response antidiabetic and antidiabetic effect.

DBEA003 in dosage up to 2000 mg/kg p.o. did not result in any physical signs of toxicity or mortality after acute treatment followed by 1000 mg/kg p.o. for 4 weeks, thus proving the extract being safe for use.

In conclusion, DBEA003 possesses potent antihyperglycemic and antidiabetic activity and it may prove to be effective in the treatment of diabetes mellitus and diabetes driven dyslipidemic condition. Study results also demonstrated DBEA003 to contain pure principle(s) responsible for antidiabetic and antidiabetic activity. Therefore, additional studies to isolate, identify and characterize the active principle(s) of DBEA003 are under way.

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