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Antidiabetic effect of various fractions of Habenaria plantaginea root in streptozotocin-induced diabetic rats

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Abstract

The aim of this study is to evaluate the antidiabetic effect of methanolic extract of Habenaria plantaginea and its various fractions in different animal models. The effect of repeated oral administration of methanolic extract along with its n-hexane, ethyl acetate and n-butanol fractions on serum lipid profile and plasma enzyme levels in diabetic rats was also examined. The effect was found to be pronounced in n-butanol fraction. In oral glucose tolerance test, reduction of fasting blood glucose levels took place from 60 min on administration of methanolic extract and its various fractions. The n-butanol fraction has almost similar effect as that of standard drug Glibenclamide (10 mg/kg b. w). After 15 days of treatment, n-butanol fraction showed maximum reduction of blood glucose levels (64.28 %). Total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels were found to be decreased by 38.98, 28.06, 56.26 and 37.77% respectively in diabetic rats whereas, cardioprotective, high density lipoprotein (HDL) was increased by 31.65%. The n-butanol fraction also restored the altered plasma enzyme such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) and alkaline phosphatase (ALP) levels to near normal. These results clearly indicate that n-butanol fraction of methanolic extract of Habenaria plantaginea possess high antidiabetic potential along with significant hypoglycaemic and hypolipidemic effects.

Keywords: Habenaria plantaginea, antidiabetic, streptozotocin.

Introduction

Diabetes mellitus (DM) is an endocrine disorder that is characterized by hyperglycemia [1]. A number of investigations, of oral antihyperglycemic agents from plants used in traditional medicine, have been conducted and many of the plants were found with good activity [2]. The World Health Organisation (WHO) has also recommended the evaluation of the plants' effectiveness in conditions where we lack safe modern drugs [3]. According to World Health Organisation projections, the diabetic population is likely to increase to 300 million or more by the year 2025 [4]. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors, glinides, which are used as monotherapy or in combination to achieve better glycaemic regulation. Many of these oral antidiabetic agents have a number of serious adverse effects [5]. Despite advances in understanding of the disorder and the management, the mortality and morbidity due to this disease is increasing [6]. The focus has been shifted to treat the various ailments through plant-derived drugs due to their safety, efficacy, cultural acceptability and lesser side effects.

Habenaria plantaginea belonging to the family Orchidaceae and is generally grown all over India, Nepal, Bangla Desh and South East Asia in tropical and sub-tropical environment [7]. It is used as folk medicines in Northern India to treat cough, asthma, helminthiasis, insanity and snake bite [8]. The plant has been used as the source of medicine for the treatment of tuberculosis and paralysis. It has been reported that various phytoconstituents such as flavonoids, cynogenetic glycosides, terpenoids and tannins are present in tuber root of Habenaria plantaginea [9].

To the best of our knowledge, no work on antidiabetic activity of of Habenaria plantaginea in streptozotocin-induced diabetic rats has been reported. Therefore, the present study was undertaken to evaluate the antidiabetic activity of Habenaria plantaginea in experimental animals.

Materials and methods

Plant material

The roots of Habenaria plantaginea were collected in the month of November from the Trisulia forest, Nayagarh, Odisha, India and were authenticated by senior taxonomist Dr. P. C. Panda, Regional Plant Resource Centre, Bhubaneswar, Odisha. A voucher specimen of the herbarium has been deposited at the Department of Pharmacognosy, Siksha 'O' Anusandhan University, Bhubaneswar, Odisha, India for future reference.

Preparation of plant extract and its fractions

The roots were dried under shade and crushed into coarse powder. Coarse powder was extracted by cold maceration method using methanol. The extract thus obtained was concentrated in rotary flash vacuum evaporator and further dried in vacuum oven. The methanolic extract was suspended in water and subjected to fractionation with n- hexane, ethyl acetate and n-butanol. The fractions were concentrated using rotary evaporator and dried in vacuum oven and utilized for animal studies.

Preliminary phytochemical screening

The methanolic extract obtained from Habenaria plantaginea was subjected to various qualitative chemical tests for the identification of various plant constituents [10].

Experimental animals

Wistar albino rats (150–200 g) and Wistar albino mice (20–25 g) of both sexes were obtained from the experimental animal facility of Siksha 'O' Anusandhan University, Bhubaneswar, Odisha. Before and during the experiment, rats were fed with standard diet. After randomization into various groups and before initiation of experiment, the rats were acclimatized for a period of 7 days under standard environmental conditions of temperature (25 ± 2° C), relative humidity (35-60%) and dark/light cycle (12/12h). Animals described as fasting were deprived of food and water for 16 h ad libitum. The conditions in the animal house and the study protocol were approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) vides registration no. 1171 / C/ 08 / CPCSEA.

Sample collection

Blood samples were collected by retro-orbital plexus puncture method and blood glucose levels were estimated using Accu-check Active Glucose Stripes in Accu-check Active Test Meter.

Acute toxicity studies

Healthy adult Wistar albino rats of either sex, starved overnight were divided into four groups (n = 6) and were orally fed with the methanolic extract of Habenaria plantaginea in increasing dose levels of 100, 500, 1000, 3000 and 5000 mg/kg body weight [11]. The rats were observed continuously for 2 h for behavioral changes and after a period of 24 and 72 h for any lethality or death.

Induction of diabetes

The Wistar albino rats of either sex of body weight of 150-200 g were kept fasting for 24 h and thereafter diabetes was induced by intra-peritoneal (i.p) injection of streptozotocin (STZ), freshly dissolved in citrate buffer (pH 4.5), immediately before use. STZ was given at a dose of 65 mg/kg body weight [12]. In order to avoid STZ induced hypoglycemic mortality, 5% glucose solution was given for 24 h to STZ treated rats [13]. After 72 h of STZ administration, the blood glucose levels were measured and the rats showing blood glucose level greater than 220 mg/dl were considered to be diabetic and were used for the present study.

Study of Habenaria plantaginea on normoglycaemic animal

Animals were divided in five groups of six rats each and treated orally once in a day as follow: group I served as control; groups II, III, IV and V were administered 200 mg/kg/day of methanolic extract, n-hexane, ethyl acetate and n-butanol fraction of Habenaria plantaginea respectively using gastric tube under the sample experimental conditions [14].

Study of Habenaria plantaginea extract and fractions on glucose loaded rats (Oral Glucose Tolerance Test)

The oral glucose tolerance test was performed as per the method of Shirwaikar [15]. In this method, rats were fasted for 16 h before and during the experiment. Rats were divided into five groups of six each and normal water (2 ml/kg, p.o), Glibenclamide (10 mg/kg, p.o) and suspensions of dried methanolic extract in 5% solution of tween80, n- hexane, ethyl acetate and and n- butanol fractions (200mg/kg, p.o) were administered to Gr. I,II, III, IV, V and VI respectively. Glucose (3 g/kg, p.o) was fed 30 min after the administration of extracts. Blood was withdrawn from retro orbital sinus. at 0, 30, 60 and 120 min of glucose administration. The blood glucose levels were estimated using glucose oxidase-peroxidase reactive strips and a glucometer (Accucheck, Roche Diagnostics, USA)

Study of Habenaria plantaginea on Streptozotocin-induced diabetic rats (Acute study)

The Wistar albino rats were divided into five groups (n=6). Group I served as solvent control which received normal water (2ml/kg, p.o) and group II received Glibanclamide (10 mg/kg, p.o) by oral route of administration. The suspensions of methanolic extract and its fractions at the dose of 200 mg/kg body weight were administered to Gr. III, IV, V and VI respectively in a similar manner. The blood samples were collected through tail vein puncturing with hypodermic needle and blood glucose levels were measured at 0, 1, 2, 4, 8 and 24 h of administration of single dose for acute study.

Study of Habenaria plantaginea on Streptozotocin-induced diabetic rats (Long term study)

Animals were divided into five groups of six rats each. The extract was administered for 15 days. Group I served as the control, which were administered normal water daily and group II diabetic rats were administered standard drug Glibenclamide (10 mg/kg, p.o) for 15 days.

Group III, IV and V diabetic rats were administered Habenaria plantaginea methanolic extract and its various fractions at the dose of 200 mg/kg respectively. The antidiabetic effect of Habenaria plantaginea extract and its fractions was determined by measuring fasting blood glucose levels on days 1, 3, 5, 7 and 15.

Estimation of biochemical parameters

Blood was collected on 15th day from retro-orbital plexus of the overnight fasted rats under light ether anaesthesia and kept aside for half an hour for clotting. Serum was separated by centrifuging the sample at 6000 rpm for 20 min. The serum was analysed for total protein [16], total cholesterol [17], TG, LDL, VLDL, HDL [18], SGPT, SGOT and ALP [19].

Statistical analysis

All the values of fasting blood glucose level and biochemical estimations were expressed as mean± standard deviation (SD) and analyzed for ANOVA followed by Dunnet's t -test. Differences between groups were considered significant at P<0.05 and P < 0.01

Results

Preliminary phytochemical screening

The phytochemical screening of methanolic extract of Habenaria plantaginea revealed the presence of flavonoids, phenolics, steroids and triterpenoides.

Acute toxicity studies

The acute toxicity study result of the methanolic extract of Habenaria plantaginea on mice showed no mortality and no significant gross behavioural changes observed even at a higher dose level of 5 g/kg b. w. Therefore, it is evidenced that the plant extracts are non-toxic in nature and hence found suitable to explore their blood glucose lowering activity in different animal models.

Antidiabetic activity in normal, glucose loaded and Streptozotocin-induced diabetic rats

The antidiabetic effect of extract of Habenaria plantaginea on the fasting blood sugar level of normal, glucose loaded and Streptozotocin-induced diabetic rats is shown in Table 1, 2, 3 and 4. In normal animals, significant (P<0.05 to p<0.01) reduction in the blood glucose levels (BGL) was observed by the methanolic extract and its different fractions as compared to the solvent control [Table 1].

Table.1. Effect of Habenaria plantaginea on blood glucose levels in normal rats.

Groups & Treatment	Blood Glucose Levels (mg/dl)					
	0 h	1 h	2 h	4 h	8 h	F value
Normal control (2ml/kg)	91.45 ±9.15	89.5 ±11.12	92.76 ±8.33	95.85 ±6.03	97.25 ±10.13	0.86
Glibenclamide (10 mg/kg	90.15 ±6.22	78.98 ±8.16*	69.78 ±5.37*	63.66 ±8.25**	58.45 ±6.32** (35.16)	178.24
Methanolic extract (200 mg/kg)	96.5 ±5.29	93.56 ±5.16	81.5 ±4.77*	78.65 ±5.59*	70.05 ±3.18** (27.40)	98.45
n-Hexane fraction (200 mg/kg)	95.36 ±6.88	98.26. ±4.53	94.25 ±4.97	86.2 ±5.29*	84.5 ±4.19* (11.38)	46.15
Ethyl acetate fraction (200 mg/kg)	89.83 ±5.11	85.5 ±5.69	76.16 ±5.52*	70.25 5.11*	66.85 ±4.92** (25.58)	97.35
n-Butanol fraction (200 mg/kg)	90.45 ±4.67	81.24 ±6.25*	71.65 ±5.68*	66.56 ±5.42**	61.35 ±5.56** (32.17)	156.45

Values expressed as mean ± SD (n=6). Treatment was done for 8 hours. The data were statistically analysed by one-way ANOVA, followed by Dunnet's t-test. p values less than 0.05 were considered significant. *: p <0.05; **: p<0.01

The maximum hypoglycaemic activity was induced by n-butanol fraction at 200 mg/kg dose level (32.17%). Hypoglycaemic activity of Glibenclamide (10 mg/kg), the reference drug, was found to be 35.16%. However, the effects of n-hexane and ethyl acetate fractions (200 mg/kg) were found to be 11.38 and 25.58% respectively.

The methanolic extract and its various fractions significantly improved the glucose tolerance test up to 120 min (Table 2). The n-butanol fraction of Habenaria plantaginea at the dose of 200 mg/kg showed maximum reduction of BGL by 32.97% from control value in 120 min. Glibenclamide improved the glucose tolerance test up to 120 min. (34.59%). The methanolic extract, n-hexane and ethyl acetate fractions significantly improved the glucose tolerance test up to 120 min (25.91, 13.25, 30.18% respectively).

Table.2. Effect of Habenaria plantaginea on blood glucose levels in glucose loaded rats.

Groups & Treatment	Blood Glucose Levels (mg/dl)				
	0 min	30 min	60 min	90 min	120 min
Normal control(2ml/kg)	94±4.22	136.5±3.95	132±5.60	128±3.28	124.85±4.65
Glibenclamide(10 mg/kg)	91.5±3.6	111.83±4.53	101.5±3.61** (23.10)	92.6±2.94** (27.65)	80.662.58** (34.59)
Methanolic extract(200 mg/kg)	89.66±4.13	118.33±8.35	110.5±6.75* (16.28)	101.33±7.45** (20.83)	92.5±5.12** (25.91)
n-Hexane fraction (200 mg/kg)	88.50±3.76	129.65±6.51	122.5±4.21* (7.19)	113.68±4.17* (11.18)	108.30±3.61** (13.25)
Ethyl acetate fraction (200 mg/kg)	90.33±7.9	116.5±4.37	107.85±4.21* (18.29)	98.67±5.17** (22.91)	87.165.70** (30.18)
n-Butanol fraction (200 mg/kg)	92.65±5.9	113.5±4.37	104.85±4.21** (20.56)	95.67±5.17** (25.25)	83.685.70** (32.97)

Values expressed as mean ± SD (n=6). The data were statistically analyzed by one- way ANOVA, followed by Dunnet's t-test. P values less than 0.05 were considered.

Table. 3. Effect of Habenaria plantaginea on blood glucose levels in Streptozotocin-induced diabetic rats (Acute study)

Groups & Treatment	Blood Glucose Levels (mg/dl)						
	0 h	1 h	2 h	4 h	8 h	24 h	F values
Normal control (2ml/kg)	231.15±8.68	235.84±17.62	238.50±8.98	234.66±17.38	235.38±15.50	250.2±16.08	0.44
Glibenclamide (10 mg/kg)	242.45±5.78	234±7.47	208.88±8.43**	174.72±10.78**	141.5±4.16** (41.63)	240.83±3.65	210
Methanolic extract (200 mg/kg)	238.45±8.75	233.76±5.93	225.55±6.04*	201.32±8.48**	182.24±8.97** (23.57)	248.88±6.18	0.70
n-Hexane fraction (200 mg/kg)	236.38±7.03	237.5±5.09	234.5±5.28	219.78±5.29*	206.35±6.30** (12.70)	241.36±6.28	25.15
Ethyl acetate fraction (200 mg/kg)	253.25±15.18	248.92±13.14	217±15.84**	188.16±13.16**	159.75±8.80** (36.92)	248.85±12.14	128.17
n-Butanol fraction (200 mg/kg)	254.46±15.18	246.5±13.14	214.66±15.84**	178.16±13.16**	152.75±8.80** (39.97)	244.76±12.14	168.75

Values expressed as mean ± SD (n=6). The data were statistically analyzed by one- way ANOVA, followed by Dunnet's t-test. P values less than 0.05 were considered significant. *: p <0.05; **: p <0.01. Figure in parenthesis indicates % fall in BGL as compared to 0 hour.

The n-butanol fraction at a dose level of 200 mg/kg b.w. produced the maximum fall of 39.97 % in the BGL of diabetic rats after 8 h of treatment (Table 3).The Glibenclamide at a dose of 10 mg/kg b.w. resulted in 41.63% fall in BGL of diabetic rats, where as methanolic extract, n-hexane and ethyl acetate fractions at the tested dose level produced the maximum fall of 25.57, 12.70 and 36.92% respectively in the BGL of diabetic rats after 8 h of treatment.

In the long term study, n- butanol fraction (200 mg/kg) registered 64.28% reduction of BGL at the end of 15 days. The standard drug, Glibenclamide (10 mg/kg) exhibited maximum reduction of BGL by 66.5%, while methanolic extract, n-hexane, ethyl acetate showed 48.53, 34.24 and 56.58% reduction of BGL. The most effective percentage reduction in BGL was observed in the animals treated by n-butanol fraction at 200 mg/kg body weight.

Table. 4. Effect of Habenaria plantaginea on blood glucose levels in Streptozotocin-induced diabetic rats (Long term study).

Groups & Treatment	Blood Glucose Levels (mg/dl)					
	Day 0	Day 3	Day 5	Day 7	Day 15	F value
Diabetic Control (2ml/kg)	235.45±10.39	238.5±9.48	228.5±9.81	232.5±7.42	241.24±8.30	1.51
Glibenclamide (10 mg/kg)	242.56±10.36	188.15±10.64**	146.67±8.15**	118.32±6.85**	81.25±10.61** (66.5)	248.84
Methanolic extract (200 mg/kg)	235.42±10.05	222.67±5.57	192.65±6.43*	157.16±4.35*	121.15±5.86* (48.53)	96.45
n-Hexane fraction (200 mg/kg)	238±6.54	232.78±7.25	207.66±6.24*	182.64±6.01**	156.5±5.24** (34.24)	75.16
Ethyl acetate fraction (200 mg/kg)	237.15±8.32	192.5±9.00**	156.33±10.30**	130.16±7.90**	102.95±8.21** (56.58)	198.64
n-Butanol fraction (200 mg/kg)	240.15±8.32	189.25±9.00**	149.33±10.30**	123±7.90**	85.76±8.21** (64.28)	214.67

Values expressed as mean ± SD (n=6). The data were statistically analyzed by one- way ANOVA, followed by Dunnet's t-test. P values less than 0.05 were considered significant. *: p <0.05; **: p <0.01. Figure in parenthesis indicates % fall in BGL as compared to day.

Biochemical parameters

The significant differences were observed in serum lipid profiles as TC,TG, HDL, LDL, VLDL, SGOT, SGPT and ALP (Table 5) in methanolic extract of Habenaria plantaginea and its different fractions treated diabetic animals at 200 mg/kg after 15 days of treatment, when compared with that of solvent control rats ($p<0.05$ to $p < 0.01$).

The n-butanol, ethyl acetate and n-hexane fraction of methanolic extract of Habenaria plantaginea reduced TC, TG, LDL and VLDL by 38.98, 28.06, 56.28, 37.77; 30.24, 22.2, 50.61, 25 and 24.86, 17.58, 44.59, 23.05% respectively. The methanolic extract of the plant registered 28.71, 21.59, 46.62 and 19.35% reduction in TC, TG, LDL and VLDL, while Glibenclamide showed the reduction of 48.55, 44.06, 63.69 and 47.27% respectively (Figure.5). There was an increase of pecentage in HDL cholesterol by 31.65, 25.21, 22.40 and 26.34 in n-butanol, ethyl acetate, n-hexane and methanolic extract treated diabetic groups respectively.

The elevated levels of SGPT, SGOT and ALP were significantly decreased by 34.81, 33.59, 33.43, 38.29; 28.02, 18.48, 13.77, 9.71and 35.4, 29.56, 24.83, 28.44% on treatment with n-butanol, ethyl acetate, n-hexane and methanolic extract respectively, where as SGPT, SGOT and ALP levels of Glibenclamide treated rats were decreased by 54.46, 31.23 and 44.57 after 15 days treatment. The most effective percentage reduction in serum lipids, SGOT, SGPT and ALP was observed in n-butanol treated rats.

Table. 5. Effect of Habenaria plantaginea on serum profile in streptozotocin -induced diabetic rats.

Groups & Treatment	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	ALP (IU/dl)	SGPT (IU/dl)	SGOT (IU/dl)
Normal (2ml/kg)	105.25±5.33	54.45±4.46	51.4±6.25	14.85±.35	12.66±4.85	126.35±8.25	21.75 ±8.24	55.5 ±4.66
Diabetic control (2ml/kg)	241.5±4.32	215±6.55	33.25±4.46	117.75±8.45	46.26.52	236.64±6.54	57.45 ±4.78	93.55 ±6.78
Glibenclamide (10 mg/kg)	124.25±5.88**	120.26±8.40**	37.35±4.25**	42.75±3.63**	24.36±5.33**	131.15±5.78*	26.16 ±6.14**	64.33 ±8.95**
Methanolic extract (200 mg/kg)	172.2428.67	168.5821.59	45.1426.34	62.8546.62	37.2619.35	169.3328.44	37.45 34.81	84.46 9.71
n-Hexane fraction (200 mg/kg)	181.46±4.57* (24.86)	177.2±6.45* (17.58)	42.85±5.75* (22.40)	65.24±6.64* (44.59)	35.55±6.24* (23.05)	177.86±8.34* (24.83)	38.15 ±5.67* (33.59)	80.66 ±6.84* (13.77)
Ethyl acetate fraction (200 mg/kg)	168.46±9.35** (30.24)	167.25±8.22** (22.20)	44.46±4.66* (25.21)	58.15±7.66* (50.61)	34.65±4.77* (25)	166.68±7.17* (29.56)	38.24 ±9.25** (33.43)	76.26 ±5.32* (18.48)
n-Butanol fraction (200 mg/kg)	147.35±9.16** (38.98)	154.67±6.08** (28.06)	48.65±7.45** (31.65)	51.48±5.66** (56.28)	28.75±4.10** (37.77)	152.85±5.74* (35.40)	35.45 ±5.75** (38.29)	67.33 ±7.24** (28.02)

Values expressed as mean ± SD (n=6). Treatment was done for 15 days. The data were statistically analysed by one-way ANOVA, followed by Dunnet's t-test. p values less than 0.05 were considered significant. *: p <0.05; **: p<0.01

Discussion

Diabetes mellitus is possibly the world's largest growing metabolic disease and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases [20]. Considerably, large number of hypoglycemic/antidiabetic plants and herbs are known through folkloric use but their introduction into modern therapy waits pharmacological testing by modern methods. The study of such medicines might offer a natural key to unlock a diabetologist's pharmacy for the future.

In the present study, treatment with Habenaria plantaginea methanolic extract and it's ethyl acetate and n-butanol fractions(200 mg/kg b.w.) in normoglycaemic rats produced significant decrease in blood glucose level. The hypoglycemic effect may be due to increased secretion of insulin from the β-cells of the pancreas, i.e., pancreatotrophic action [21]. The results were comparable with that of Glibenclamide, which acts by stimulation of insulin release, thus, further confirming that the extract lowers the blood glucose by a pancreatotrophic action [22].

In normal and glucose loaded rats, hypoglycaemic action of the n-butanol fraction of methanolic extract is more than the other fractions and has almost similar effect as of synthetic drug Glibenclamide especially during OGTT and Streptozotocin-induced diabetes.

Prolonged administration of methanolic extract of Habenaria plantaginea leads to significant reduction in blood glucose level, which is in agreement with other study [23].

The mechanism by which Streptozotocin brings about its diabetic state includes selective destruction of pancreatic β-cells which make the cells less active leading to poor sensitivity of insulin for glucose uptake by the tissues [24,25]. The increased levels of plasma glucose in STZ-induced diabetic rats were lowered by the administration of Habenaria plantaginea extracts. The reduced glucose levels suggested that the extracts might exert insulin-like effect on peripheral tissues by either promoting glucose uptake metabolism by inhibiting hepatic gluconeogenesis or by absorption of glucose into the muscle and adipose tissues through the stimulation of revitalisation of the remaining β-cells [26,27]. Another possible mechanism by which Habenaria plantaginea brings about its hypoglycemic action may be by potentiating the insulin effects of plasma by increasing either the pancreatic secretion of insulin from the existing β-cells or by its release from the bound form [28].

The total protein content in serum was significantly lowered in the solvent (water) treated group, but it returned to nearly normal in the extract treated groups. Normal liver functioning was affected due to decreased protein synthesis in diabetic rats while it was

almost restored in the plant extract treated animals. Serum enzymes including SGPT, SGOT and ALP were used in the evaluation of hepatic disorders. An increase in these enzyme activities reflects active liver damage. Inflammatory hepatocellular disorders result in extremely elevated transaminase levels [29].

The increase in the availability of plasma SGPT, SGOT and ALP indicated liver dysfunction [30]. Therefore, an increase in the presence of SGPT, SGOT and ALP in plasma might be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream [31]. On the other hand, treatment of the diabetic rats with the subjected plant extracts caused reduction of these enzymes in plasma when compared to the solvent group and consequently reduce the liver damage. A significantly ($p < 0.01$) elevated activity of SGPT was observed in the solvent control rats suggesting hepatic dysfunction in these animals. Treatment of methanolic, n-hexane, ethyl acetate and n-butanol fractions and Glibenclamide significantly reduced hepatic dysfunction ($p<0.01$).

Diabetes is associated with hyperlipidemia [32]. High levels of TC and more importantly LDL cholesterol are major coronary risk factors whereas several studies have showed that an increase in HDL cholesterol is associated with a decrease in coronary risk. Most of the drugs that decrease total cholesterol also decrease HDL cholesterol [33]. However, it is interesting to found that in the present study the dose of 200 mg/kg of body weight of the methanolic and n-butanol fractions not only lowered the TC, TG, and LDL, but also enhanced the cardio protective lipid HDL after 15 days treatment. This would definitely reduce the incidence of coronary events, which is the major cause of morbidity and deaths in diabetic subjects [34].

Under normal conditions, the enzyme lipoprotein lipase hydrolyses triglycerides. Diabetes mellitus results in failure to activate this enzyme thereby causing hypertriglyceridemia. As the test extracts reduced the VLDL, TC and TG, hence it might be presumed that the methanolic extracts are responsible for the enhancement of transcription of lipoprotein lipase similar to that of insulin.It is well known that LDL plays an important role in arteriosclerosis and that hypercholesterolemia is associated with a defect relating to the lack of LDL receptors. The decrease of total cholesterol and LDL levels achieved by administration of methanolic extract, demonstrates a possible protection against hypercholesterolemia and the harm this condition brings about.

Conclusions

The results obtained in the study indicate that n-butanol fraction of methanolic extract of Habenaria plantaginea showed significant blood glucose lowering activity, both in the normal as well as STZ-induced diabetic rats. The administration of methanolic extract and its various fractions to the diabetic rats for 15 days not only significantly lowered the fasting blood glucose level of the diabetic animals but also lowered the lipid profiles, SGOT and SGPT levels. The blood glucose lowering and hypolipidemic effects are thus protective mechanism against the development of atherosclerosis, hyperlipidaemia and hyperglycemia, which are common in diabetes. It also caused reversal of the damage of liver observed in diabetic animals by lowering of the SGOT and SGPT levels. Among methanolic extract and its all tested fractions, n-butanol fraction exhibited most potent antidiabetic and hypolipidemic effect. It was found to have a high margin of safety and thus Habenaria plantaginea seems to have a promising value for the development of a potent phytomedicine for diabetes. Further studies are needed to isolate and characterize the chemical constituents of the methanolic extract of Habenaria plantaginea that may be responsible for the hypoglycemic and hypolipidemic activity.

Author's contributions

Goutam Ghosh has carried out the antidiabetic activity study. Debajyoti Das has designed the study protocol and helped in drafting the manuscript. Agnimitra Dinda has performed the statistical analysis. Paidisethy Sudhir Kumar carried out the biochemical study and has helped review of the research article.

Declaration: Conflict of interest

The author(s) declare that they have no conflicts of interest to disclose.

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IAEC: Institutional Animal Ethical Committee
OECD: Organization for Economic Corporation and Development
ANOVA: Analysis of variance
i.p: Intraperitonial
min: Minutes
mg: Miligram
g: Gram
º C: Degree centigrade
h: Hour
OGTT: Oral glucose tolerance test
p.o: Per oral
STZ: Streptozotocin
WHO: WHO World health organization
TC: Total cholesterol
TG: Triglyceride
LDL: Low density lipoprotein
VLDL: Very low density lipoprotein
HDL: High density lipoprotein
SGOT: Serum glutamic oxaloacetic transaminase
SGPT: Serum glutamic pyruvic transaminase
ALP: Alkaline phosphatase