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Evaluation of Anti-Hyperglycaemic activity of Ethanolic Extract of Stems of *Bryophyllum pinnatum* against Type 2 Diabetic rats

Research Article

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Abstract

To evaluate antidiabetic activity of stems of *Bryophyllum pinnatum* in Alloxan male Wistar rats cause diabetes. Thirty albino rats were divided into distinct five groups (n=6). By Intraperitoneal injection caused the onset of diabetes of Alloxan (120mg/Kg). vehicle control, negative control, 200, and 400mg/kg ethanolic extracts were given orally in 28 days as single administrations to the vehicle control, diabetic control, normal group, and test groups. Glucose, (fasting and post prandial for 1st and 21st day) serum cholesterol, Triglyceride, HDL (High density lipoprotein), LDL (Low density lipoprotein) Serum creatinine and urea were estimated with blood tests. The student's t test was performed to evaluate the results. In diabetic rats, a low dose of ethanol extract 200mg/kg significantly decreased all biochemical markers of diabetes. A 400mg/kg of ethanol extract was given to diabetic rats and showed substantial decreases in blood glucose and biochemical parameters. Both ethanol high and low dose extracts show antidiabetic activity in Alloxan induced diabetes.

Key Words: Antidiabetic, Wistar rats, Ethanol, Glucose, Biochemical markers.

Introduction

Diabetes is a metabolic disorder disease of sugars, fats and proteins. Typically affecting a wide demographic, it is the largest problem throughout the world. Diabetes is not a specific condition, but rather a metabolic disorder that occurs with a variety of causes, including insulin resistance. The main symptoms of diabetes mellitus are increased appetite, increased urinary production, ketonemia and ketonuria, which occur due to defects in the metabolism of carbohydrates, fats, and proteins. It is called ketoacidosis when ketones are found in the blood or urine, so medication should be begun as soon as possible, if not, it might exacerbate the condition. Diabetes mellitus has resulted in microvascular (retinopathy, neuropathy and nephropathy) and macrovascular (heart attack, stroke and peripheral artery disease) complications. (1,2) The rapid increase in unhealthy habits, urbanisation and ageing is largely due to diabetes. Reactive oxygen species (ROS) that causes lipid peroxidation and membrane damage are caused by hypoglycemia which is a consequence of diabetes mellitus. ROS plays a significant role in the development of nerve disease, kidney disease and cataracts in diabetes. Antioxidants shield β-cells from oxidation and thus play a significant role in diabetes prevention. Plants which are rich in antioxidants including tannins, flavonoids, vitamin C and E will maintain the role of β-cells and prevent the development of ROS caused by diabetes. Polyphenols, which are classified into many different groups, such as flavonoids, tannins and stilbenes, are considered to have numerous health-promoting effects, including antioxidant, anti-inflammatory, antidiabetic and antienzyme activity. As a main enzyme, aldose reductase catalyses Glucose to sorbitol is derived from glucose and the molecule is linked to diabetes complications such as peripheral neuropathy and retinopathy. Studies have demonstrated the efficacy of aldose reductase inhibitors and alpha-glucosidase inhibitors in treating diabetic complications. (3) The study aims to include accessible data on diabetes mellitus, its epidemiology, causes of diabetes, pathophysiology, available cures, diagnostic criteria, primary screening model available, herbal approach to being treated for diabetes with medicinal plants.

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Materials and Methods

Plant Details

Even though this is not the focus of this paper, the plant *Bryophyllum pinnatum* is a species of semiprostrate tropical plant that fills the rain forest of Guatemala and Mexico. The hematolly plant is a perennial plant that grows in the wild and is used as a common medicinal plant in tropical Africa, China, Australia, and Ayurvet. It has been used in Asia as a Traditional Medicine as far back as the 5,000 B.C. It is now being used in countries all over the world for cures of different illnesses. The leaf extracts of this plant have been used as a cure for illnesses including infectious,

fungal and viral diseases. The herb *Bryophyllum pinnatum*, or the tiny "love plant" plant, aids anxiety by raising high in the day, and bringing itself down low in the night. It is referred to by many names, such as Ephedra, and is known to grow in many locations in nature, including in many different types of plants. Also, not to be forgotten, like Ayurveda medicine, and other ways of medicine around the world, there has been a long history of using ephedrine in medicine. The different medicinal ingredients in this plant are used to cure multiple diseases, ranging from bacterial infections to viral infections in both humans and animals. (4-5)

Collection of plant materials

The fresh stem of the plant *Bryophyllum Pinnatum* was collected from Kachrapalayam Hills, Kallakurichi District, Tamil Nadu, India. The collected plant material was washed with tap water for 3 times and sterilized by spraying with 70% alcohol. The sterilized plant material was shade dried at room temperature to avoid chemical changes and frequently observed for any fungal contamination as the plant material rich in water content. When the plant material was completely dried, it is subjected to prepare fine powder with the help of pestle and motor. The fine powder is collected and used for extraction of crude drug in ethanol solvents by Soxhlet extraction method. (6)

Extraction of the plant material

300 g of finely powdered stems powder was defatted with 2L ether in a soxhlet apparatus for 48 h. After extraction the extracts were separately concentrated by distillation and dried at room temperature until solid mass is formed. The collected extracts were weighed and stored at room temperature for further verification. The percentage yield was calculated by using following formula

$$\text{Yield (\%)} = (W1*100)/W2$$

Where, W1= weight of the extract residue obtained after solvent removal;

W2= weight of the plant powder

Experimental animals

Male Wistar rats of 6-8 weeks of age, weighing between 150-250 g was obtained from Animal House department of pharmacy, Annamalai University. The rats are kept in normal conditions of temperature and humidity (25^{0}C ± 50^{0}C), relative humidity (55 ± 10%), and 12/12 h light/dark cycle. Animals were fed commercial pellets and water, although it was not known how much was fed. The research protocols were approved by the Institutional Animal Care Committee (IAEC), and were in compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines (Approval No: AU-IAEC/1265/11/19/M.Pharm/ 11/2019-20).

Experimental induction of diabetes

The animals were partitioned into five groups of six each. Grouping of animals was listed in Table 1. The animals will be They studied overnight fasting and confirmed the initial blood glucose of a rat tail at the end of it. Alloxan was dissolved in regular saline and then diluted with citrate buffer. Insulin-deficient diabetes was induced in overnight fasted rats with a single intraperitoneal injection dose of 120 mg/kg alloxan. It was found with blood glucose levels at 72 h. The animals were studied with blood glucose concentrations exceeding 250 mg/dl. (7)

The vehicle (saline), Metformin and extracts was administered to the appropriate animals and followed for 28 days. Metformin and the extracts were freshly distributed in standard saline and sterile water before it was administered. The fasting body weight, blood glucose level, was calculated 1st and 21st day, from the rat tail vein.

Table 1: Grouping of animals for alloxan induced diabetic model

Groups	No. of animals	Group specification	Treatment
Group I	6	Vehicle control	Administered with portable water for 28 days
Group II	6	Negative control	Receives alloxan 120 mg/kg/P.o. (*i.p*) at the day of induction
Group III	6	Test2 (Extract)	Diabetic rats to treated with *B.pinnatum* high dose 400 mg/kg, *p.o* for 28 days
Group IV	6	Test1 (Extract)	Diabetic rats to treated with *B. pinnatum* low dose 200 mg/kg, metformin low dose 150 mg, *p.o* for 28 days
Group V	6	Standard	Diabetic rats were treated with metformin for 28 days (150 mg/kg).

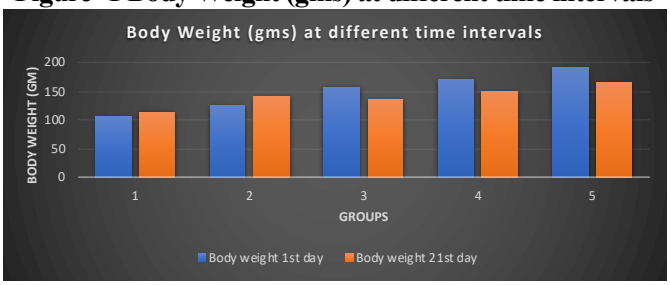
Body weight analysis

The body weights of the experimental animals are assessed on the first and 21st day of the required treatment showed in Table-2.

Table 2: Body weight (gms) at different time intervals

Groups	Treatment	Body weight	
		1st day	21st day
Group I	Vehicle control	108.1 ±3.26	115.8 ±2.04
Group II	Negative control	125.5 ±2.64	142.8 ± 3.74
Group III	High dose of ethanol extract	158.0 ± 2.08	138.0 ± 1.22
Group IV	Low dose of ethanol extract	172.5 ± 1.91	151.0 ± 3.32
Group V	Standard	189.9± 2.96	166.2 ± 1.25

Figure -1 Body Weight (gms) at different time intervals



Study protocol

All 5 groups of rats on 1st day and 21st days blood was collected. The parameter like blood glucose level for fasting and post prandial, biochemical parameters are observed and tabulated.

Statistical analysis

The findings were expressed with the mean ± SEM. Student's t-test was used to analyse the outcomes of all classes of animals.

Sample collection

In this study, all the groups were treated with respective extracts and standard drugs. After the administration of the procedure, the animals were anaesthetized by ketamine hydrochloride and blood was obtained from retro-orbital sinus. The serum was allowed to clot at room temperature and then was centrifuged at 10000 rpm for 10 minutes.

Results and Discussion

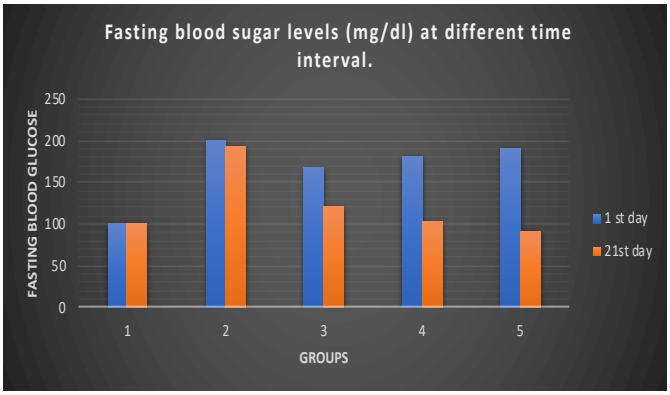
The ethanol extract of *Bryophyllum Pinnatum* stem are subjects to antidiabetic activity in albino rats. Where in alloxan was used as the diabetogenic agent. The mean ± SEM Fasting blood glucose level in normal control rats was on 1st day 77.2 ± 3.42 and 21th day was 81.34 ± 1.86. A substantial increase in blood glucose levels was reported in a diabetic control group that was 185 ± 4.34. Whereas metformin treated group showed progressive reduction in blood glucose levels at 1st day 188.3 ± 4.65 and last day was 87.5 ± 4.1. The extract plus low dose of standard drug (metformin) was reduced the fasting blood glucose level at 1st day 178.4 ± 1.95 and last day was103 ± 2.72. and the third group i.e. only high dose of extract was significantly reduced blood glucose level at 1st day 160.3 ±3.12 and the last day was 109 ±3.98.

Table: 3 Data showing fasting blood sugar levels (mg/dl) at different time intervals.

Groups	Treatment	Fasting blood sugar level (mg/dl)	
		1st day	21st day
Group I	Vehicle control	77.2±3.42	81.34± 1.86
Group II	Negative control	179.7 ± 1.95	185 ± 4.34
Group III	High dose of Ethanol extract	160.3 ± 3.12*	109 ± 3.98**
Group IV	Low dose of ethanol extract	178.4 ± 1.93*	103.4 ±2.72*
Group V	Standard	188.3 ± 4.65*	87.5 ± 4.1**

Where n=6 * P<0.05, **P<0.01 compare to negative control.

Figure 2: Fasting Blood sugar levels (mg/dl) at different time intervals



The Table 3 showed a reduction in fasting blood glucose levels in both metformin and extract treated group when comparable with diabetic control. The data were statistically analysed by Student 't' test. It indicates a significant difference among the groups at 1st and 21th days of treatment.

The ethanol extract of *Bryophyllum Pinnatum* stem is subjected to antidiabetic activity in albino rats. Where in alloxan was used as the diabetogenic agent. The mean ±SEM Post prandial blood glucose level in normal control rats was on 1st day 102 ± 2.86 and 21th day was 100 ± 2.96. A market reduce in the blood glucose level of the diabetic control group was assessed that was 194 ± 2.21. Whereas metformin treated group showed progressive reduction in blood glucose levels at 1st day 190 ± 0.91 and last day was 91 ± 2.21. The extract plus low dose of standard drug (metformin) was reduced the fasting blood glucose level at 1st day 182.6 ± 4.08 and last day was 104.4 ± 2.32 and the third group i.e. only high dose of extract was significantly reduced blood glucose level at 1st day 168.3 ± 3.34 and the last day was 120.8 ± 3.17.

The Table-4 showed a reduction in post prandial blood glucose levels in both metformin and extract treated group when comparable with diabetic control.

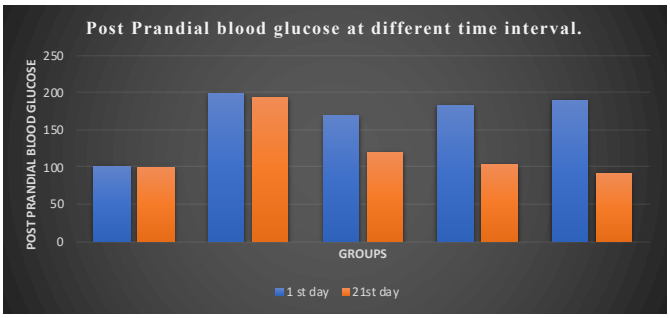
The result was analyzed by Student's' test. Student 't' test indicates a significant difference among the groups at 1st and 21st days of treatment.

Table: 4 Data showing post prandial blood glucose at different time interval.

Groups	Treatment	Post Prandial blood sugar level (mg/dl)	
		1st day	21st day
Group I	Vehicle control	102 ± 2.86	100 ± 2.96
Group II	Negative control	200 ± 2.32	194 ± 2.21
Group III	High dose of ethanol extract	168.3 ± 2.34*	120.8 ± 3.17**
Group IV	Low dose of ethanol extract	182.6 ± 4.08*	104.4 ± 2.32*
Group V	Standard	190 ± 0.91*	91 ± 2.21**

Where n=6 * P<0.05, **P<0.01 compare to negative control

Figure-3 Post Prandial blood glucose at different time interval



Biochemical parameters studies

Serum cholesterol

A marked rise in serum cholesterol levels were observed in diabetic control group that was 244.2 ± 6.54. Whereas ethanol extract group have shown a large decrease in cholesterol level at 179.8 ± 1.98. And ethanol extract plus metformin low dose group have shown a substantial lowering of blood cholesterol at 158.4 ± 2.52. Whereas the fifth group (received metformin) have shown a substantial drop in cholesterol level in the serum at 155.5 ± 3.72.

The student 't' test indicates a significant difference in serum cholesterol among the metformin and ethanol extract treated groups.

Serum triglycerides

A marked rise in serum triglycerides levels were observed in diabetic control group that was 166.8 ± 7.17. Whereas ethanol extract group have shown a drop in triglyceride levels at 129.8 ± 3.15 and ethanol extract plus metformin low dose group have shown a large decrease in triglycerides level at 106 ± 5.65. Whereas the fifth groups (received metformin) have shown a signification reduction of serum triglycerides level at 98.5 ± 1.95.

The student's' test indicates a significant difference in serum triglycerides among the metformin and ethanol extract treated groups.

Serum HDL

An improvement in HDL was seen in the diabetic control group that was 38.2 ± 0.65. Whereas ethanol extract group have shown a major decrease of HDL in the serum at 47.8 ± 1.93. And ethanol extract plus metformin low dose group have shown a drop in the amount of HDL serum at 53.8 ± 1.15. Whereas the fifth groups (received metformin) have shown a significant reduction of serum HDL level at 54.5 ± 1.95

The student 't' test indicates a significant difference in serum HDL among the metformin and ethanol treated groups.

Serum LDL

We measured a marked increase in serum LDL levels in diabetic group that was 168.8 ± 3.59. Whereas ethanol extract group have shown A substantial decrease in serum LDL level at 127.8 ± 2.90. And ethanol extract plus metformin low dose group have shown a significant reduction in serum LDL level at 101 ± 2.90. And ethanol extract plus metformin low dose group have shown a major reduction in serum LDL level at 101 ± 3.58. Whereas the fifth group (received metformin) have shown a significant reduction of serum LDL level at 98.5 ± 2.16.

The student 't' test indicates a significant difference in serum LDL among the metformin and ethanol extract treated group.

Serum Creatinine

A marked rise in serum creatinine levels were observed in diabetic control group that was 1.32 ± 0.05. Whereas ethanol extract group have shown a substantial drop in serum creatinine concentration at 0.82 ± 0.02. And ethanol extract plus metformin low dose group have shown a substantial decrease in serum creatinine level at 0.732 ± 0.028. Whereas the fifth group (received metformin) have shown a significant reduction of serum creatinine level at 0.67 ± 0.021.

The student' t' test indicates a significant different in serum creatinine among the metformin and ethanol extract treaded group.

Serum urea

A marked rise in serum urea levels were observed in diabetic control group that was 67 ± 2.43. Whereas ethanol extract group have shown a significant reduction in serum urea level at 47.2 ± 3.79. And ethanol extract plus metformin low dose group have shown a significant reduction in serum urea level at 45.8 ± 3.22. Whereas the fifth group (received metformin) have shown a significant reduction of serum urea level at 39.2 ± 1.70.

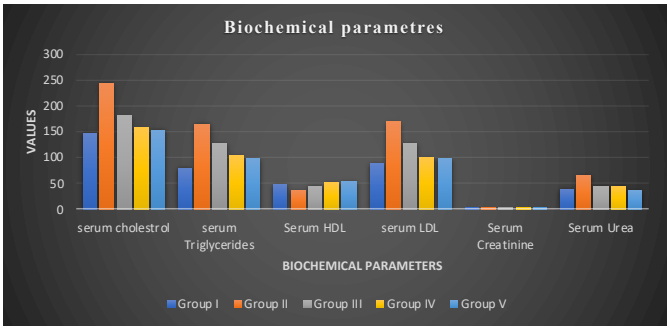
The student's' test indicates a significant different in serum urea among the metformin and ethanol extract treated group**.** The Biochemical parameters are expressed in Table-5.

Table: 5 Data showing bio-chemical parameters after 21 days treatment.

Groups	Treatment	Serum Cholesterol	Serum Triglycerides	Serum HDL	Serum LDL	Serum Creatinine	Serum Urea
Group I	Vehicle control	149 ± 3.77	82.3 ± 5.4	50.3±2.95	90.6±2.90	0.54 ±0.03	42±3.60
Group II	Negative control	244.2±6.54	166.8±7.17	38.2±0.65	168±3.59	1.32±0.05	67±2.43
Group III	High dose of ethanol extract	179.8±1.98 *	129.8±7.17 *	47.8±1.93**	127.8±2.90*	0.82±0.02 *	47.2±3.79 **
Group IV	Low dose of ethanol extract	158.4±2.52 **	106±5.65*	53.8±1.15**	101±3.58*	0.732±0.028 *	45.8±3.22 *
Group V	Standard	155±3.72*	98.5±1.95*	54.5±1.95**	98.5±2.16*	0.67±0.021*	39.2±1.70 *

Where n=6 * P<0.05, **P<0.01 compare to negative control

Figure 4 - Bio-chemical parameters after 21 days treatment



Conclusion

Ethanol stem extract of *Bryophyllum Pinnatum* has got significant anti-diabetic activity by its antihyperglycemic action and insulin sensitizing activity greater than control and lesser than reference drug metformin. In addition to regular antidiabetic drugs, *Bryophyllum Pinnatum* stems can also be considered as an adjuvant agent in the treatment of diabetes management. More studies are required to confirm the anti-diabetic activities of individual phytoconstituents of *Bryophyllum Pinnatum* in human studies were warranted. Therapeutic potentials of *Bryophyllum Pinnatum* may helpful in the management of diabetes with minimum complications. It may useful in the prevention of diabetes and other degenerative diseases and to be subjected for further research.

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