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Pharmacological Modeling and Study for Antidiabetic Activity of *Praecitrullus fistulosus* Leaves Extracts

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ABSTRACT

Phytochemical and pharmacological investigations of leaves of *Praecitrullus fistulosus* for the antidiabetic activity have been done in our research work encompassed in depth and systematic screening of plant leaves and further extraction, characterization and bioevaluation. The research was envisaged for antidiabetic activity of different extracts procured by successive extraction methods and to find out or isolate the most possible active compounds from the active extracts showing the best activity. The antidiabetic activity of all extracts has been evaluated by STZ induced diabetes. The isolated compounds have been evaluated by *in-vitro* and *in-vivo* models. The alcohol soluble extractives values were found to be higher than water soluble extractive value. Alcohol being a moderately non polar solvent, able to extract polar and non-polar components yields higher extractive value. The ethanol extract shows significant enhancement in glucose tolerance in glucose fed hyperglycemic normal rats and produced a marked decrease in blood glucose levels at 200 mg/kg and 400 mg/kg body weight in streptozotocin-diabetic rats after 21 days treatment.

Keywords: Praecitrullus fistulosus, Streptozotocin and Glibenclamide, diabetes, Pharmacological Evaluation

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INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by a loss of glucose homeostasis, with disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[1] It is one of the common metabolic disorders with micro and macro vascular complications that results insignificant morbidity and mortality. It is considered as one of the five leading causes of death in the world.[2] Antioxidants have been shown to prevent the destruction of β-cells by inhibiting the peroxidation chain reaction and thus they may provide protection against the development of diabetes.[3] The worldwide prevalence of diabetes for all age groups was estimated to be 2.8% in 2000 and it is projected to be 5.4% in 2025. Currently available therapies for diabetes include insulin and various oral antidiabetic agents such as sulfonylureas, biguanides, α-glucosidase inhibitors and glinides. Despite considerable progress in the treatment of diabetes by oral hypoglycaemic agents, search for newer drugs continues because the existing synthetic drugs have several limitations and harmful effects. In the Indian traditional system of medicine several drugs of plant, mineral, and animal origin are described for their antidiabetic properties. There is an increasing demand by patient to use the natural product with antidiabetic activity. Medicinal and herbal plant products are traditionally used from long ago in many countries for the treatment of diabetes mellitus. So the traditional herbal medicines are mainly used which are obtained from plants, it plays important role in the management of diabetes mellitus.[4] Herbal products or plant products are rich in phenolic compounds, favonoids, terpenoids, coumarins, and other constituents which show reduction in blood glucose levels.[5] Several species of herbal drugs have been described in the scientific and popular literature as having antidiabetic activity. Due to their perceived effectiveness, fewer side effects in clinical experience and relatively low costs, herbal drugs are prescribed.[6]

Praecitrullus fistulosus is reported to contain polyphenols, flavonoids, ascorbic acid, tannin, alkaloid, saponin, phytosterol, diterpines, thiamin, and carotene, proteins, carbohydrates and cardiac glycosides. As per the literature review, it has been observed that *Praecitrullus fistulosus* is listed among the various medicinal plants widely been used

as an antimicrobial, antihelmintic, antioxidant and in treatment of diabetes mellitus. Sometimes used for gastric problems, appetizer, dyspepsia, weakness and colic in stomach and taken for blood pressure.Despite the rich presence of antioxidant principles and traditional use of *Praecitrullus fistulosus* leaves, systematic and scientific studies are lacking to delineate the antidiabetic activity of the plant leaves and it effect of hyperglycemia induced oxidative stress.[7-11] For resolving the problem of Diabetes mellitus, proposed a study in which plant *Praecitrullus fistulosus* has been selected for the antidiabetic activity.

MATERIAL AND METHODS

The leaves of *Praecitrullus fistulosus* were collected and the material was authenticated and identified. The correctly identified plant leaves were washed thoroughly in tap water, shade dried at room temperature for 10 days, coarsely powdered, and the powder was passed through sieve No.60 and used for extraction. This was extracted with different solvents in order to their increasing polarity to get the correct and dependable retention factor. Drug was subjected to extraction with ethanol (90%) as well as methanol in soxhlet apparatus, the extraction was completed in 25 cycles for two different solvents i.e. ethanol and methanol respectively. The extracts were dried & stored in dark place.

Experimental animals Wistar Albino rats of either sex (150 to 200 g) were purchased from the CPCSEA approved vendor and maintained at Animal House of the Institute, under standard laboratory conditions. In our studies we selected 1/10th and 1/5th dose i.e. 200 and 400 mg/kg dose (OECD Guideline 2001).[12] Doses equivalent to 200 mg and 400 mg of the crude drug per kg body weight were calculated, and suspended in 1% w/v Tween 80 solutions for the experiment.

Oral Glucose Tolerance Test: A method called Normal Glucose Oral Glucose Tolerance Test [NG-OGTT] is chosen for the effective analysis of drug so as to reduce the usage of a greater number of animals. The glycemic levels of rats withheld from food and water, had been determined and then was indeed administered the extract and standard. The animals had been divided into respective groups of 6 rats in each. Solutions of control, standard and the test samples (200 & 400 mg/kg) had been administered orally. The blood glucose levels had been determined in the following pattern: 0 min and 30 min to access the effect of test samples on normal blood glucose rats. The rats were then administered orally with 2g/kg glucose and the glucose levels were determined at 60, 90, 120, 150 and 180 min after glucose load. Blood was collected from the tip of the tail vein and fasting blood glucose level was calculated utilizing single touch glucometer (Accu check active) which was designed based on the glucose oxidase technique (Chattopadhyay, 1999).[13]

Group 1: received 1% w/v Tween 80 solution (5ml/kg body weight)

Group 2: received Glibenclamide 5mg/kg body weight

Group 3: Treated with ethanol extract of Praecitrullus fistulosus (200 mg/kg body weight) Group 4: Treated with ethanol extract of Praecitrullus fistulosus (400 mg/kg body weight) Group 5: Treated with methanol extract of Praecitrullus fistulosus (200 mg/kg body weight) Group 6: Treated with methanol extract of Praecitrullus fistulosus (400 mg/kg body weight)

Streptozotocin (Stz) Induced Diabetes In Rats:

After fasting 18 hours, the rats were injected intra-peritoneal injection through tail vein with a single dose of 50 mg/kg Streptozotocin, dissolved in freshly prepared .1 M citrate buffer (pH 4.5) immediately before use. After injection, the rats had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock. The diabetes was confirmed by estimating the blood glucose level after 72 hours by glucometer based on glucose oxidation method. Rats having blood glucose level more than 250 mg/dl were selected for further study (Jaiswal et al., 2014, Edwin et al., 2003, Chattopadhyay et al., 1997).[14-16]

Experimental Design: In order to assess the anti-diabetic activity, the animals were divided in fifteen groups of six animals in each group.

Group 1: Normal control rats (0.9% NaCl treated)

Group 2: Diabetic control, STZ -treated rats (50 mg/kg body weight)

Group 3: Treated with ethanol extract of Praecitrullus fistulosus (200 mg/kg body weight) Group 4: Treated with ethanol extract of Praecitrullus fistulosus (400 mg/kg body weight) Group 5: Treated with methanol extract of Praecitrullus fistulosus (200 mg/kg body weight) Group 6: Treated with methanol extract of Praecitrullus fistulosus (400 mg/kg body weight) Group 7: Standard drug (Glibenclamide) treated rats (5 mg/kg body weight)

The test drug and reference drug was administered orally at two dose level for a period of 21 days from starting day of diabetes (Yasodamma et al., 2013, Verma et al., 2013).[17-18] The blood was withdrawn by tail vein puncturing method. The samples of blood were obtained just before inducing diabetes and after drug administration on 3rd, 7th, 14th and 21st day. Blood glucose levels were determined by using glucometer (Rahar et al., 2011).[19]

RESULTS AND DISCUSSION

The total ash content was 5 times greater than acid insoluble ash, the presence of calcium oxalate crystals or acid soluble inorganic matter. The water and volatile content of a crude drug were determined by test for loss on drying[20]. In our investigation the percentage yield of loss on dying was found to be 6.71% (w/w). Extractive values are chiefly used for the determination of exhausted or adulterated drug. The alcohol soluble extractives values were found to be higher than water soluble extractive value. Alcohol being a moderately non polar solvent, able to extract polar and nonpolar components yields higher extractive value.

Table 1: Determination of Physicochemical Parameters

S. No.	Determination	% ash content
1	Total ash	12.03 ± 0.03
2	Acid insoluble ash	2.83 ± 0.02
3	Water soluble ash	4.86 ± 0.21
4	Alcohol soluble extract value	5.20 ± 0.21
5	Water soluble extract value	3.80 ± 0.04
6	Loss on drying	6.71 ± 0.03

Oral Glucose Tolerance Test

Ethanol extracts (EA) at the dosage levels of 200 & 400mg/kg body weight shown significant hypoglycemic effect in overnight fasted normal rats. Methanol extracts (MT) also shown significant activity in treated rats as compared to normal rats, but the effect was comparatively lesser than ethanol extract[21].

Table 2: Effect of different extracts on oral glucose tolerance test in normal rats

Group No.	Test sample (mg/kg)	Blood glucose levels (mg/dL)						
		0 min	30 min	60min (glucose load)	90min	120min	150min	180min
I	Normal Control	81.43±3.70	81.80±1.31	81.38±2.96	184.10±4.12	154.05±3.49	144.05±3.49	138.26±3.60
II	Standard	82.54±3.17	69.21±3.90**	67.69±1.14***	153.28±5.20**	117.28±6.20**	92.98±5.19***	67.38±2.12***
III	ET 200 mg/kg	80.23±2.70	72.20±2.11**	69.48±1.96***	162.30±4.32	132.04±2.49**	120.16±3.60***	90.56±2.60***
IV	ET 400 mg/kg	81.33±5.70	69.30±1.61**	67.68±2.52***	155.10±4.12	122.05±3.49***	108.46±5.50***	81.56±6.50***
V	MT 200 mg/kg	81.33±3.40	79.23±1.51*	76.18±1.26*	169.30±7.12	151.20±7.19	146.16±1.60	138.16±1.60
VI	MT 400 mg/kg	81.13±8.40	76.40±5.21*	72.58±1.56**	165.10±7.12	141.23±8.19**	135.26±3.60**	133.16±3.60**

Streptozotocin Induced Antidiabetic Activity

The induction of diabetes with streptozotocin increases the blood glucose level significantly (p<0.001) in group II rats as compared to normal rats. In 21 day study glibenclamide the standard drug restored the blood glucose highly significant with the p<0.001 in 14 days whereas ethanol extract (200 & 400 mg/kg) reduced the glucose level significant with the p<0.01 & p<0.001. Methanol extracts had significant effects (p<0.01) on 14th and 21st days, but the effect was comparatively lesser than ethanol extract.

Table 3: Effect of different extracts on blood glucose level in streptozotocin induced diabetic rats

Group No.	Group	Blood Sugar level				
		Long Term Study (Days)				
		Before inducing Diabetes	3	7	14	21
I	Normal control	80.3± 0.46	82.2±0.17	81.4 ± 1.7	81.9± 0.57	80.11± 0.18
II	Diabetic control	82.4 ±0.81	242.7±1.79	273.8±1.53	268.3 ±4.04***	298.1±0.22***
III	Ethanol extract (200 mg/kg)	84.27±3.09	243.4±4.05	216.2±2.39***	200.8±2.08***	196.2±0.29***
IV	Ethanol extract (400 mg/kg)	83.78±1.09	241.6±4.09	205.2±4.75***	193.6±5.02***	175.3±0.82***
V	Methanol (200 mg/kg)	83.4± 0.94	242.6±1. 76	218.9 ± 3.19	214.8±2.99**	211.6±1.30**
VI	Methanol extract (400 mg/kg)	78.4± 0.22	240.6± 1.39	214.2± 3.18	211.8±2.28**	210.6±2.210***
VII	Glibernclamide (5 mg/kg)	82.25± 0.97	240.8±2.54	192.4 ±3.32**	166.3 ±1.47***	161.8±0.34***

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups

The ethanol extract produced a marked decrease in blood glucose levels at 200 mg/kg and 400 mg/kg body weight in streptozotocin-diabetic rats after 21 days treatment[22,23]. The antidiabetic effect Praecitrullus fistulosus may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after glibenclamide administration.

CONCLUSION

The present investigation comprises of the phytochemical and pharmacological investigations of leaves of *Praecitrullus fistulosus* for the antidiabetic activity. In preliminary phytochemical assessment, phytoconstituents like carbohydrate, glycosides, alkaloids, phytosterol, triterpinoids, amino acid, phenolic compound, flavonoids and saponins were showed positive tests in the different extracts. In present study, the ethanol extract of *Praecitrullus fistulosus* produced a marked decrease in blood glucose levels at 200 mg/kg and 400 mg/kg body weight in streptozotocin-diabetic rats after 21 days treatment. The antidiabetic effect may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after glibenclamide administration. In conclusion, the results of part of the study of selected topic demonstrate that *Praecitrullus fistulosus* plant leaves have good antidiabetics activity and it opens a new path for further research to continue work in this field so that herbal products or medicines could be brought in market for the benefit of society.

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