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ANTI-DIABETIC ACTIVITY OF ETHANOL EXTRACT OF *PRAECITRULLUS FISTULOSUS* LEAVES ON STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT: Objective: The present study was designed to evaluate the anti-diabetic activity of ethanol extract of *Praecitrullus fistulosus* leaves on streptozotocin induced diabetic rats. **Materials and Methods:** The anti-diabetic activity of plant leaves was investigated in streptozotocin induced diabetes model. Effect of ethanol extract of *Praecitrullus fistulosus* (EPF) leaves on normal blood glucose levels and oral glucose tolerance test were studied in normoglycemic rats while antidiabetic effect was evaluated in streptozotocin-induced hyperglycemic rats. EPF (200 and 400 mg/kg) was administered orally for 21 days. Glibenclamide (5mg/kg, orally for 21 days) was used as reference standard. **Results:** Administration of the EPF caused significant dose-dependent reduction in blood glucose in both normoglycemic and hyperglycemic rats and also improved glucose tolerance test. EPF reduced glycosylated hemoglobin, lactate dehydrogenase and creatinine kinase levels in streptozotocin treated animals. The extract also ameliorated oxidative stress Parameters -TBARS, catalase and superoxide dismutase activity and glutathione content. **Conclusion:** The ethanol extract of *Praecitrullus fistulosus* (EPF) leaves exhibited antidiabetic activity possibly through increased secretion of insulin and the effect may be attributed to the presence of flavonoids and phenolic compounds present in extract.

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. The World Health Organization predicted that DM affects approximately 171 million people worldwide and the number is expected to reach to 366 million in 2030 ³. In diabetes, hyperglycaemia generates reactive oxygen species (ROS) which in turn cause lipid peroxidation and membrane damage and thus, plays an important role in the production of secondary complications in diabetes mellitus such as kidney, eye, blood vessel, and nerve damage. 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 ⁴.

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India has the more than 63 million of diabetic persons⁵. 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. Herbal medicines have ever been used and claimed as antidiabetic agents but very less are available on commercially formulated forms⁶. *Praecitrullus fistulosus* commonly known as 'Tinda' in Hindi, belonging to the family Cucurbitaceae, is cultivated as a vegetable in India⁷. *Praecitrullus fistulosus* is reported to contain polyphenols, flavonoids, ascorbic acid, tannin, alkaloid, saponin, phytosterol, diterpenes, thiamin, and carotene that possess antidiabetic, antioxidative and anthelmintic activity⁸⁻¹¹. 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 its effect on hyperglycemia induced oxidative stress.

MATERIALS AND METHODS:

Plant Material and Extraction: The leaves on *Praecitrullus fistulosus* were collected during the month of May 2013 from the outfield near to Gwalior (Madhya Pradesh). The material was authenticated and identified at raw material herbarium by Dr K.K. Nagaich, Professor, Department of Horticulture, College of Agriculture, Gwalior (Madhya Pradesh). The plant material was shade dried at room temperature for 10 days, coarsely powdered, and the powder was passed through sieve No.60 and used for extraction. The powdered material was extracted separately using 90% ethanol by Soxhlet extraction method. The extract was concentrated using rotary vacuum evaporator. The dried extract was stored in airtight container & placed in cool place. The percent yield of the ethanol extracts of *Praecitrullus fistulosus* was found 12.65 % (w/w) with Blackish-red color.

Preliminary Phytochemical Tests: The crude ethanol extract of *Praecitrullus fistulosus* was subjected to qualitative tests for identification of different constituents like alkaloids, flavonoids, terpenoids, phenolics, glycosides, saponins and tannins by using standard qualitative methods described by Trease and Evans¹². Ethanol extracts revealed the presence of alkaloids, phenolics, flavonoids, terpenoids and tannins.

Drugs and chemicals: Streptozotocin (STZ) was purchased from Sigma, St. Louis, Mo, USA. Glibenclamide was procured as gift sample from Nicholas Piramal Pvt. Ltd, Mumbai. All chemicals used were of analytical grade.

Animals: Wistar albino rats of either sex (150- 200 g) were used for the present investigation and maintained at Animal House of the Institute. They were randomly distributed into various groups and housed in propylene cages under standard laboratory conditions i.e. temperature $25 \pm 2^\circ \text{C}$, relative humidity ($50 \pm 15\%$) and 12-hour light-dark cycle and fed with a standard commercial pellet diet and water *ad libitum*. The experimental protocols were approved on 30/09/2014 by Institutional Animal Ethical Committee and performed as per guidelines of CPCSEA, New Delhi. (Proposal no IPS/COP/IAEC/03)

Toxicity evaluation: Acute oral toxicity study was carried out as per the acute toxic class procedure given in OECD Guideline No. 423. The ethanol extract of *Praecitrullus fistulosus* (EPF) at a single oral dose of 2000 mg/kg body weight was given to three animals. The animals were continuously observed for 2 weeks for mortality and general behavior. The test was repeated in another three rats to confirm the acute toxic class of LD₅₀ determination.

Oral glucose tolerance test (OGTT): The oral glucose tolerance test was performed in overnight fasted (18 h) normal rats. They were divided into four groups (n = 6). Group I served as normal control which received 1% w/v Tween 80 solution and Group II and Group III received ethanol extract of *Praecitrullus fistulosus* (EPF) leaves orally at the doses of 200 and 400 mg/kg respectively, whereas Group IV received glibenclamide (5mg/kg). The blood glucose levels were determined in the following pattern: 0 min. and 30 min to assess 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 and 180 min after glucose load. Blood was collected from the tip of the tail vein and fasting blood glucose level was measured using single touch glucometer (Accu Check Active).

Experimental design:

Induction of diabetes: The STZ was dissolved in freshly prepared 0.1 M citrate buffer (pH 4.5) immediately before use and was administered by intra-peritoneal route at the dose of 50 mg/kg body weight for each rat and their blood glucose levels were checked after 72 hr. The rats whose blood glucose levels were more than 250 mg/dl were considered as diabetic and they were divided into five groups of six rats each¹³.

Experimental procedure: In experiment, total 30 rats were used (24 diabetes surviving rats, 6 normal control rats) for the execution of the experiment^{14, 15}. The rats were divided as follows into five groups:

- Group I: Normal control rats (0.9% NaCl-treated).
- Group II: Diabetic control (1% Tween 80 solution)
- Group III: EPF 200mg/kg body weight.
- Group IV: EPF 400mg/kg body weight.
- Group V: Glibenclamide 5mg/kg body weight

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 glucometer¹⁶.

Estimation of biochemical parameters: On 22nd day, other biochemical parameter were assessed in blood/serum. Glycosylated hemoglobin (HbA1c) was estimated in blood by the methods of Drabkin and Austin and Sudhakar and Pattabiraman. Serum creatinine kinase was assayed by the method of Tomas and lactate dehydrogenase (LDH) in serum was determined by the method of Wroblewski. Animals were sacrificed by cervical dislocation.

Pancreases were collected and washed with phosphate buffered saline (pH 7.4). Pancreatic tissue homogenate (10%) was prepared in 0.15M ice-cold KCl. TBARS (thiobarbituric acid-reactive substances), a marker for lipid per oxidation, Catalase, superoxide dismutase (SOD) activity and glutathione content were assessed by the standard methods. Tissue protein was estimated using conventional Biuret test.

Statistical evaluation: All the data are presented as mean \pm SEM, n= 6. The differences between groups were evaluated by one-way analysis of variance (ANOVA) followed by Dunnet's test to determine the statistical significance. $p < 0.05$ was chosen as the level of significance.

RESULTS:

Toxicity evaluation: Acute oral toxicity showed that ethanol extract did not produce any significant changes in the behavioral or neurological responses in the dose of 2000 mg/kg body weight till the observation period of 14 days. Acute oral toxicity studies revealed the no mortality or moribund stage of the ethanol extract of *Praecitrullus fistulosus* (EPF) leaves.

Effect of EPF on oral glucose tolerance test: The extract treatment (200 & 400mg/kg) caused significant reduction in normal blood glucose within 30 min of administration. (Table 1) Administration of glucose (2g/kg) produces significant increase in blood glucose of normal rats. Treatment with EPF 200mg/kg, 400mg/kg and Glibenclamide (5mg/kg) exhibited significant reduction ($*p < 0.05$, $**p < 0.01$, $***p < 0.001$ respectively) in blood glucose level over the period of 120 min as compared to normal control group as shown in Table 1. EPF showed significant reduction in blood glucose level at 120 minutes in comparison with the control. (Table 1)

TABLE 1: EFFECT OF ETHANOL EXTRACTS ON ORAL GLUCOSE TOLERANCE TEST IN NORMAL RATS

Gr.	Treatment	Blood glucose levels (mg/dL)					
		0 min	30 min	60min (glucose load)	90min	120min	180min
I	Normal Control	81.43 \pm 3.70	81.80 \pm 1.31	81.38 \pm 2.96	184.10 \pm 4.12	154.05 \pm 3.49	138.26 \pm 3.60
II	Standard	82.54 \pm 3.17	69.21 \pm 3.90**	67.69 \pm 1.14***	153.28 \pm 5.20**	117.28 \pm 6.20**	67.38 \pm 2.12***
III	Ethanollic 200mg/kg	80.23 \pm 2.70	72.20 \pm 2.11**	69.48 \pm 1.96***	162.30 \pm 4.32	132.04 \pm 2.49**	90.56 \pm 2.60***
IV	Ethanollic 400mg/kg	81.33 \pm 5.70	69.30 \pm 1.61**	67.68 \pm 2.52***	155.10 \pm 4.12	122.05 \pm 3.49***	81.56 \pm 6.50***

Values are mean \pm SEM (N= 6), $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ compared with Normal control group.

Effect of EPF on STZ induced hyperglycemia:

Blood glucose levels of the STZ treated rats were significantly higher than those in normal rats. In STZ (50 mg/kg) induced rats, the blood glucose level significantly increased from 82.4±0.81 to 298.1±0.22 mg/dl in diabetic control rates. Ethanol extracts were given up to 21 days at a dose of 200

mg/kg b.w and 400 mg/kg b.w. upto 21 days decreases blood glucose levels from 243.4±4.05 to 196.2±0.29 (19%) and 241.6±4.09 to 175.3±0.82 (28%) mg/dl, respectively. Standard drug Glibenclamide treatment also decreased the blood glucose levels from 240.8±2.54 to 161.8±0.34 (33%) mg/dl. (**Table 2**)

TABLE 2: EFFECT OF ETHANOL EXTRACTS ON GLUCOSE LEVEL IN STREPTOZOTOCIN INDUCED DIABETIC RATS

Group No	Group	Blood Sugar level			
		Long Term Study (Days)			
		3	7	14	21
I	Normal control	82.2 ± 0.17	81.4 ± 1.7	81.9 ± 0.57	80.11 ± 0.18
II	Diabetic control	242.7 ± 1.79	273.8 ± 1.53***	268.3 ± 4.04***	298.1 ± 0.22***
III	Ethanol extract (200 mg/kg)	243.4 ± 4.05	216.2 ± 2.39***	200.8 ± 2.08***	196.2 ± 0.29***
IV	Ethanol extract (400 mg/kg)	241.6 ± 4.09	205.2 ± 4.75***	193.6 ± 5.02***	175.3 ± 0.82***
V	Glibenclamide (5 mg/kg)	240.8 ± 2.54	192.4 ± 3.32**	166.3 ± 1.47***	161.8 ± 0.34***

Values are mean ± SEM (N= 6), *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control group.

Effect of EPF on Glycosylated haemoglobin (HbA_{1C}), Creatinine Kinase (CK) and Lactate dehydrogenase (LDH): STZ treatment increased glycosylated hemoglobin along with serum levels of creatinine kinase and lactate dehydrogenase. Treatment with ethanol extract of *Praecitrullus*

fistulosus, in both doses decreases the level of glycosylated hemoglobin and attenuated decrease in CK and LDH activity. Glibenclamide treatment also showed similar significant ($p < 0.01$) decrease in blood HbA_{1C} levels, CK and LDH activity when compared to group II. (**Table 3**)

TABLE 3: EFFECT OF ETHANOLIC EXTRACTS ON HBA_{1C}, SERUM CK, SERUM LDH

Group No	Group	Whole blood HbA _{1C} (%)	Serum Creatinine Kinase (CK), (IU/L)	Serum LDH (IU/L)
I	Control	5.12 ± 0.22	69.14 ± 2.88	190.12 ± 5.40
II	Diabetic control	14.33 ± 0.32***	151.32 ± 2.91**	315.44 ± 7.11***
III	Ethanol extract (200 mg/kg)	9.20 ± 0.34**	130.18 ± 0.65**	261.31 ± 8.23**
IV	Ethanol extract (400 mg/kg)	7.54 ± 0.27***	120.18 ± 0.64***	253.21 ± 8.30***
V	Glibenclamide (5 mg/kg)	5.17 ± 0.43***	88.62 ± 2.67***	231.76 ± 9.34***

Values are mean ± SEM (N= 6), *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control group.

Effect of EPF on oxidative stress parameters: STZ treatment increased TBARS formation along with reduction in level of CAT, SOD and Glutathione. Treatment with ethanol extract of *Praecitrullus fistulosus*, in both doses decreases the level of TBARS whereas increases CAT, SOD

activity and Glutathione content. Glibenclamide treatment also showed similar significant ($p < 0.01$) decrease in TBARS levels with increased CAT, SOD activity and Glutathione content when compared to group II. (**Table 4**)

TABLE 4: EFFECT OF ETHANOL EXTRACTS ON OXIDATIVE STRESS PARAMETERS

Group No	Group	TBARS (nmol MDA/mg protein)	CAT (nmol H ₂ O ₂ - consumed/min/mg protein)	SOD (IU/mg protein)	GSH (level of phosphorous liberated/ min/mg protein)
I	Control	0.489 ± 0.037	4.33 ± 0.089	3.59 ± 0.069	52.4 ± 0.945
II	Diabetic control	4.521 ± 0.562***	0.69 ± 0.020***	0.232 ± 0.025***	10.58 ± 1.034***
III	ethanol extract (200 mg/kg)	2.813 ± 0.451*	2.19 ± 0.132**	3.34 ± 0.272**	41.24 ± 1.116***
IV	ethanol extract (400 mg/kg)	0.723 ± 0.011***	2.71 ± 0.070**	3.62 ± 0.091***	41.12 ± 1.129***
V	Glibenclamide (5 mg/kg)	1.123 ± 0.011***	3.19 ± 0.156***	3.48 ± 0.087***	42.65 ± 1.651***

Values are mean ± SEM (N= 6), *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control group.

DISCUSSION: The present study demonstrates the antidiabetic effect of leaves of *Praecitrullus fistulosus* in STZ induced diabetes and oxidative stress. Streptozotocin (STZ) administration causes the destruction of β -cells after three days and reaches its peak at three to four weeks in rats¹⁷. β -cells are particularly sensitive to damage by nitric oxide and free radicals because of their low levels of free radical scavenging enzymes¹⁸. The improved glycemic control in oral glucose tolerance tests by the extracts of *Praecitrullus fistulosus* shows that the extracts also lowered the blood glucose levels even in normal rats. The effect of lowering blood glucose levels in normal rats may be due to the increased efficiency of the peripheral tissues for the uptake of glucose from blood. Thus the extracts can also be useful in patients with type II diabetes.

In the present study, results of the experiment indicated the significant antidiabetic and antioxidant activity of EPF (200 & 400 mg/kg b.w.). Since, the experiment focused on exploring the competence of ethanol extract of *Praecitrullus fistulosus* for the treatment of diabetes and related complications like oxidative stress to substantiate traditional claim. The elevated glucose level was successfully controlled by the EPF extract and glycosylated hemoglobin to be used as an indicator control of diabetes since glycohemoglobin levels approach normal values in diabetics in metabolic control¹⁹. EPF decreased the level of glycosylated hemoglobin indicating control of diabetes. Streptozocin-induced oxidative stress in diabetes is also a predictor of cardiac damage. Since LDH and CK are specific cardiac marker enzymes, increased serum LDH and CK levels were considered as marker of oxidative stress-induced cardiac damage²⁰.

Streptozotocin induced diabetic rats are associated with hyperlipidemia and increased levels of serum creatinine²¹. The ethanol extract treated rats at a dose level of 200 mg/kg and 400 mg/kg showed reduced levels of LDH and creatinine in serum when compared with the diabetic control group.

The lowering of LDH and creatinine in serum of EPF treated rats is presumed mainly to be a manifestation of lowering of blood glucose level. The increase in the levels of lipid peroxidation

indicates a decrease in the enzymatic antioxidant defence mechanism²². Several studies showed that oxygen free radicals are generated in diabetic state, and reduction in the activity of antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) also contributes to the development of oxidative stress in DM²³⁻²⁴. In the present study, it was observed that the EPF extract increased the SOD, CAT activity and GSH content in the pancreatic tissues of diabetic rats. This indicates that amelioration of oxidative damage by EPF extract in diabetic rats. The activities of both SOD and CAT were augmented in diabetic rats which could be attributed to the strong antioxidative properties.

The antidiabetic potential of ethanol extracts of *Praecitrullus fistulosus* may be due the presence of the secondary metabolites (flavonoids, alkaloids, phenolics, glycosides, and terpenes) present in varied concentrations in *Praecitrullus fistulosus*. These secondary metabolites are reported to possess antidiabetic potential differently and are responsible for observed antidiabetic effect.

CONCLUSION: In conclusion, ethanol extract of *Praecitrullus fistulosus* leaves exhibited antidiabetic effect against diabetes and also ameliorated the oxidative damage in pancreas. The effects may be attributed to presence of antioxidant phytochemical present in *Praecitrullus fistulosus*. Further mechanistic studies are required to suggest the appropriate mechanism for the antidiabetic effect of the plant.

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CONFLICT OF INTEREST: The authors of this article declare no conflict of interest in this study.

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