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Evaluation of Anti-diabetic Activity of *Bambusa vulgaris* leaves in Streptozotocin Induced Diabetic Rats

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ABSTRACT
The leaves of *Bambusa vulgaris* possesses several bioactivities and is used in traditional medicinal systems. However, its anti-diabetic activity has not been scientifically investigated so far. The present study was carried out to evaluate the anti-diabetic activity of petroleum ether extract of *Bambusa vulgaris* (family: Poaceae) leaves in Streptozotocin induced diabetic rats. The preliminary phytochemical study showed the presence of phytosterols and tannins. From the toxicity study it was observed that petroleum ether extract of *Bambusa vulgaris* (PEBV) was nontoxic up to the dose of 2000 mg/kg body weight. In this study, animals received continuous oral administration of PEBV for a period of 15 days at the doses of 200 mg/kg and 400 mg/kg body weight. The control group was administered distilled water for the same duration. The blood glucose level was determined by GOD-POD kit method. The effect of PEBV was compared with oral dose of 0.5mg/kg Glibenclamide. The results showed that the PEBV significantly lowered the fasting blood sugar level of hyperglycemic rats in a dose dependent manner and it was also comparable to that of the standard drug Glibenclamide.

Keywords: Anti-diabetic, *Bambusa vulgaris,* Glibenclamide, Streptozotocin.

INTRODUCTION

Diabetes mellitus is a major and growing public health problem throughout the world. According to WHO projections, the prevalence of diabetes is likely to increase by 35%. Currently there are over 150 million diabetic patients worldwide. Recent estimates project that the number of patients diagnosed with Type II diabetes will more than double to 300 million before 2025**.** India has more than 30 million people with diabetics. It is estimated that by 2025, the number of diabetics will rise to 57 million in India, the highest number of diabetics in the world. [1-2]

There are several drugs in clinical practice for the treatment of diabetes mellitus. Many of these oral anti-diabetic agents have been reported to show serious adverse effects. It is apparent that due to the side effects of the currently used drugs, there is a need for a potent drug with minimal adverse effects, which can be taken for long durations. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug. Throughout the world many traditional plant treatments for diabetes exist. However, few have received scientific or medical scrutiny and the WHO has recommended that traditional plant treatments for diabetes warrant further evaluation. [3-5]

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Bambusa vulgaris, commonly known as golden bamboo is taxonomically a grass, but its habit is tree-like. It occurs throughout India in areas up to 2100 m elevation. Bamboo leaves have been claimed to be used as astringent, ophthalmic solution, sudorific and febrifuge. In Nigerian folklore medicine, bamboo is claimed to be used as an emmenagogue, abortifacient, appetizer and for managing respiratory diseases as well as gonorrhea. Leaves are used in ayurvedic medicine in ptosis and paralytic complaints.The leaves have been used in Indian folk medicine to treat various inflammatory conditions. [6-8] Though the plant and its extracts have been used in the folklore medicine extensively, there is no scientific evidence for such activities available in established scientific journals of repute. Keeping this in view, the present study has been undertaken to investigate the anti-diabetic potential of petroleum ether extract of *B. vulgaris* in Streptozotocin induced diabetic rats.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) (purchased from Sigma, St Loius, MO, USA) and glucose oxidase/peroxidase (GOD/POD) reagent (glucose kits were obtained from Randox Laboratories Ltd, UK). All other chemicals used were of analytical grade.

Plant material

The leaves of *Bambusa vulgaris* was collected from ABS Botanical & Research Institute, Karipatty, Tamil Nadu, India. The collected plant material was identified and authenticated

by the Botanical Survey of India, Southern circle, TNAU Campus, Coimbatore.

Preparation of plant extract

The collected leaves of *Bambusa vulgaris* were dried in shade at room temperature and reduced to coarse powder using a mechanical grinder. The dried powder material was extracted with petroleum ether (60-80°C) by hot continuous extraction in a Soxhlet's apparatus for 48 h. The extract was concentrated under reduced pressure and stored in an air tight container.

Phytochemical screening

The petroleum ether extract was screened for the presence of various phytoconstituents like steroids, alkaloids, tannins, flavonoids, and glycosides by employing standard phytochemical tests. [9]

Animals

Swiss albino mice of female sex weighing 20-25 g and male albino rats of Wistar strain weighing around 160-180 g were procured from Agricultural University, Manuthy, Trissure. They were acclimatized to animal house condition, fed with commercial pellet raw chow (Hindustan Lever Ltd, Bangalore) and have free access to water. The experimental protocol was approved by the IAEC (Institutional Animal Ethical Committee).

Toxicity study

Acute oral toxicity study was performed in mice by following Organization for Economic Co-operation and Development (OECD) guidelines AOT No. 425. [10]

Induction of diabetes

Streptozotocin (STZ) induced hyperglycaemia has been described as a useful experimental model to study the activity of hypoglycaemic agents. After overnight fasting (deprived of food for 16 hours, had been allowed free access to water), diabetes was induced in rats by intraperitoneal injection of STZ dissolved in 0.1 M sodium citrate buffer pH 4.5 at a dose of 50 mg/kg body weight. After the injection they have free access to food and water. The animals were allowed to drink 5 % glucose solution overnight to overcome the hypoglycaemic shock. The development of diabetes was confirmed after 48 h of the Streptozotocin injection. The animals having fasting blood glucose levels more than 200 mg/dL were considered as diabetic rats and used for experimentation.

Experimental protocol

In the diabetic rat, 5 days after the induction of diabetes, animals were divided into four groups each having 6 rats. Non diabetic animals are grouped for control. Total of 5 groups of animals of six each were used for study.

Group I: Received 2% w/v gum acacia 1ml/kg orally served as control group (non diabetic control).

Group II: Served as STZ induced diabetic control received 2% w/v gum acacia 1ml/kg orally for 15 days.

Group III: Streptozotocin induced diabetic animals received petroleum ether extract of *Bambusa vulgaris* leaf 200 mg/kg body weight once daily orally for 15 days.

Group IV: Streptozotocin induced diabetic animals received petroleum ether extract of *Bambusa vulgaris* leaf 400 mg/kg body weight once daily orally for 15 days.

Group V: Streptozotocin induced diabetic animals received the standard drug Glibenclamide 0.5 mg/kg body weight once daily orally for 15 days.

All the group of animals received the treatment for 15 days. Rats were fasted overnight and the blood was withdrawn from the retro-orbital plexus on the 5th day of induction of diabetes and 15th and 20th day post induction (that is 10 and 15 days of drug treatment) to determine the blood glucose level by glucose oxidase - peroxidase (GOD/POD) method. The change in body weight was observed throughout the treatment period in experimental animals.

Statistical analysis

All the grouped data were expressed as mean ± SD. Difference between the control and treatment groups were tested for significance using ANOVA followed by Dunnett's test. $P<0.05$ were considered significant.

RESULTS

Phytochemical screening

The preliminary phytochemical studies showed the presence of phytosterols and tannins in petroleum ether extract of *Bambusa vulgaris* leaves.

Acute toxicity studies

In acute oral toxicity study, the petroleum ether extract of *Bambusa vulgaris* leaves did not produced lethality up to the dose level of 2000 mg/kg**.**

Anti-diabetic activity

Fasting blood glucose level (FBS) was measured on 5th, 15th and 20th day of post induction. The FBS level of Group III (PEBV 200 mg/kg) and Group IV (PEBV 400 mg/kg) on 15th day of post induction (10 days of treatment) were 196.11 ± 1.3 mg/dL and 162.16 ± 0.45 mg/dL respectively. The FBS level of Glibenclamide (0.5 mg/kg) treated animal was 112.11 ± 0.92 mg/dL on 15th day of post induction. The FBS level of Group III (PEBV 200 mg/kg) and Group IV (PEBV 400 mg/kg) on 20th day of post induction (15 days of treatment) were 175.41 ± 0.94 mg/dL and 144.75 ± 0.31 mg/dL respectively. The FBS level of Glibenclamide (0.5 mg/kg) treated animal was 107.21 ± 0.75 mg/dL on 20th day of post induction.

The FBS levels in STZ treated rats were significantly high ($P < 0.001$) when compared to the non diabetic control. Oral administration of PEBV at the dose of 200 mg/kg body weight showed a significant decrease in FBS level (248.02 ± 1.9 to 175.41 ± 0.94 mg/dL, $P < 0.01$), as compared with the diabetic control group. Oral administration of PEBV at the dose of 400 mg/kg body weight showed a significant decrease in FBS level (250.14 ± 1.6 to 144.75 ± 0.31 mg/dL, $P < 0.001$), as compared with the diabetic control group. Glibenclamide treated group showed a significant decrease in the FBS levels (249.09 ± 2.1 to 107.21 ± 0.75 mg/dL, $P<0.001$), as compared to the diabetic control. The continuous oral administration of PEBV for a period of 15 days at the doses of 200 mg/kg and 400 mg/kg body weight showed a significant decrease in fasting blood sugar (FBS) level, which was dose dependant and also comparable to that of the standard drug Glibenclamide.

DISCUSSION

Any drug that is effective in diabetes will have the ability to control the rise in glucose level by different mechanisms and the ability of the extracts to prevent hyperglycaemia could be determined by hyperglycaemic animal model. In animals, diabetes can be induced by partial pancreatectomy or by the administration diabetogenic drugs such as streptozotocin, alloxan, ditizona and anti-insulin serum. Streptozotocin is a naturally occurring nitrosourea product of *Streptomyces achromogenes,* and it is widely used to induce diabetes in

experimental animals. Usually, the intraperitoneal injection of a single dose (50 mg/kg body weight) of it exerts direct toxicity on β cells resulting in necrosis within 48-72 h and causes a permanent hyperglycemia. Streptozotocin was used in the present study for the induction of diabetes in rats.

Table 1: Effect of petroleum ether extract of *Bambusa vulgaris* on fasting blood sugar (mg/dL) level in STZ induced diabetic rats.

Groups	Post Induction Days		
	5th	15th	20th
Group I Non diabetic control	67.65 ± 0.96	68.12 ± 0.94	69.82 ± 1.1
Group II Diabetic control	252.04 ± 2.1***	239.11 ± 1.6***	238.23 ± 1.2***
Group III Diabetic control + MEBV (200 mg/kg)	248.02 ± 1.9	196.11 ± 1.3**	175.41 ± 0.94**
Group IV Diabetic control + MEBV(400 mg/kg)	250.14 ± 1.6	162.16 ± 0.45***	144.75 ± 0.31***
Group V Diabetic control + Glibenclamide (0.5mg/kg)	249.09 ± 2.1	112.11 ± 0.92***	107.21 ± 0.75***

All the values are expressed as mean ± SEM, n=6 in each group. Values are significantly different from control. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$

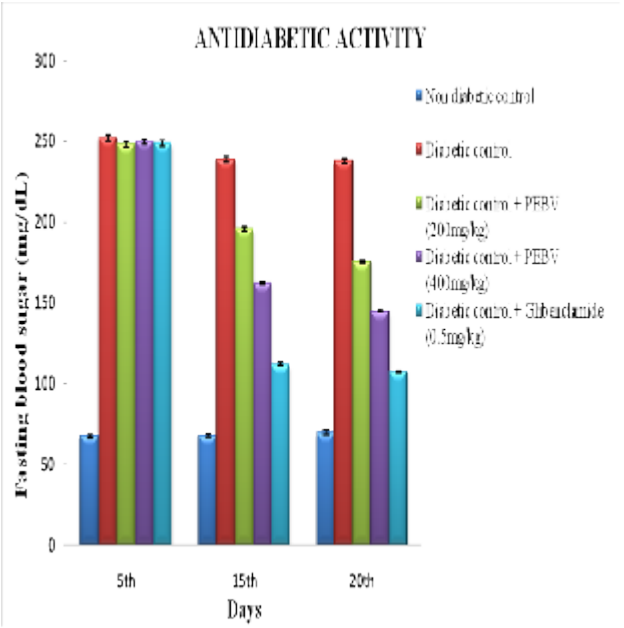

Fig. 1: Effect of petroleum ether extract of *Bambusa vulgaris* on fasting blood sugar (mg/dL) level in STZ induced diabetic rats

Our observations are in well agreement with the reports by several workers that STZ induced diabetes mellitus and insulin deficiency leads to increased blood glucose. [11] The blood glucose data obtained clearly indicate that the petroleum ether extract of *Bambusa vulgaris* produce significant anti diabetic effects in STZ induced diabetic rats.

The mechanism of anti-diabetic activity of the extracts of *Bambusa vulgaris* cannot be proposed by the present study. The possibility exists that the plant may act by either a pancreatic or extra-hepatic mechanism or both mechanisms. A pancreatic mode of action is feasible because in mild diabetes not all the beta cells of the pancreas are destroyed by STZ. Surviving beta cells retain the capacity to synthesize and secrete insulin. [12-13]

In conclusion, the petroleum ether extract of *Bambusa vulgaris* leaves showed a significant anti-diabetic activity in streptozotocin induced diabetic rats. The phytosterols present in petroleum ether extract may be possibly responsible for the anti-diabetic activity. However, we suggest that further work should be carried out at cellular and molecular levels to find out the absolute mechanism of action of the plant in experimental diabetes. The present investigation has also opened avenues for further research to the development of potent phytomedicine for diabetes mellitus from the *Bambusa vulgaris.*

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