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Original Research Article

Experimental Evaluation of Antidiabetic Activity of *Piper betle* Leaf Extract in Streptozotocin Induced Diabetic Albino Rats

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Conflict of interest: Nil

Abstract

Aims: The present study evaluates the antidiabetic activity of *Piper Betle* leaves extract on the blood glucose level of streptozotocin induced diabetic rat models.

Objective: To study the antidiabetic activity of aqueous extract of *Piper betle* in streptozotocin induced diabetes in albino rats. ii) To compare the antidiabetic activity of *Piper Betle* extracts with that of standard drug glibenclamide used in the treatment of type 2 diabetes mellitus.

Materials and Methods: In the present study 24 male albino wistar rats divided into 6 groups with 6 animals were taken. One group as control was given normal saline for 15 days daily. Other 3 groups were induced diabetes. Standard and test groups were fed with glibenclamide (0.5mg/kg) and aqueous extract (50, 100, 150mg/kg) daily for 15 days respectively caused a significant ($P<0.01$) reduction in blood glucose levels in diabetic rats. The body weight of diabetic animals was also improved after daily administration of extracts. The extract also improved other altered biochemical parameters associated with diabetes.

Result: The results were analysed with student t-test.

Keywords: Antidiabetic, Glibenclamide, *Piper betel*, Streptozotocin.

Introduction

Diabetes mellitus is a major health problem in the modern world. It is a metabolic disorder in which the body does not produce or properly use insulin. The more common form of diabetes mellitus is Type 2 Diabetes mellitus or Non-Insulin Dependent Diabetes Mellitus (NIDDM) and is characterized by insulin resistance, impaired insulin secretion and hyperglycemia. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 [1] (Sarah Wild etal 2004). According to the International Diabetes federation, India has been declared as the Diabetes capital of the world. Plants have been used as sources of drugs for Diabetes in developing countries where the cost of conventional medicine is a burden to the population. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes. World Health Organization (WHO) has suggested that the evaluation of the potential of plants as effective therapeutic agents, especially in areas in which we lack safe modern drugs (Vijay

Kumar Dadi etal). [2] Although many drugs and interventions are available to manage diabetes, these are expensive for the large diabetic population of developing countries, apart from their inherent adverse effects. So it is necessary to look for new cheap alternatives to manage this major health problem (Park K. Diabetes mellitus). [3]

The Betel is the leaf of wine belonging to the Piperaceae Family. It is mostly available in Asian countries. It is valued both as a Mild stimulant and for its medicinal properties [4]. The *piper*

betle leaf extract contains large number of bioactive molecules like polyphenol, alkaloids, steroids, saponin, and tannins and are used in traditional medicinal systems [5]. However, anti-diabetic activity has not been scientifically investigated so far. The aim of this study therefore, was to investigate the anti-diabetic activity of *Piper betel* leaves [6].

Aim: To evaluate the Antidiabetic effect of *Piper betle* leaf extract in Streptozotocin induced diabetic albino rats.

Materials and Metodsh

The study was carried out in research laboratory of the Department of Pharmacology, Chalmeda Anand Rao Institute of Medical Sciences, Karimnagar, after obtaining ethical clearance from the Institutional Animal Ethics Committee (IAEC).

Materials

Plant

Piper betle leaves were collected from local market (Karimnagar, Telangana, India). These were Identified and Authenticated by the Department of Botany, S.R.R Govt College, and Karimnagar. The Authenticated plant leaves were used for the preparation of Extract.

Preparation of Extract

Fresh leaves of *Piper betle* were collected and air dried in shade at room temperature. The dried leaves were crushed by using mortar and pestle and extraction was carried out in soxhlet apparatus using 50% ethanol[7]. The extract was evaporated under reduced pressure and a dark brown mass was obtained.

Experimental design [7] (H.Gerhard Vogel): Albino rats of either sex weighing between 150 to 250 gms were used. The rats were divided into 6 groups. Each group consists of 6 animals. They were allowed free access to food (standard pellets) and water *ad libitum*. Experimental protocols and procedures used in this study were approved by the Institutional Animal Ethics Committee of Chalmeda Anandarao Institute of Medical Sciences, Karimnagar and Telangana state.

Induction of diabetes(Mabel Parimala et al.2014) [8]: STZ was stored at 4 - 8°c [9]. It was dissolved in normal saline. It was always prepared freshly for immediate use. All rats were fasted overnight before diabetes was induced. STZ [9] was given in the dose of 50mg/kg body weight, single intra peritoneal injection. The animals were observed to be diabetic from the 3rd day onwards. The animals showing a blood glucose level of 250mg/dl and above were considered diabetic and were included for the study. [10]

Grouping of Animals: Group I Normal control group recieved distilled water orally. Group II STZ induced rats which served as a diabetic control group orally daily. Group III STZ induced diabetic rats which served as a standard group and were given Glibenclamide at a dose of 0.5mg/kg body weight orally daily. Group IV STZ induced diabetic rats which served as a test group-1 and were given *Piper betle* leaf extract at a dose of 50mg/kg body weight orally daily. Group V STZ induced diabetic rats which served as test group-2 and were given *Piper betle* leaf extract at a dose of 100mg/kg body weight orally daily. Group VI STZ induced diabetic rats which served as test group-3 and were given *Piper betle* leaf extract at a dose of 150mg/kg body weight orally daily.

Collection of blood sample

Blood was drawn from tail vein of the rat through a sterile tuberculin syringe. Very gentle aspiration was done, in order to avoid vein collapse.

Estimation of blood glucose

Blood glucose estimation was done by using one touch – Horizon glucometer. The test strip was inserted in to the glucometer and the sample was directly placed on the test strip. The result i.e., the blood glucose level will appear on the screen within five seconds in mg/dl.

Standard drug administration

Glibenclamide was administered orally through oral feeding tube in diabetic rats for 15 consecutive days at a dose of 0.5mg/kg body weight. The blood glucose concentrations were monitored on 0, 5, 10, and 15days.

Test drug administration

The ethanolic extract of Piper betel was administered orally at a dose of 50mg/kg, 100mg/kg, and 150mg/kg body weight to groups IV, V, and VI respectively through oral feeding tube for 15 consecutive days. The blood glucose concentrations were monitored on 0, 5, 10, and 15days.

Statistical analysis

The data of all the groups were expressed as Mean ± SD., standard error of mean and percentage reduction were calculated. The test of significance was done by using student t-test.

Observation and Results

The present study was carried out to evaluate the Anti diabetic effect of *Piper betle* leaf extract in Streptozotocin induced diabetic rats.

The rats were divided into six groups.

Group I: The group of animals received distilled water orally and the blood glucose levels were measured on 0, 5, 10 and 15days, the results of which are shown in Table and the corresponding graphical representation is shown in Graph. **Group II:** The diabetes induced animals in this group were given distilled water and they served as diabetic control group. The blood glucose levels measured on 0, 5, 10, and 15days are shown in Table and the corresponding graphical representation is shown in Graph. **Group III:** The diabetes induced animals in this group were given Glibenclamide (0.5 mg/kg body weight) and they served as diabetic standard group. Their blood glucose levels are shown in Table. The percentage reduction in blood glucose levels of these animals when compared with diabetic control group was about 16.87% on the 5th day, 48.68% on the 10th day, and on day 15, it was 64.26% as shown in the Table and the corresponding graphical representation is shown in Graph. **Group IV:** The diabetic animals in this group were treated with *Piper betle* leaf extract at a dose of 50mg/kg body weight. The percentage reduction in blood glucose levels was about 1.654% on 5th day, 21.93% on the 10th day and 42.28% on 15th day and the values are shown in Table and are graphically represented in Graph. **Group V:** The diabetic animals in this group were treated with *Piper betle* leaf extract at a dose of 100mg/kg body weight. The percentage reduction in blood glucose levels was about 1.214% on 5th day, 26.10% on the 10th day and 50.64% on 15^{th} day and the values are recorded and represented in Table and Graph respectively. **Group VI:** The diabetic animals in this group were treated with *Piper betel* leaf extract at a dose of 150mg/kg body weight. The percentage reduction in blood glucose levels was about 4.19% on 5th day, 29.27% on the 10th day and 54.35% on 15th day and the values recorded and represented in Table and Graph respectively.

The blood glucose levels of all groups with Mean±SD, standard error of mean and percentage reduction are compared together in table and the differences are shown graphically in Graph.

Body weight: The mean body weight of the animals of all the groups for days 0, 5, 10 and 15 are recorded in Table and is graphically compared in Graph.

Table 1: Mean Blood glucose levels of all the groups

Group	Parameters	Day-0	Day-5	Day-10	Day-15
Group-I Normal control	Mean±SD	96.83 ± 19.71	92.83 ± 4.708	94.00 ± 6.387	90.83 ± 7.333
	SEM	8.047	1.922	2.608	2.994
Group-II Diabetic control	Mean±SD	275.17 ± 33.701	302.17 ± 31.852	335.83 ± 25.506	376.83 ± 16.364
	SEM	13.758	13.004	10.413	6.680
Group-III Diabetic Standard (Glibenclamide 0.5mg/kg)	Mean±SD	310.67± 50.409	251.17± 41.513	172.33 ± 41.433	134.67 ± 32.445
	SEM	20.579	16.948	16.915	13.246
	% reduction	-	16.87	48.68**	64.26**
Group-IV Diabetic test-1 *(Piper betle* leaf extract 50mg/kg)	Mean±SD	347.17 ± 32.45	307.17 ± 16.67	262.17 ± 32.090	217.50 ± 19.460
	SEM	13.25	6.809	13.101	7.945
	% reduction	-	1.654	21.93*	42.28**
Group-V Diabetic test-2 (*piper Betle* leafextract100mg/kg)	Mean±SD	332.83 ± 33.127	298.50 ± 24.370	248.17 ± 33.67	186.00 ± 23.332
	SEM	13.524	9.949	13.749**	9.525**
	% reduction	-	1.214	26.10	50.64
Group-VI Diabetic test-3 (*piper betle* leaf extract150mg/kg)	Mean±SD	341.17 ± 30.195	289.50 ± 17.986	237.50 ± 46.285	172.00 ± 45.259
	SEM	12.327	7.343	18.896	18.477
	% reduction	-	4.19	29.27*	54.35**

n = 6 animals in each group: *P value<0.001, **P value<0.0001

Table 2: Mean body weight of all the groups

Group	Parameters	Day-0	Day-5	Day-10	Day-15
Group-I Normal control	**Mean±SD**	**211.67 ± 20.14**	**213.33 ± 18.36**	**211.33 ± 20.35**	**214.33 ± 17.41**
	SEM	**8.22**	**7.50**	**8.31**	**7.11**
Group-II Diabetic control	**Mean±SD**	**208.00 ± 11.52**	**187.33 ± 10.63**	**166.67 ± 8.73**	**145.33 ± 8.55**
	SEM	**4.71**	**4.34**	**3.57**	**3.50**
Group-III Diabetic Standard (Glibenclamide 0.5mg/kg)	**Mean±SD**	**210.33 ± 15.92**	**191.33 ± 17.19**	**184.33 ± 18.17**	**181.00 ± 18.41**
	SEM	**6.50**	**7.02**	**7.42**	**7.51**
Group-IV Diabetic test-1 (*Piper betle* leaf extract 50mg/kg)	**Mean±SD**	**213.33 ± 21.72**	**199.00 ± 7.12**	**182.67 ± 10.01**	**166.00 ± 8.40**
	SEM	**3.53**	**2.91**	**4.09**	**3.43**
Group-V	**Mean±SD**	**211.00 ±**	**194.00 ±**	**183.00 ±**	**172.00 ±**

Diabetic test-2 (*piper betle* leaf extract 100mg/kg)		**21.72**	**16.54**	**14.79**	**8.94**
	SEM	**8.87**	**6.75**	**6.04**	**3.65**
Group-VI Diabetic test-3 (*piper betle* leaf extract 150mg/kg)	**Mean±SD**	**209.67 ± 16.90**	**194.33 ± 14.72**	**186.67 ± 12.50**	**174.67 ± 9.85**
	SEM	**6.90**	**6.01**	**5.10**	**4.02**

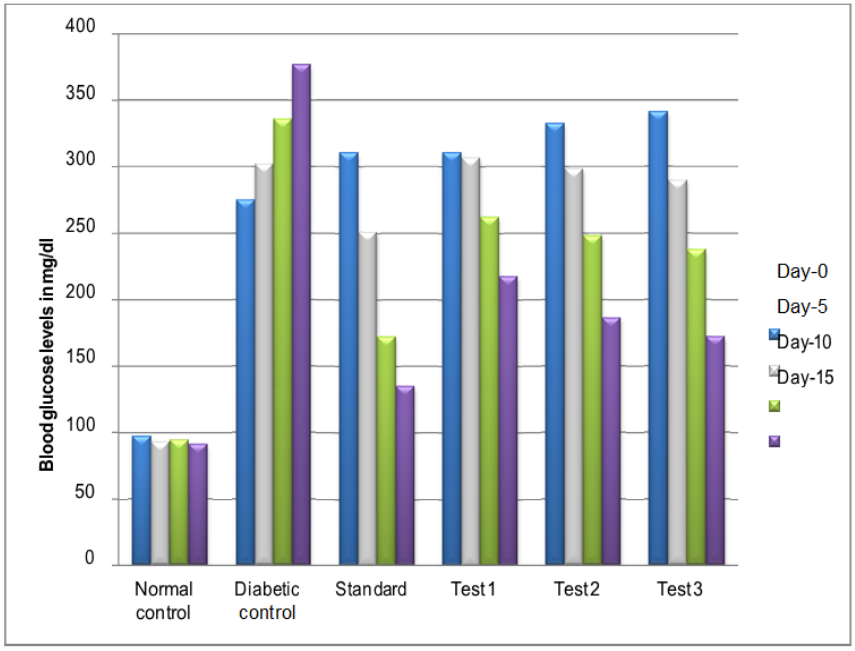

Figure 1: Mean blood glucose levels of all the groups

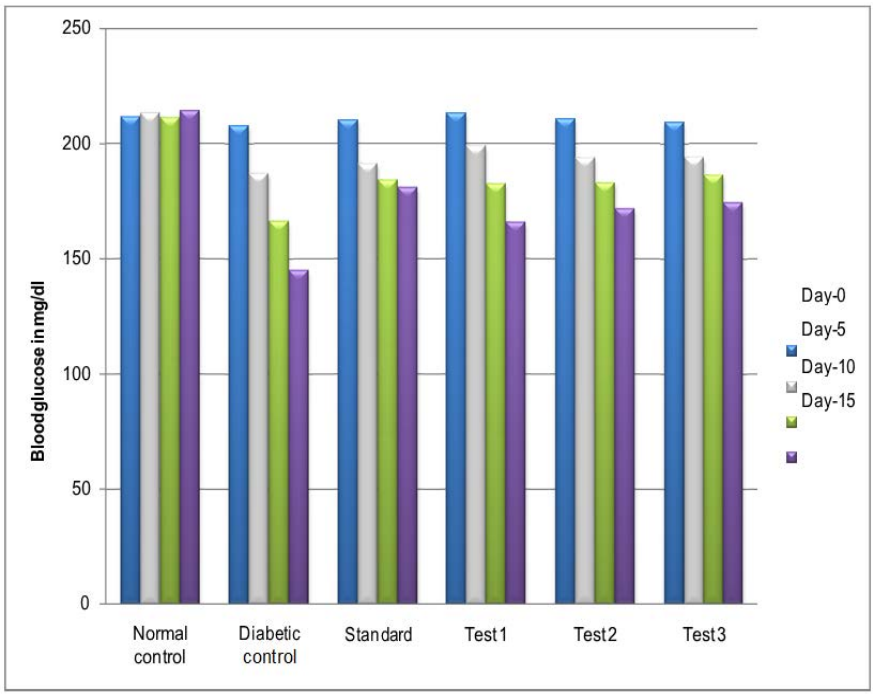

Figure 2: Mean body weight of all the groups

Discussion

Diabetes mellitus (DM) refers to a group of common metabolic disorders that share the phenotype of hyperglycaemia. Several distinct types of DM are caused by a complex interaction of genetics and environmental factors. The factors contributing to hyperglycaemia include reduced insulin secretion, decreased glucose utilization, and increased glucose production. The complications associated with diabetes involve multiple organ systems and impose a tremendous burden on the individual with diabetes and on the health care system.

Plants have played a major role in the development of new therapeutic agents. But there is still an extensive demand for new oral anti-diabetic drugs which are cheaper and have lesser side effects. Different indigenous drugs are used in the traditional systems of medicine for the treatment of diabetes mellitus but they lack scientific investigations for their efficacy in a laboratory setting.

One such plant, *Piper betle* (leaf) is also known as paan. Betel leaves is used by many people in Asia as a medicinal plant, which can cure many diseases [11]. Leaves have been traditionally used for chewing purposes along with other condiments. Betel leaves possess activity like anti diabetic, anti ulcer, anti platelet aggregation, respiratory depressant and anti helminthic property (Tripathi shalini etal.,2012) [12]. Hence the present study was carried out to evaluate the anti hyperglycemic effect of extracts of leaves of *piper betle* in streptozotocin induced diabetic rats.

In the present study, 50% ethanolic extract of dried leaves piper betel was given to different groups of animals at a dose of 50, 100 and 150 mg/kg body weight (groups IV, V and

VI) and the blood glucose lowering effect of these groups were compared with the diabetic control group.

The observations of this study were showed that the test drug (Piper betle leaf extract) and Standard drug (glibenclamide 0.5mg/kg body weight) reduces blood glucose level from the 5th day onwards.

The percentage reduction in blood glucose concentrations in streptozotocin induced diabetic rats of Piper betle leaf extract was 42.28%, 50.64% and 54.35% in corresponding test groups IV, V and VI (50, 100 and 150mg/kg body weight)respectively. A standard anti-diabetic drug glibenclamide (0.5mg/kg body weight), was observed to lower the blood sugar by 64.26%when compared to diabetic control rats. Thus the anti-diabetic activity of extract can be comparable to glibenclamidde, at least at the highest dose of the extract that is 150mg/kg body weight.

The statistical P-value in reducing blood glucose level was highly significant in standard group (glibenclamide 0.5mg/kg) and in group V (*Piper betle* leaf extract of 100mg/kg) and significant in group IV and VI (50 and 150mg/kg).

In addition, protection against body weight loss of diabetic animals was also observed with Standard drug Glibenclamide and Test drug Piper betle leaf extract.

The observation reduction of blood sugar by the extract can be attributed to any of three mechanisms. Any bio-active compounds may lower blood sugar either by potentiating the pancreatic secretion of insulin or increasing the glucose uptake [13] (Farjou etal., 1987; Nyunai etal.,2009) alternatively compounds may inhibit glucose absorption in gut [14] (Bhowmik etal.,2009). In either of these mechanisms or a combination of these mechanisms, the resultant effect will be reduction of sugar level in the blood [15].

Other Studies revealed that, the Piper betle leaf extract reduces the blood glucose level, glycosylated haemoglobin and decreased

activities of liver glucose-6-phasphatase and fructose- 1,6-bis phasphatase, whereas liver hexokinase increased in Streptozotocin induced diabetic rats. The ability of lowering blood glucose level of Streptozotocin induced diabetic rat's gives a suggestion that the extract have the insulinomimetic activity [16].

In this study, the anti-diabetic activity of ethanolic extract of Piper betle leaves, we have not explored the actual mechanism behind the reduction of blood sugar in streptozotocin induced diabetic rats, but further experiments are on the way to elucidate the actual mechanism. [17-19]

Conclusion

The following conclusions may be drawn, in the present study

- *Piper betle* leaf extract has dose dependent anti-diabetic effect in Streptozotocin induced diabetic rats.
- Anti-diabetic effect of *Piper betle* leaf extract was effective as comparable to standard (Glibenclamide 0.5mg/kg).

The anti-diabetic activity of *Piper betle* leaf extract at a dose of 50mg/kg and 100mg/kg body weight was comparatively less than the standard drug (Glibenclamide) where as 150mg/kg body weight of *Piper betle* leaf extract was comparable to standard drug Glibenclamide.

Further studies are required to confirm Piper betle leaf extract anti-diabetic activity and to find out exact mechanism of action.

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