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Research Article

ANTIDIABETIC AND ANTIHYPERLIPIDEMIC EFFCETS OF THE ETHANOLIC EXTRACT OF ALOCASIA INDICA RHIZOMES IN HIGH FAT DIET/STREPTOZOTOCIN AND STREPTOZOTOCIN/NICOTINAMIDE-INDUCED TYPE 2 DIABETIC RATS

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ABSTRACT

Objective: The investigate the antidiabetic and antihyperlipidemic effect of ethanolic extract of *Alocasia indica* (EEAI) rhizomes in high-fat diet/streptozotocin (HFD/STZ) and STZ/nicotinamide-induced Type 2 diabetic rats.

Methods: Diabetes was induced in male Wistar rats by the administration of a HFD for 15 days/STZ (35 mg/kg b.w., i.p.) and STZ (60 mg/kg b.w., i.p.)/nicotinamide (110 mg/kg b.w., i.p.). EEAI (100 and 200 mg/kg b.w., p.o.) was administered to diabetic rats for 28 days in HFD/STZ-induced Type 2 diabetic rats and for 15 days in STZ/nicotinamide-induced Type 2 diabetic rats. The effect of EEAI on blood glucose and body weight was studied in Type 2 diabetic rats. All these effects were compared with glibenclamide (5 mg/kg b.w., p.o.) as a reference antidiabetic drug.

Results: The administration of the EEAI (100 and 200 mg/kg b.w., p.o.) resulted in a significant decrease in blood glucose level and significant increase in body weight in the HFD/STZ and STZ/nicotinamide-induced Type 2 diabetic rats. Further EEAI showed antihyperlipidemic activity as evidenced by significant decrease in serum total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), very LDL-C levels coupled together with elevation of high-density lipoprotein cholesterol level in diabetic rats in the HFD/STZ and STZ/nicotinamide-induced Type 2 diabetic rats.

Conclusion: The results suggest that the EEAI rhizomes possess a promising effect on the HFD/STZ and STZ/nicotinamide-induced Type 2 diabetes.

Keywords: Antidiabetic, Antihyperlipidemic, *Alocasia indica*, High-fat diet, Streptozotocin, Nicotinamide.

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by chronic hyperglycemia with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both [1]. According to the International Diabetes Federation, worldwide the estimated prevalence of diabetes and projection for year 2030 will be 350 million [2]. Defects in carbohydrate mechanism and steady efforts of the physiological systems to correct the imbalance in carbohydrate metabolism create an over action on the endocrine system, which leads to the worsening of endocrine control. Continuing deterioration of endocrine control augments the metabolic disturbances by altering carbohydrate metabolic enzymes and leads to hyperglycemia [3]. Diabetes mellitus can be controlled by diet, exercise, and chemotherapy. Insulin and synthetic hypoglycemic agents may produce serious adverse effects and also they are not appropriate for use during pregnancy [4]. The medicinal plants provide a natural source of oral hypoglycemic agents for the development of new leads, as well as a nutritional supplement to existing therapies [5]. Some of the plants, which are being used for the treatment of diabetes, have received scientific or medicinal search and even the WHO expert committee on diabetes recommends that this area demand further consideration [6]. Herbal drugs are in fact effective, produce minimal or no adverse effects clinically and have relatively low costs as compared to synthetic oral hypoglycemic agents [7]. India has about 45,000 plant species and numerous of them have medicinal properties. More than 800 plants are reported to be used for the treatment of diabetes as traditional medicines [8].

Alocasia indica (Araceae), commonly called Makachu, is a traditional medicine against diabetes. Powdered dry leaves of the plant are the known traditional medicine for the treatment of 'Madhumeha' (diabetes) [9]. It is an annual herb that grows at the foothills of the Himalayas, around the inner Tarai region (east of Koshi River) in Eastern Nepal. Pharmacologically this plant has been investigated for its antioxidant [10], hepatoprotective [11], antidiarrheal [12], anthelmintic [13], and antimicrobial [14]. This plant contains cynogenetic glycosides, flavonoids, gallic acid, ascorbic acid, mallic acid, alocasin, amino acids, oxalic acid, β-lectines, and succinic acid [15].

However, in spite of the various bioactive phytoconstituents and miscellaneous medicinal activities accredited to this plant, no biochemical studies have been carried out to shed light on the role of this plant in diabetes. In the light of the above, the present study was undertaken to explore its role on blood glucose, body weight, and serum lipid profile (total cholesterol [TC], triglycerides [TG], high-density lipoprotein cholesterol [HDL-C], low-density lipoprotein cholesterol [LDL-C] and very LDL-C [VLDL-C]) in high-fat diet/streptozotocin [HFD/STZ] and STZ/nicotinamide-induced Type 2 diabetic rats.

METHODS

Plant material

The rhizomes of the plant *A. indica* were collected from the local market of Lucknow, and its identification and authentification were done from National Botanical Research Institute, Lucknow, India (Ref. No: NBRI/CIF/260/2011). The rhizomes were split into small pieces and dried in the shade. The dried material was then grounded separately into coarse powder by a mechanical grinder. The resulting powder was then used for soxhlet extraction.

Preparation of ethanolic extract

The ethanolic extract of rhizomes of *A. indica* (EEAI) was prepared by soxhletion. The powdered plant material was defatted with petroleum

ether (60-80°C) in a soxhlet apparatus. The defatted marc (250 g) was repeatedly extracted with 500 ml 95% ethanol in a soxhlet apparatus. The reflux time for complete extraction was 40 cycles. The extracts were cooled at room temperature (RT), filtered, and evaporated to dryness under reduced pressure in a rotary evaporator. The resultant extract was used for further studies.

Preliminary phytochemical screening

An attempt was made to observe the presence and absence of diverse phytochemical constituents in the EEAI, viz., alkaloids (Wagner's test), flavonoids (Shinoda test), tannins (Ferric chloride test), steroids and triterpenes (Liberman-Burchard's test), and saponins (Foam test) according to standard methods [16].

Chemicals

STZ was purchased from Himedia (Mumbai, India). TG, TC, and HDL kits were purchased from Span Diagnostics (Gujarat, India). All other chemicals used were of analytical grade.

Animals

The experiments were carried out with male Wistar rats weighing 180-200 g obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow, India. Research on experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care (1088/07/CPCSEA). They were kept in polyacrylic cages (22.5 cm^2 × 37.5 cm) and were maintained under standard housing conditions (RT, 24-27°C, and humidity, 60-65%) with a 12 hrs light/12 hrs dark cycle. Food and water were available *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee, which follow the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and conform to the international norms of the Indian National Science Academy. Ethical norms were strictly followed during all experimental procedures [hygia/M.Pharm./12/2011-12].

Induction of experimental diabetes in rats

Model 1: HFD and low dose STZ induced diabetes

Type 2 Diabetes was induced in male Wistar rats weighing 180-200 g. The rats were fed with HFD (composition of HFD is given in Table 1) for 15 days except normal control rats and then injected with low dose of STZ (35 mg/kg b.w., i.p.) dissolved in freshly prepared 0.01 M citrate buffer (pH 4.5). 5 days after injection, the rats were fasted, and the blood glucose level was estimated; rats having blood glucose levels ≥300 mg/dl were selected and used for the study. The rats were fed with HFD throughout the experimental period [17].

Model 2: STZ and nicotinamide induced diabetes

Type 2 diabetes was induced in overnight fasted animal by an intraperitoneal administration of 60 mg/kg b.w. of STZ dissolved in freshly prepared 0.01 M citrate buffer of pH 4.5, and thereafter nicotinamide (120 mg/kg b.w., *i.p.*) dissolved in normal saline after 5 minutes hyperglycemia was confirmed by the elevated glucose level (≥250 mg/dl) in the blood and was determined on day 0 and 10 after injection. The rats found with permanent Type 2 Diabetes were selected for antidiabetic study [18].

Table 1: Composition of HFD used in the experiment

Ingredients	HFD (%)
Raw beef fat	40
Casein	30
Glucose	10
Wheat flour	7
Salt mixture	6
Bran	4
Vitamin mixture	3

HFD: High fat diet

Experimental design

Assessment of antidiabetic effect of EEAI on HFD/STZ-induced Type 2 diabetic rats

The animals were divided into five groups and each group consisted of six rats (n=6).

Group 1: Normal control	Normal rats+vehicle
Group 2: Diabetic control	HFD for 15 days+streptozocin (35 mg/kg b.w., i.p.)+vehicle
Group 3: Treated group	HFD for 15 days+streptozocin (35 mg/kg b.w., i.p.)+EEAI (100 mg/kg b.w., p.o.) for 28 days
Group 4: Treated group	HFD for 15 days+STZ (35 mg/kg b.w., i.p.)+ EEAI (200 mg/kg b.w., p.o.) for 28 days
Group 5: Standard group	HFD for 15 days+STZ (35 mg/kg b.w., i.p.)+glibenclamide (5 mg/kg b.w., p.o.)

The vehicle and drugs were administered orally using an intragastric tube once daily for 28 days, continuously. The blood glucose level and body weight of rats was measured on day 0, 14 and 28 after treatment. At the end of the 28th day, lipid profile was measured. Blood samples were collected retro orbitally from the inner canthus of the eye under mild ether anesthesia using capillary tubes for biochemical estimations.

Assessment of antidiabetic effect of EEAI on STZ/nicotinamide-induced Type 2 diabetic rats

The animals were divided into five groups and each group consisted of six rats (n=6).

Group 1: Normal control	Normal rats+vehicle
Group 2: Diabetic control	Streptozocin (60 mg/kg b.w., i.p.)+ nicotinamide (120 mg/kg b.w., i.p.)
Group 3: Treated group	Streptozocin (60 mg/kg b.w., i.p.)+ Nicotinamide (120 mg/kg b.w., i.p.)+ EEAI (100 mg/kg b.w., p.o.) for 15 days
Group 4: Treated group	Streptozocin (60 mg/kg b.w., i.p.)+ nicotinamide (120 mg/kg b.w., i.p.)+ EEAI (200 mg/kg b.w., p.o.) for 15 days
Group 5: Standard group	Streptozocin (60 mg/kg b.w., i.p.)+ nicotinamide (120 mg/kg b.w., i.p.)+ glibenclamide (5 mg/kg b.w., p.o.)

The vehicle and drugs were administered orally using an intragastric tube once daily for 15 days, continuously. Fasting blood glucose level was estimated on overnight fasted rats on day 1, 5, 12, and 15 after treatment. Body weight of all the animals was recorded prior to the treatment and after treatment. At the end of the 15th day, lipid profile was measured. Blood samples were collected retro orbitally from the inner canthus of the eye under mild ether anesthesia using capillary tubes for biochemical estimations.

Biochemical estimations

Blood glucose estimation

Blood glucose was estimated by ultra-touch two glucometer (Johnson and Johnson, India).

Serum lipid profile estimation

Serum TC, TG, and HDL-C were estimated using commercially available kits (Span Diagnostics, Gujarat, India). VLDL-C and LDL-C were calculated by Friedewald's formula [19].

$$VLDL=TG/5$$

$$LDL=TC-(HDL-C+VLDL-C)$$

Body weight

Body weight of the animals was taken gravimetrically.

Statistical analysis
The experimental results were expressed as a mean±standard error of mean and were statistically analyzed using one-way ANOVA followed by Dunnet's multiple test. p<0.05 were considered significant.

RESULTS

Effect of EEAI on blood glucose levels in HFD/STZ-induced Type 2 diabetic rats
The effect of EEAI on the blood glucose levels of diabetic rats is given in Table 2. HFD/STZ-treated diabetic rats showed significant increase in the levels of blood glucose when compared to normal rats. Oral administration of EEAI at 100 and 200 mg/kg b. w. did not showed any significant effect on blood glucose levels (Table 2). Standard drug glibenclamide showed significant decrease (p<0.01) in the blood glucose level.

Effect of EEAI on blood glucose levels in STZ/nicotinamide-induced Type 2 diabetic rats
The effect of EEAI on the blood glucose levels of diabetic rats is given in Table 3. STZ/nicotinamide-treated diabetic rats showed significant increase in the levels of blood glucose when compared to normal rats. Oral administration of EEAI at 100 and 200 mg/kg b. w. showed significant decrease (p<0.05 and p<0.01) in the blood glucose levels. Standard drug glibenclamide showed significant decrease (p<0.01) in the blood glucose level.

Effect of EEAI on body weight in HFD/STZ-induced Type 2 diabetic rats
In HFD-low STZ induced diabetic rats a significant increase in body weight were observed. After 28 days of treatment with EEAI at 100 and 200 mg/kg, the body weight was significantly increased by 196.25±1.25 g, p<0.05 and 208.75±3.14 g, p<0.05, respectively, when compared to the first day. The diabetic control and glibenclamide treated rats showed increase in body weight by 187.5±4.78 g and 211.25±3.75 g, p<0.01 (Table 4).

Effect of EEAI on body weight in STZ/nicotinamide-induced Type 2 diabetic rats
Body weight of animals in all groups was recorded at 0, 7th, and 15th day and change in body weight was also mentioned. The highest change in body weight during the study period was found to be in the diabetic control group which decreases up to 191.2±0.374 g. In all treatment group with 100 mg/kg of EEAI and 200 mg/kg of EEAI body weight was increased up to 202.6±0.4 g and 211±1.029 g, p<0.01, respectively (Table 5).

Effect of EEAI on serum lipid profile in HFD/STZ-induced Type 2 diabetic rats
EEAI at a dose of 100 mg/kg b.w. and 200 mg/kg b.w., significantly decreases TC, TG, LDL-C, and VLDL-C (p<0.01). Glibenclamide 5 mg/kg b.w., significantly decreases TC, TG, LDL-C, and VLDL-C (p<0.01). HDL-C increased significantly in diabetic rats treated with EEAI 100 mg/kg b.w. (p<0.05), 200 mg/kg b.w., as well as in glibenclamide 5mg/kg, b.w. (p<0.01) (Table 6).

Effect of EEAI on serum lipid profile in STZ/nicotinamide-induced Type 2 diabetic rats
EEAI at a dose of 100 mg/kg b.w. and 200 mg/kg b.w., significantly decreases TC, TG, LDL-C, and VLDL-C (p<0.01). Glibenclamide 5 mg/kg b.w., significantly decreases TC, TG, LDL-C, and VLDL-C (p<0.01). HDL-C increased significantly in diabetic rats treated with EEAI 100 mg/kg b.w. (p<0.05), 200 mg/kg b.w., as well as in glibenclamide 5 mg/kg, b.w. (p<0.01) (Table 7).

Table 4: Effect of EEAI on body weight in HFD/STZ-induced Type 2 diabetic rats

Groups	Body weight (g)		
	0 day	14th day	28th day
Control	185.20±1.04	200.31±3.44	215.11±2.21
Diabetic control	182.50±1.44	182.50±4.33	187.50±4.78
Treated group (EEAI 100 mg/kg b.w.)	190.00±3.77	185.00±2.04	196.25±1.25*
Treated group (EEAI 200 mg/kg b.w.)	186.25±2.39	202.50±4.78*	208.75±3.14*
Standard group	188.75±3.75	201.25±5.54*	211.25±3.75**

Each value is mean±SEM of 6 rats in each group. *p<0.05, **p<0.01 and ***p<0.001 in comparison to diabetic control. EEAI: Ethanolic extract of *Alocasia indica*, HFD: High fat diet, STZ: Streptozotocin, SEM: Standard error of mean

Table 2: Effect of EEAI on blood glucose levels in HFD/STZ-induced Type 2 diabetic rats

Groups	Plasma glucose levels (mg/dl)		
	0-day	14th day	28th day
Control	117.54±2.85	116.54±5.80	115.70±5.70
Diabetic control	337.62±8.80	343.75±13.73	403.93±14.42
Treated group (EEAI 100 mg/kg b.w.)	338.21±5.62	325.86±9.70	288.96±7.29**
Treated group (EEAI 200 mg/kg b.w.)	339.27±6.93	297.64±7.61*	238.15±9.60**
Standard group	319.76±8.39	270.81±6.72**	192.10±9.04**

Each value is mean±SEM of 6 rats in each group. *p<0.05, **p<0.01 and ***p<0.001 in comparison to diabetic control, SEM: Standard error of mean, EEAI: Ethanolic extract of *Alocasia indica*, HFD: High fat diet, STZ: Streptozotocin

Table 5: Effect of EEAI on body weight in STZ/nicotinamide-induced Type 2 diabetic rats

Groups	Body weight (g)	
	Initial weight	Final weight
Control	187.25±0.83	221.88±1.48
Diabetic control	212.41±1.16	191.24±1.37
Treated group (EEAI (100 mg/kg b.w.)	198.36±1.54	202.65±1.23
Treated group (EEAI 200 mg/kg b.w.)	204.83±1.19*	211.82±1.29**
Standard group	195.28±1.16**	205.83±1.66**

Each value is mean±SEM of 6 rats in each group. *p<0.05, **p<0.01 and ***p<0.001 in comparison to diabetic control. EEAI: Ethanolic extract of *Alocasia indica*, STZ: Streptozotocin, SEM: Standard error of mean

Table 3: Effect of EEAI on fasting blood glucose levels in STZ/nicotinamide-induced Type 2 diabetic rats

Groups	Fasting blood glucose levels (mg/dl)			
	Day 1	Day 5	Day 12	Day 15
Control	71.82±0.66	75.22±0.58	73.67±0.74	75.68±0.75
Diabetic control	307.22±2.49	321.23±2.23	360.86±3.02	365.27±3.15
Treated group (EEAI 100 mg/kg b.w.)	282.86±1.86	253.44±1.88**	193.67±1.74	141.08±1.42*
Treated group (EEAI 200 mg/kg b.w.)	271.45±1.44	246.47±1.87	173.69±1.74**	119.62±1.05**
Standard group	263.22±1.01	241.88±1.66	165.49±1.32**	114.85±1.23

Each value is mean±SEM of 6 rats in each group. *p<0.05, **p<0.01 and ***p<0.001 in comparison to diabetic control. SEM: Standard error of mean, EEAI: Ethanolic extract of *Alocasia indica*, STZ: Streptozotocin

Table 6: Effect of EEAI on TC, TG, HDL-C, LDL-C and VLDL-C levels in HFD/STZ-induced Type 2 diabetic rats

Lipid level	Control	Diabetic control	Treated group (EEAI 100 mg/kg b.w.)	Treated group (EEAI 200 mg/kg b.w.)	Standard group
TC (mg/dl)	92.50±6.56	186.25±7.66	103.22±5.62	99.74±5.28	94.64±4.88
TG (mg/dl)	64.40±4.73	198.36±7.28	92.60±5.82	78.86±4.26**	72.27±4.16
HDL-C (mg/dl)	42.58±1.32	26.20±0.86	32.02±0.71*	36.84±1.16	40.28±1.76**
LDL-C (mg/dl)	37.04±1.22	120.38±6.76	52.68±4.75**	42.87±2.75	39.91±1.63**
VLDL-C (mg/dl)	12.88±0.94	39.67±1.45	18.52±1.16	15.77±0.85	14.45±0.83

Each value is mean±SEM of 6 rats in each group. *p<0.05, **p<0.01 and ***p<0.001 in comparison to diabetic control. EEAI: Ethanolic extract of *Alocasia indica*, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, High fat diet, STZ: Streptozotocin, SEM: Standard error of mean

Table 7: Effect of EEAI on TC, TG, HDL-C, LDL-C and VLDL-C levels in STZ/nicotinamide-induced Type 2 diabetic rats

Lipid level	Control	Diabetic control	Treated group (EEAI 100 mg/kg b.w.)	Treated group (EEAI 200 mg/kg b.w.)	Standard group
TC (mg/dl)	71.60±5.68	166.20±6.66	97.20±5.73	94.47±5.68	83.64±4.68
TG (mg/dl)	74.40±5.89	176.60±6.87	102.60±6.87	91.82±5.66**	85.02±4.05
HDL-C (mg/dl)	25.60±1.53	10.20±0.66	15.02±0.71*	20.26±0.66	23.82±0.57**
LDL-C (mg/dl)	31.12±1.75	120.68±5.68	61.66±3.75**	55.85±0.87	42.82±0.83**
VLDL-C (mg/dl)	14.88±1.18	35.32±1.37	20.52±1.37	18.36±1.13	17.00±0.81

Each value is mean±SEM of 6 rats in each group. *p<0.05, **p<0.01 and ***p<0.001 in comparison to diabetic control. EEAI: Ethanolic extract of *Alocasia indica*, TG: Triglyceride, HDL-C: High-density lipoprotein cholesterol, LDL-C: Low-density lipoprotein cholesterol, VLDL-C: Very low-density lipoprotein cholesterol, High fat diet, STZ: Streptozotocin, SEM: Standard error of mean

DISCUSSION

The simulation of experimental diabetes in rats using chemicals, which selectively destroy pancreatic β-cells is very suitable and easy to use. STZ and alloxan are most usual substances to induce diabetes in rats. STZ is lethal to β-cells. STZ produces massive reduction of the β-cells of the islets of Langerhans and induces hyperglycemia [20]. The present investigation reports the antidiabetic and antihyperlipidemic activities of the EEAI rhizomes in HFD/STZ and STZ (STZ)/nicotinamide-induced Type 2 diabetic rats. The possible mechanism of action of plants extracts could be correlated with the plasma insulin levels.

It is generally accepted that glibenclamide (a sulfonylurea family drug) causes reduction of blood glucose mainly by stimulation of insulin release from pancreatic β-cells. Furthermore during long-term treatment, an insulin-independent blood glucose-decreasing mechanism may work [21].

EEAI rhizomes at a dose of 100 and 200 mg/kg b.w., (p.o.) showed significant decrease in blood glucose level and significant increase in body weight in both the experimental models. Further EEAI showed antihyperlipidemic activity as evidenced by significant decrease in serum TC, TG, LDL-C, and VLDL-C levels coupled together with elevation of HDL-C level in diabetic rats in HFD/STZ and STZ/nicotinamide-induced Type 2 diabetic rats. In our study, it is evident from the above results that the EEAI at a dose of 100 and 200 mg/kg b.w., (p.o.) showing antidiabetic and antihyperlipidemic activities.

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