Research Article **ISSN:2456-1622**

Asia Pacific Journal of Pharmaceutical and Applied Sciences

PHYTOCHEMICAL ANALYSIS AND ANTIDIABETIC POTENTIAL OF *Ipomea reniformis* in RATS

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ABSTRACT

Diabetes is a significant global health concern, and India faces a particularly alarming epidemic. This study investigated the antidiabetic potential of *Ipomoea reniformis* extracts in streptozotocin-induced diabetic rats. Various extracts, including petroleum ether, ethanol, and ethyl acetate fractions, were prepared and subjected to phytochemical analysis. Acute toxicity studies confirmed the safety of the extracts. Diabetic rats treated with ethyl acetate insoluble fractions of ethanolic extracts exhibited significant reductions in blood glucose levels, with the 600 mg/kg dose demonstrating the most potent effect. The extracts also positively impacted serum lipid profiles, improving parameters such as triglycerides, total cholesterol, HDL, LDL, and VLDL. These findings suggest that *Ipomoea reniformis*, particularly its ethyl acetate insoluble fractions, holds promise as a potential source of antidiabetic agents, warranting further investigation.

Keywords: *Ipomoea reniformis*, Diabetes, HDL, Insulin, LDL, VLDL, etc.

Submitted: 21 October 2024; Revised: 13 November 2024; Accepted: 25 November 2024

INTRODUCTION

Diabetes is a significant endocrine disorder that contributes to high morbidity and mortality rates worldwide [1]. The issue is particularly critical in India, where numerous studies have highlighted an increased ethnic predisposition to diabetes among migrant Asian Indians. Recent epidemiological research has identified a growing diabetes epidemic in the country [2]. According to the latest Diabetes Atlas published by the International Diabetes Federation (IDF), India currently has the largest population of individuals with diabetes, totaling 40.9 million. This number is projected to rise to 69.9 million by 2025 [3].

Diabetes is a chronic condition marked by elevated blood glucose levels caused by either an absolute or relative deficiency in circulating insulin [4]. It encompasses a group of metabolic disorders characterized by hyperglycemia due to defects in insulin secretion, insulin action, or both. Today, diabetes is a leading degenerative disease worldwide, affecting millions of individuals and associated with severe complications such as hypertension, atherosclerosis, and microcirculatory disorders[5]. Diabetes is broadly classified into two primary types: Type 1 diabetes (insulin-dependent diabetes mellitus), characterized by insufficient insulin production due to the destruction of insulin-producing beta cells in the islets of Langerhans within the pancreas, and Type 2 diabetes (non-insulin-dependent diabetes mellitus), which results from insulin resistance or reduced insulin sensitivity, coupled with relatively diminished insulin secretion that may progress to an absolute deficiency. The impaired response of body tissues to insulin is thought to involve defects in insulin receptors on cell membranes. Globally, Type 2 diabetes is the most prevalent form of the disease, with developing countries being disproportionately affected by this epidemic [6,7].

Current treatments for diabetes include insulin and a range of oral hypoglycemic agents such as sulfonylureas, metformin, alpha-glucosidase inhibitors, and troglitazone[8]. In conventional therapy, Type 1 diabetes is managed with exogenous insulin, while Type 2 diabetes is treated using oral hypoglycemic drugs like sulfonylureas, either as monotherapy or in combination, to achieve improved glycemic control. However, these treatments have limitations and are often associated with serious side effects [9]. Consequently, the search for safer, more specific, and effective hypoglycemic agents remains a critical area of research. Natural extracts from widely available traditional medicinal plants show significant potential for the development of new antidiabetic drugs.

Plants have long been an outstanding source of medicinal drugs, with many current pharmaceuticals being derived directly or indirectly from them. Ethnobotanical studies have documented over 800 plant species that may possess antidiabetic properties [10]. Numerous

herbs have demonstrated antidiabetic effects when evaluated using modern experimental techniques. A wide range of plant-derived active compounds, encompassing various chemical classes, has shown promising activity, supporting their potential use in the treatment of diabetes mellitus [11].

Ipomoea reniformis Chois (family Convolvulaceae) is a highly branched, creeping herb that roots at its nodes. The leaves are kidney-shaped or ovate-cordate, up to 1.9 cm long, broader than they are long, and toothed. The flowers are axillary, yellow, and accompanied by hairy sepals, blooming predominantly during the rainy season. This plant is widely distributed across India, particularly in damp regions such as the upper Gangetic plains, Gujarat, Bihar, Chhattisgarh, West Bengal, the Western Ghats (up to 900 meters in altitude), Goa, Karnataka, as well as in Ceylon and Tropical Africa [12].

Extracts of *Ipomoea reniformis* have been found to contain resin, glycosides, and reducing sugars. Chemical investigations have revealed the presence of esterified forms of caffeic, p-coumaric, ferulic, and sinapic acids. Traditionally, this medicinal plant has been used in folkloric medicine for managing diabetes and is reported to exhibit numerous therapeutic properties. In indigenous medicine, it is utilized for treating conditions such as cough, neuralgia, rheumatism, inflammation, diuresis, nasal disorders, fever related to liver enlargement, kidney diseases, and epileptic seizures [13].

MATERIALS AND METHODS

Collection and authentication of plant material

In the present study, the leaves of *Ipomoea reniformis* were collected from local area of Jaipur, Rajasthan. The plant of *Ipomoea reniformis* has been authenticated from Botanical Survey of India, Jodhpur, and Rajasthan, India. A voucher specimen has been deposited in the herbarium for future reference.

Extraction of *Ipomoea reniformis*

200 g coarse powdered leaves were defatted with 800 ml petroleum ether (60-80^{0}C) using Soxhlet apparatus. Extraction was continued until a drop of solvent from siphon tube, when evaporated on filter paper, did not leave a greasy spot (approximately 10-12 cycles). After the defattation, mark was taken out from extractor and spreaded as a bed on a clean paper and dried till evaporation of petroleum ether. Mark was kept for ethanolic extraction. The light brown colored petroleum ether extract was collected and kept for phytochemical analysis.

Phytochemical screening of *Ipomoea reniformis* extracts

Phytochemical screening of *Ipomoea reniformis* extracts were carried out on the basis of

qualitative chemical tests.

Animal study

Selection of animal species

Healthy young male Wistar rats (n=3) weighing between 150 to 220 g (8 to 12 weeks old) were used for acute toxicity study to determine LD_{50} of various extracts. The animals were divided in to 9 groups and each groups with three animals were used for assessing the toxicity study.

Acute oral toxicity

The acute oral toxicity studies of extracts were carried out as per the guidelines of Organization for Economic Co-operation Development (OECD) guidelines, draft guidelines 423 adopted on 17th December 2001 received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy young male Wistar rats were selected and their weight were recorded. Animals were divided into nine groups containing three rats. Extracts were prepared as a suspension by triturating with 2% Tween-80. The different dose of extracts solution of (5, 50, 200, 300, 500, 1000, 1500, 2000 and 5000 mg/kg b.wt) were given orally. Animals were observed at regular time intervals at least once during the first 30 minutes of initial dosing during the first 24 hrs (with special attention given during first 4 hours), and daily then after for total 14 days. As per anneure-2a of OECD 423 guideline following methodology was used. Animals were observed at regular time intervals at least once during the first 30 minutes of initial dosing during the first 24 hrs. In all the cases no death was observed in treated groups with in first 24 hrs. Additional observations like behavioral changes in skin, fur, eyes, mucous membranes, respiratory, circulatory, autonomic and central nervous systems and somato motor activity and behavior pattern were observed in each group of rats. Attention was also given to observation of tremors and convulsions. Acute toxicity study of various extracts of *Ipomoea reniformis.*

Pharmacological investigation of various crude extracts for antidiabetic activity

Adult Wistar rats (150-180 g) of either sex was procured and housed in the animal house of IDMA Lab, with 12 hrs light and 12 hrs dark cycles. Standard pellets obtained from Hafed, Rohatak, India, were used as a basal diet during the experimental period. The control and experimental animals were provided food and drinking water ad *libitum*. After randomization into various groups, the rats were acclimatized for a period of seven days under standard environmental conditions of temperature, relative humidity, and dark/light cycle. All animal experiments were approved by the Institutional Animal Ethics Committee (IAEC) of IDMA

Lab.

Streptozotocin induced diabetic model

STZ was dissolved in freshly prepared 0.1 M cold citrate buffer (pH 4.5) and administered by intraperitoneal route (45 mg/kg) to the overnight fasted rats. Diabetes was confirmed 72h after induction by measurement of tail vein blood glucose levels using glucometer by glucose oxidase-peroxidase method using strips. Diabetic rats were kept 14 days under standard laboratory condition for the stabilization of blood glucose levels. After 14 days induction of diabetes, blood glucose was again determined and animals with a blood glucose level greater than 250 mg/dl were selected for the study.

Experimental Design

In the experiment, a total of 36 albino rats were used. The rats were divided into 6 groups, comprising of 6 rats in each group. Experimental design for preliminary antidiabetic activity of various crude extracts of *Ipomoea reniformis*.

Groups	Treatment
I	Normal albino control rats received distilled water (NC)
II	Diabetic control albino rats (DC)
III	Albino rats received standard drug glibenclamide 10 mg/kg b.wt (Standard 10)
IV	Albino rats received ethyl acetate insoluble fraction of ethanolic extracts of *Ipomoea reniformis*150 mg/kg b.wt (EAIEE 150)
V	Albino rats received ethyl acetate insoluble fraction of ethanolic extracts of *Ipomoea reniformis* 300 mg/kg b.wt (EAIEE 300)
VI	Albino rats received ethyl acetate insoluble fraction of ethanolic extracts of *Ipomoea reniformis* 600 mg/kg b.wt (EAIEE 600)

Sample collection

Blood samples of all groups of rats were collected from tail vein before and 2, 4, 6, 8 and 24 hr after treatment. Blood glucose levels were determined in each group of rats. Blood glucose levels were measured by using Gluco-meter.

Body weight measurement

Body weights were determined at 0, 7, 14, 21 and 28 days. The increases in percentage of body weight in experimental diabetic rats were calculated. The result were compared with that of the standard drug.

Biochemical parameter (Serum lipid profile)

The serum was analyzed for total cholesterol (TC), triglycerides (TG), high density

lipoproteins (HDL), low density lipoproteins (LDL) and very low-density lipoproteins (VLDL).

Statistical Analysis

The result of the study was subjected to one way analysis of variance followed by student's t-test for multiple comparisons. Values with $P < 0.05$ were considered significant.

RESULTS AND DISCUSSION

The percentage yield, consistency and colour of different extracts / fraction of *Ipomoea reniformis* were tabulated in table 1.

Table 1. Extracts/fractions colour, and percentage yield

S. no.	Extraction /fraction	Colour	Consistency	(% w/w) Yield
1	Petroleum ether extract	Light brown	Sticky mass	1.79%
2	Ethanol extract	Dark brown	Sticky mass	7.36%
4	Ethyl acetate soluble fraction	Dark brown	Sticky mass	24.46 % of ethanolic extract
5	Ethyl acetate insoluble fraction	Dark brown	Sticky mass	52.10 % of ethanolic extract

The percentage yield of petroleum ether and ethanolic extract of *Ipomoea reniformis* leaves was found to be 1.79 %, 7.36 %, whereas ethyl acetate soluble fraction and ethyl acetate insoluble fraction of ethanolic extract were found 24.46 %, 52.10 %, respectively. These extracts and fractions were stored in airtight container for further studies.

Phytochemical Test

The result of qualitative chemical tests of different extracts of *Ipomoea reniformis* were tabulated in table 2.

Table 2. Qualitative phytochemical analysis of *Ipomoea reniformis*

Tests	Petroleum ether extract	Ethanol extract	Ethyl acetate soluble ethanolic extract	Ethyl acetate insoluble ethanolic extract
Steroid	-	+	-	+
Triterponoids	-	+	+	-
Glycosides	-	+	+	+
Carbohydrates	-	+	-	+
Alkaloids	-	+	+	+
Flavonoids	-	+	+	+
Tannins	-	+	-	+

Proteins and amino acid	-	+	-	+
Lipids	+	-	-	-

- absent and + present

Petroleum ether extract leaves showed presence of steroid, ethyl acetate soluble fraction showed the presence of triterponoids, glycoside, alkaloid and flavonoid whereas ethanolic extract and ethyl acetate insoluble fractions leaves showed the presence of various phytochemical groups alkaloids, flavonoids, phytosterols, glycosides, tannins and proteins.

Acute toxicity Studies

Table 3: Acute toxicity study of ethanol extracts of *Ipomoea reniformis*

S. No.	Dose (mg / kg b. wt.)	Numbers of animals	Observation
1	5	3	All animals survived
2	50	3	All animals survived
3	200	3	All animals survived
4	300	3	All animals survived
5	500	3	All animals survived
6	1000	3	All animals survived
7	1500	3	All animals survived
8	2000	3	All animals survived
9	5000	3	All animals survived

Table 4: Acute toxicity study of ethyl acetate soluble fraction of ethanolic extracts of *Ipomoea reniformis*

S. no.	Dose (mg / kg b. wt)	Numbers of animals	Observation
1	5	3	All animals survived
2	50	3	All animals survived
3	200	3	All animals survived
4	300	3	All animals survived
5	500	3	All animals survived
6	1000	3	All animals survived
7	1500	3	All animals survived
8	2000	3	All animals survived
9	5000	3	All animals survived

Table 5: Acute toxicity study of ethyl acetate insoluble fraction of ethanolic extracts of *Ipomoea reniformis*

S. no.	Dose (mg / kg b. wt)	Numbers of animals	Observation
1	5	3	All animals survived

2	50	3	All animals survived
3	200	3	All animals survived
4	300	3	All animals survived
5	500	3	All animals survived
6	1000	3	All animals survived
7	1500	3	All animals survived
8	2000	3	All animals survived
9	5000	3	All animals survived

Mortality was not observed in any groups of rats up to the dose level of 5000 mg/kg body weight. As per OECD guideline, these extracts come under the category -5 Hence, these are safe. The LD_{50} as per OECD guideline, this extract under category was found to be 2500 mg/kg. The LD_{50} of these extract should be effective 1/4 to 1/20. Therefore, dose of ethanolic extract, ethyl acetate soluble fraction of ethanolic extract, ethyl acetate insoluble fraction of ethanolic extract of *Ipomoea reniformis* for hypoglycemic activity was found to be 300, 600 mg/kg b. wt.

Blood glucose level

Table 6. Effect of different extract of *Ipomoea reniformis* on blood glucose level in streptozotocin induced diabetic rats

Groups	Treatment	Blood glucose level mg/dl					
		0hr	**2hrs**	**4hrs**	**6hrs**	**8hrs**	**24hrs**
I	NC	68.16 ±3.44	68.66 ±3.62	69.16 ±3.74	71.83 ±4.44	72.13 ±3.44	72.83 ±3.44
II	DC	379.83 ±8.04	384.66 ±8.61	388.33 ±9.84	392.16 ±9.83	396.83 ±9.48	398.00 ±9.06
III	Standard 10	395.00 ±9.83	257.33 ±6.80**	220.16 ±7.47**	157.50 ±6.76**	229.00 ±11.23**	322.66 ±10.47
IV	EAIEE 150	401.50 ±8.33	375.66 ±10.49	319.50 ±8.47	231.50 ±8.56*	279.66 ±9.95*	368.33 ±11.08
V	EAIEE 300	392.00 ±8.41	356.00 ±8.15	274.16 ±7.47*	215.16 ±9.74*	268.83 ±8.32*	358.50 ±10.38
VI	EAIEE 600	392.83 ±8.07	292.33 ±7.28**	218.66 ±6.88**	188.50 ±6.14**	253.00 ±7.46**	348.00 ±9.31

n=6, *****p<0.05- significant, ******p<0.01-more significant v/s diabetic control, SEM= standard error mean, SD = standard deviation, n= number of animals

NC - Normal control , DC - Diabetic control , Standard 10- Glibenclamide10 mg/kg b.wt , EAIEE 150- Ethyl acetate insoluble fraction of ethanolic extract 150 mg/kg b.wt , EAIEE 300- Ethyl acetate insoluble fraction of ethanolic extract 300mg/kg b.wt , EAIEE 600- Ethyl acetate insoluble fraction of ethanolic extract 600mg/kg b.wt

On the basis of blood glucose level in streptozotocin induced model in rats, control group treated rats showed the normal blood glucose level 68.26 ± 3.44, 68.96 ± 3.62, 70.16 ± 3.74, 71.83 ± 4.44, 72.13 ± 3.44 on 0, 7, 14, 21 and 28 days respectively. In streptozotocin induced diabetic animals were found elevated level of blood glucose 379.83 ± 8.04, 384.66 ± 9.66, 389.66 ± 11.52, 393.16 ± 10.87, 402.83 ± 10.30 on 0, 7, 14, 21 and 28 days respectively. As expected the administration of glibenclamide 10 mg/kg b.wt reduced the blood glucose level from 395.00 ± 8.63 to 183.83 ± 6.94 (53.46 %) after 21 days and to 145.00 ± 5.65 (63.29 %) after 28 days. Ethyl acetate insoluble fraction of ethanolic extract of *Ipomoea reniformis* was less effective at the dose at dose of 150 and 300 mg/kg b.wt but ethyl acetate insoluble fraction of ethanolic extract of *Ipomoea reniformis* was such as effective as the reference drug. At the dose of 600 mg/kg b. wt *Ipomoea reniformis* reduced the blood glucose level from 295.33 ± 8.36 to 218.33 ± 5.39 (44.32 %) and to 165.66 ± 5.31 (57.79 %) after 28 days.

Biochemical Parameter

On the basis of biochemical parameters in streptozotocin induced model in rats control group rats showed normal level of all TG (80.10 ±0.41), TC (78.26 ±1.67), HDL (26.28±0.73), LDL (40.18±1.18), VLDL (16.07 ± 0.24). In streptozotocin induced diabetic rats were found to be elevated TG (152.17 ± 1.47), TC (146.28 ± 1.87), LDL (90.10 ±1.01), VLDL (30.09 ± 0.42), except HDL (14.14 ±0.48) compared to normal rats group. Glibenclamide (standard group) treated animals group showed almost normal level compare to normal group treated rats TG (93.84 ±1.19), TC (88.10 ±1.21), HDL (23.07±0.78), LDL (62.15±0.63), VLDL (18.82 ± 0.59). Ethyl acetate insoluble fraction of ethanolic extract of *Ipomoea reniformis* at the dose of 600 mg/kg b.wt reduce the elevated level TG (99.13±1.13), TC (96.16±1.87), LDL (68.19±0.72), VLDL (20.25±0.57), except HDL (20.54±0.26) compared to normal treated rats group.

Table 7. Effect of different extract of *Ipomoea reniformis* on serum lipid profile in streptozotocin induced diabetic rats

Groups	Treatment	Serum lipid profile levels mg/dl				
		TG	**TC**	**HDL**	**LDL**	**VLDL**
I	NC	80.10 ±0.41	78.26 ±1.67	26.28 ±0.73	40.18 ±1.18	16.07 ±0.24
II	DC	152.17 ±1.47	146.28 ±1.87	14.14 ±0.48	90.10 ±1.01	30.09 ±0.42
III	Standard 10	93.84 ±1.19**	88.10 ±1.21**	23.07 ±0.78**	62.15 ±0.63**	18.92 ±0.59**

IV	EAIEE 150	120.09 ±1.16 ^{ns}	111.16 ±1.55^{ns}	17.00 ±1.55^{ns}	79.08 ±1.09^{ns}	25.26 ±0.38*
V	EAIEE 300	113.19 ±1.17*	106.28 ±1.31*	18.62 ±0.47**	71.24 ±0.60*	24.27 ±0.35**
VI	EAIEE 600	99.13 ±1.13**	96.16 ±1.87**	20.54 ±0.26**	68.19 ±0.72**	20.25 ±0.57**

n=6, *****p<0.05- significant, ******p<0.01-more significant v/s diabetic control, SEM= standard error mean, SD = standard deviation, n= number of animals

NC - Normal control , DC - Diabetic control , Standard 10- Glibenclamide10 mg/kg b.wt , EAIEE 150- Ethyl acetate insoluble fraction of ethanolic extract 150 mg/kg b.wt , EAIEE 300- Ethyl acetate insoluble fraction of ethanolic extract 300mg/kg b.wt , EAIEE 600- Ethyl acetate insoluble fraction of ethanolic extract 600mg/kg b.wt.

CONCLUSION

This study highlights the significant antidiabetic potential of *Ipomoea reniformis*, a plant traditionally used for managing diabetes. The researchers conducted a detailed investigation of various plant extracts and found that the ethyl acetate-insoluble fractions of ethanolic extracts were particularly effective in lowering blood glucose levels in diabetic rats. Notably, this effect, especially at a dose of 600 mg/kg, was comparable to that of the standard antidiabetic drug glibenclamide. Additionally, these extracts positively influenced the serum lipid profile by improving parameters such as triglycerides, total cholesterol, HDL, LDL, and VLDL, which are often disrupted in diabetes. Future research should focus on isolating and characterizing the specific bioactive compounds responsible for these antidiabetic effects, assessing the long-term efficacy and safety of *Ipomoea reniformis* extracts through pre-clinical and clinical studies, and investigating the mechanisms underlying its antidiabetic properties. Such studies could validate the therapeutic potential of *Ipomoea reniformis* and contribute to the development of novel and effective treatments for diabetes.

CONFLICTS OF INTEREST

The author had no competing interests.

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