



## ANTIHYPERGLYCEMIC ACTIVITY OF YOUNG SHOOTS OF *Calamus inermis*- AN *in vivo* STUDY

### Physiology

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### ABSTRACT

Diabetes mellitus (DM) is a metabolic syndrome characterized by hyperglycemia. Medicinal plants being an important therapeutic aid, there is an exponential growth in this field of research. *Calamus inermis* belongs to the Family Arecaceae and is considered beneficial antidiabetic plant in folk medicine. Present study evaluated the antihyperglycemic activity of hydroethanolic extract of young shoots of *Calamus inermis* in streptozotocin (STZ) induced diabetic rats.

Qualitative phytochemical analysis revealed the presence of flavonoid, saponin, tannin, phenol, alkaloid, anthocyanin and terpenoid. Quantitative test for total phenol and total flavonoid was estimated. There was a significant ( $p < 0.05$ ) decrease in the blood glucose and increase in body weights in the experimental groups treated with the extract as compared to the diabetic control group. Administration of extracts at 200 mg/kg b.w. and 400 mg/kg b.w. showed dose dependent decrease in blood glucose level.

### KEYWORDS

Antihyperglycemic, *Calamus inermis*, *in vivo*

### INTRODUCTION

Diabetes mellitus is a metabolic disorder caused by impaired insulin insensitivity or insulin secretion (1). Numbers of synthetic drugs like Sulfonylureas, Thiazolidinediones,  $\alpha$ -Glucosidase inhibitors are available to treat diabetes but drug discovery is diverted towards natural sources in quest to achieve safer and cost effective agents (2, 3). Thus, several medicinal plants are being explored for possible antihyperglycemic activities (4).

*Calamus* is a genus of the palm Family Arecaceae. *Calamus inermis* also known as *Calamus latifolius* is distributed from Eastern Nepal to Indo-China and Malaysia. Stems may grow to lengths of 200 meters (5, 6, 7). Pharmacological properties like central nervous system depressant, analgesic, anti-inflammatory and immuno-suppressive activity have been reported in some of the spices of *Calamus*. (8, 9). Based on ethnobotanical survey, the degree of use of *Calamus inermis* as antidiabetic is found to be high among the population residing at remote areas of Sikkim. Its young shoot which is bitter in taste is often consumed as wild vegetable. Pharmacological properties including antihyperglycemic activity of *Calamus inermis* have not been scientifically investigated to the best of our knowledge. Hence, the present study aims to explore the antihyperglycemic activity in the animal model.

### MATERIALS AND METHODS

The plant material collected from South District of Sikkim was identified by the Taxonomist of Department of Botany, University of North Bengal, Siliguri, India and the herbarium (accession number: 05817) was deposited. All the chemicals used in the study including Streptozotocin (STZ) and glibenclamide were purchased from Sigma-Aldrich. The hydroethanolic extract was prepared by the method describe by Kokate et al., 2001(10) with slight modification. The young shoots of *Calamus inermis* were shed dried and powdered. Hydroethanol was used for extract preparation using soxhlet apparatus and was concentrated using rotary evaporator (IKA Germany, RV 3 V). Extract was stored at 4°C for further use. For the detection of different phytoconstituents of the extract, phytochemical tests were performed by standard methods (11). Total phenolic content was estimated by Folin-Ciocalteu method (12) and total flavonoid content was estimated by Aluminium chloride colorimetric method (13).

Experiment on male albino Wistar rats was conducted in accordance with the guidelines of the CPCSEA, (MC/SMIMS/IAEC/05/2016). The experimental protocol was approved by the Institutional Animal Ethics Committee of the Sikkim Manipal Institute of Medical Sciences (SMIMS), Gangtok, Sikkim, India. Rats were housed in the animal house of SMIMS and were fed a standard rat pellet diet and water. The extract was administered orally at different dose levels of 200 mg/kg,

400 mg/kg, 600 mg/kg, 1200 mg/kg, and 2000 mg/kg of body weight in order to carry out Acute oral toxicity study according to OECD guideline (14).

### Experimental design

Male albino Wistar rats weighing 150-200 g were assigned into five groups with six animals in each group. Group I (Normal control): Untreated normal rats, Group II (Diabetic control): Diabetic animals fed with normal diet, Group III (Positive control): Animals treated with Glibenclamide, Group IVA and IVB: Animals treated with the plant extract (200 mg/kg b.w. and 400 mg/kg b.w. respectively). Glibenclamide was given at a dose of 0.5 mg/kg b.w. The oral administration of hydroethanolic extracts in two different concentration was continued once daily at the same time for 28 days (15). Body weight and blood glucose levels were estimated on day 0, 7th, 14th, 21st and 28th day (16). STZ reconstituted in freshly prepared cold citrate buffer (pH 7.4) was administered at the concentration of 60 mg/kg b.w. for the induction of diabetes. Glucose levels were measured in blood samples collected from tail vein after 72 h of STZ administration. Rats with fasting glucose levels of  $\geq 200$  mg/dl were selected for experiment (17). After 72 h of STZ injection extract was orally administered, which continued once daily for the period of 28 days. In overnight fasted Wistar rats blood samples were collected from tail vein puncture and glucose level was measured using One Touch (Verio Flex) glucometer (18). The effect of the hydroethanolic extract of *Calamus inermis* on body weight was also determined using single pan balance (19). Statistical analysis was carried out by using ANOVA by means of Graph pad prism statistical software. A value of  $p < 0.05$  was considered to be significant.

### RESULTS

Preliminary phytochemical screening showed the presence of anthocyanin, saponin, alkaloid, phenol, tannin, flavonoid and terpenoid. The quantitative estimation determined the total phenolic content and total flavonoid content of  $21.83 \pm 0.02$  mg GAE/g and  $28.76 \pm 0.66$  mg RE/g of extract respectively.

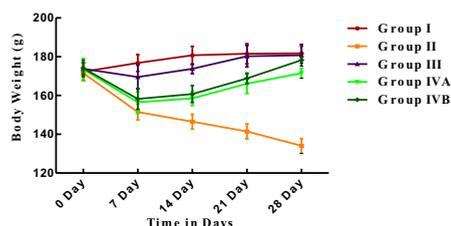
The hydroethanolic extract of *Calamus inermis* at the doses of 200 mg/kg b.w. and 400 mg/kg b.w. were evaluated for antihyperglycemic activity. The effect of the extract and the reference drug glibenclamide on blood glucose level of diabetic rats is depicted in Table 1. There was a significant ( $p < 0.05$ ) decrease in the blood glucose level after 14 days in the experimental groups treated with the extract as compared to the diabetic control group. Experimental animals treated with glibenclamide exhibited significant ( $p < 0.05$ ) decrease in the blood glucose level after 7 days of treatment as compared to diabetic control group.

**Table 1: Effect of hydroethanolic extract of *Calamus inermis* on blood glucose levels in streptozotocin induced diabetic rats**

Groups	Fasting blood glucose levels (mg/dl)				
	Day 0	Day 7	Day 14	Day 21	Day 28
Group I (Normal control)	87.5±4.93	91.75±4.71	90.75±3.5	34.25±2.87	90±1.15
Group II (Diabetic control)	92.5±7.93	305.75±8.53	354.75±5.90	362.00±6.05	375.05±5.12
Group III (Positive control)	91.5±5.74	240.5±4.65*	129.75±4.11*	101.5±6.55*	90.75±7.04*
Group IVA Extract (200 mg/kg b.w.)	97.5±8.50	298.00±7.34	243.75±6.23*	211.00±3.36*	180.5±9.91*
Group IVB Extract (400 mg/kg b.w.)	94.75±4.91	296.5±4.29	236.5±2.51*	196.5±6.45*	146.5±4.79*

Data represents mean ± SD (n=6). \*p<0.05, when groups III, IVA and IVB were compared with group II (diabetic control group).

Prior to STZ administration, there were no significant differences in the average body weights of all the 5 groups of experimental animals. By the end of the first week after DM was experimentally induced, the weights of groups II, III, IVA, and IVB were significantly reduced. Weight loss continued for four weeks in diabetic control animals. Administration of hydroethanolic extract of *Calamus inermis* extracts at 200 mg/kg b.w. and 400 mg/kg b.w. and glibenclamide (0.5 mg/kg b.w.) significantly increased the body weights in the experimental animals treated with the extract and the antidiabetic drugs as compared to diabetic control groups.



**Figure 1: Body weight of different experimental groups treated with the hydroethanolic extract of *Calamus inermis***

## DISCUSSION

Phytochemicals are a good source for finding new therapy for diabetes (20). The present study on animal models shows that *Calamus inermis* possess antihyperglycemic property. The exact mechanism is unknown; however normalization of bodyweight indicates its positive effect against hyperglycemia by restoring disturbance in lipid and protein metabolism (21). Its antihyperglycemic activity may have been achieved by decreasing carbohydrate absorption from small intestine, inhibiting gluconeogenesis in liver, enhancing glucose uptake by tissues or by protecting beta cells against degeneration (22). Phytochemicals including, phenolic compounds, anthocyanin, saponin, alkaloid, tannin, flavonoid and terpenoid were present in the extract which may be responsible for antihyperglycemic activity. These compounds are also well known for antioxidant property and can be beneficial in diabetic microvascular complications as well as cardiovascular disorders (23). To sum up, the present study proves that *Calamus inermis* is beneficial in diabetes but well-designed mechanistic approach should be performed in this area.

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