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Original Research Article

Evaluation of hypoglycemic activity of methanolic extract of *Acorus calamus* (linn). roots in alloxan induced diabetes rat model

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ABSTRACT

Background: To evaluate hypoglycemic activity of methanolic extract of roots of *Acorus calamus* (AC) in alloxan induced diabetic albino rats and to compare with standard oral hypoglycemic drug glibenclamide.
Methods: A total of 54 rats were used for this study. The study was done in two phases. In phase I, oral glucose tolerance test was done in 4 groups at 0, 30, 60 and 120 minutes after administration of AC in 3 different doses – 100, 150 and 200mg/kg to 3 different groups, with control being the fourth group. The dose of AC which caused maximal blood glucose lowering was selected for phase II. In phase II, rats were divided into 5 groups. First 2 groups were non diabetic groups which were given distilled water (DW) and AC respectively. Next 3 groups were alloxan induced diabetic groups which were given DW, AC and Glibencamide 0.5mg/kg po respectively. All drugs were given for 28 days and FBS was measured on 0, 3, 7, 14, 21, and 28th days.
Results: In phase I, both AC 150 and 200mg/kg lowered blood glucose but their effect was comparable and thus lower dose - 150mg/kg was selected for phase II. In phase II, among non-diabetic groups, AC 150 mg/kg produced significant hypoglycemia in comparison with control group. Among diabetic groups, both AC 150 mg/kg and glibenclamide 0.5 mg/kg produced significant hypoglycemia in comparison with control group on all days. On days 3 and 7, hypoglycaemic action of AC 150mg/kg was not as much as Glibenclamide ($p < 0.05$), but on days 14, 21 and 28, the hypoglycaemic action of AC 150 mg/kg was comparable to Glibenclamide 0.5mg/kg. ($P > 0.05$).
Conclusions: AC 150mg/kg causes hypoglycemia in alloxan induced diabetic rats as well as nondiabetic rats.

Keywords: Acorus calamus, Alloxan, Anti diabetic, Diabetes, Glibenclamide

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder that occurs due to deficiency of insulin action. There are various causes of diabetes mellitus - destruction of beta cells of pancreas (Type 1 DM) or resistance to insulin action (Type 2 DM), etc. Irrespective of the cause, diabetes mellitus is characterized by hyperglycemia and altered metabolisms of carbohydrates, lipids and amino acids.[1]

Diabetes mellitus has been one of the greatest public health problem over the years throughout the world. It is one of the leading contributor to death and is ranked at 3rd position. International Diabetes Federation predicts that approximately 592 million individuals will have diabetes by 2035.[2,3]

Diabetes mellitus if not controlled results in complications like obesity, renal failure, retinopathy, neuropathy, etc. Strict maintenance of euglycaemia is mandatory to prevent complications which can be attained by exogenous insulin and/or oral hypoglycaemic drugs. All the current medications for diabetes mellitus are not devoid of adverse effects, as a result patient are prone to develop a new disease at the cost of getting treated for diabetes mellitus. Resistance to these drugs over prolonged periods adds

another problem where even combination of > 4 drugs may not be sufficient to maintain euglycaemia.[2,4]

Ancient and medieval literatures have mentioned many herbal products, minerals and metals for the cure of diabetes mellitus. Many of these compounds are yet to be explored. Researchers have documented more than 150 plants which have hypoglycaemic action.[5,6]

Acorus calamus also called as Sweet Flag is an indigenous plant of south east Asia. Ethanomedicine has documented numerous medicinal properties of roots and leaves of this plant like spasmolytic, anti diarrhoeal, aphrodisiac, anti-inflammatory, anti-microbial, anti-ulcer, anti-oxidant and also anti-diabetic.[7-10]

In this study we explored the hypoglycaemic action of methanolic extract of roots of *Acorus calamus* in alloxan induced diabetic rats and have compared its action with a standard oral hypoglycaemic drug – Glibenclamide.

METHODS

This study was done at department of pharmacology, JJM medical college, Davangere.

Animals

54 Swiss albino rats of either sex weighing 170-200gm were used for this study. These animals were bred and housed in central animal house of JJM medical college, Davangere. The temperature in animal house was maintained at 24±2^{0}C with 12:12 hour light dark cycle. Utmost care was taken for proper ventilaton, and nutrition. Rats having fasting blood glucose between 80-115mg/dl were selected.[11]

Chemicals

Crude powder of roots of *Acorus calamus* was obtained from Natural Remedies, Bangalore. Soxhlet apparatus was used to prepare methanolic extract of this powder. Extract was stored in dark bottle and refrigerated. Fresh solution of *Acorus calamus* extract was used on each day of experiment. Acorus calamus was administered orally in doses of 100/150/200mg/kg body weight.[12-14]

Alloxan (Sigma-Aldrich) solution in distilled water (Cadila labs) was freshly prepared on day of administration. It was administered in dose of 150mg/kg i.p. Glibenclamide (Cadila labs) was administered 0.5mg/kg orally.[11,15,16]

Instrument

Blood glucose levels were measured using Glucometer (Abbott).[17]

The study was done in two phases.[12,13,16,18]

In phase I, Oral Glucose Tolerance Test (OGTT) was done with 3 different doses of the methanolic extract of *Acorus calamus* (100, 150 and 200mg/kg). 24 healthy euglycaemic rats were divided into 4 groups (n=6). All rats were fasted overnight for 12 hours. They had free access to water. Group I received 1ml distilled water. Groups II, III and IV received *Acorus calamus* in doses of 100, 150 and 200mg/kg b.w. respectively. After 30 minutes of administration of *Acorus calamus* / distilled water, glucose was administered orally in a dose of 2g/kg. Blood samples for measuring glucose levels were collected from tail vein before administering glucose and after 30, 60 and 120 minutes of glucose administration. The dose of *Acorus calamus* which produced maximum blood glucose reduction in euglycaemic rats was selected for next phase of study (Table 1).

Table 1: Comparison of Mean±SD values of blood glucose levels (mg/dl) in different groups (Phase I).

	Fasting	0 Min	30 Min	60 Min	120 Min
Distilled Water	84.33±2.94	83.83±3.25	97.17±2.79	137.17±5.42	108.33±5.01
ACORUS 100mg/kg	86.33±4.13	84.67±1.86	93.17±4.71	133.17±8.11	105.00±2.28
ACORUS 150mg/kg	85.17±3.19	83.33±2.66	84.67±1.97	125.33±3.14	93.33±2.66
ACORUS 200mg/kg	85.17±2.32	83±1.79	83.67±2.50	123.67±1.86	92.50±2.07

Phase II was done to compare the effect of *Acorus calamus* with Glibenclamide in Alloxan induced diabetic rats. Dose of *Acorus calamus* for this phase was selected from phase I which produced maximal hypoglycaemia.

30 rats were grouped into 5 groups having 6 rats in each group. Only those rats having Fasting Blood Sugar in range of 80-115mg/dl were selected.[10,12] Groups I and II served as non-diabetic groups to whom Alloxan was not given. Alloxan 150mg/kg i.p. was administered to groups III, IV and V to induce diabetes. Only those rats which developed FBS >250mg/dl after 7 days of alloxan administration were taken for study.[6,11,15,17,19] Rats were grouped as follows (Table 5).

- Non-diabetic groups:
  - *Group-1 (Control):* Distilled water 1ml p.o.

- *Group-2 (Test):* Methanolic extract of *Acorus calamus* p.o. (dose with maximum reduction in blood glucose in phase 1).

- Alloxan induced diabetic groups:

  - *Group-3 (Control):* Distilled water 1 ml p.o.
  - *Group-4 (Standard):* Glibenclamide 0.5mg/kg body wt p.o.in distilled water.
  - *Group-5 (Test):* Methanolic extract of Acorus calamus p.o. (dose with maximum reduction in blood glucose in phase 1).

All drugs viz. - distilled water, glibenclamide and *Acorus calamus* were given for 28 days.[6,11,12,15,19]

Fasting blood glucose (FBS) was measured on 0, 3, 7, 14, 21, and 28th days.[6,11,12,15,17,18]

Statistical analysis

Blood sugar levels were taken as Mean±SD for each group. Comparison of means across groups at every occasion was done using one way ANOVA with LSD post hoc test. P value <0.05 was considered as statistically significant. SPSS v20 (IBM) was used for statistical analysis.

RESULTS

In phase I of this study, we observed that *Acorus calamus* 100 mg/kg lowered blood glucose levels only at 120th minute but its effect was comparable to that of distilled water and the difference was not statistically significant (p = 0.09) (Table 2). Hence this dose was excluded for further study.

Table 2: Comparison of blood sugar lowering action of Acorus calamus 100mg/kg with distilled water (Phase I).

	Distilled water	Acorus 100mg/kg	p value
Fasting	84.33±2.94	86.33±4.13	0.29
0 MIN	83.83±3.25	84.67±1.86	0.56
30 MIN	97.17±2.79	93.17±4.71	0.04
60 MIN	137.17±5.42	133.17±8.11	0.19
120 MIN	108.33±5.01	105.00±2.28	0.09

Table 3: Comparison of blood sugar lowering action of Acorus calamus 150mg/kg with distilled water (Phase I).

	Distilled water	Acorus 150mg/kg	Acorus 200mg/kg	p value of DW vs Acorus150	p value of DW vs Acorus 200
Fasting	84.33±2.94	85.17±3.19	85.17±2.32	0.66	0.658
0 Min	83.83±3.25	83.33±2.66	83±1.79	0.73	0.565
30 Min	97.17±2.79	84.67±1.97	83.67±2.50	<0.05	<0.05
60 Min	137.17±5.42	125.33±3.14	123.67±1.86	<0.05	<0.05
120 Min	108.33±5.01	93.33±2.66	92.50±2.07	<0.05	<0.05

Acorus calamus 150mg/kg and 200mg/kg produced remarkable blood sugar reduction at 30, 60 and 120 minutes (Table 1).

Blood sugar lowering action of *Acorus calamus* 150 and 200mg/kg at 30, 60 and 120 minutes was statistically significant when compared to control group (Table 3).

Meanwhile, Blood glucose lowering action of *Acorus calamus* 150mg/kg and 200mg/kg were almost similar and there was no statistically significant difference between these two groups (Table 4).

Hence, we decided to take the lower dose (150mg/kg) for phase II.

In phase II, among the non-diabetic groups, it was observed that *Acorus calamus* 150mg/kg produced hypoglycemia on days 3, 7 and 14. Later blood glucose level gradually raised to normal levels from day 21 to day 28. This hypoglycaemic action of *Acorus calamus* 150mg/kg on days 3, 7, 14 and 21 was statistically significant when compared to distilled water (p <0.05) (Table 6).

Table 4: Comparison of blood sugar lowering action of Acorus calamus 150mg/kg with 200mg/kg (Phase I).

	Acorus 150mg/kg	Acorus 200mg/kg	p value
Fasting	85.17±3.19	85.17±2.32	1.00
0 Min	83.33±2.66	83±1.79	0.81
30 Min	84.67±1.97	83.67±2.50	0.59
60 Min	125.33±3.14	123.67±1.86	0.59
120 Min	93.33±2.66	92.50±2.07	0.66

Table 5: Comparison of Mean±SD values of blood glucose levels (mg/dl) in different groups (Phase II).

	Non diabetic groups		Diabetic groups		
	Distilled water	**Acorus 150mg/kg**	**Distilled water**	**Acorus 150mg/kg**	**Glibenclamide 0.5mg/kg**
Day 0	295.17±5.85	84.83±3.06	295.17±5.85	295.83±4.62	297.50±3.45
Day 3	304.67±4.59	53.17±4.02	304.67±4.59	262.33±4.13	243.83±5.31
Day 7	314.67±6.19	56.83±2.31	314.67±6.19	241.83±5.95	225.67±5.99
Day 14	305.33±5.96	64.67±3.08	305.33±5.96	213.17±7.34	206.50±5.86
Day 21	295.67±5.12	73.83±3.19	295.67±5.12	192.17±5.60	186.83±5.12
Day 28	285.83±5.60	82.33±3.20	285.83±5.60	176.50±5.68	169.83±8.33

Table 6: Comparison of hypoglycaemic action of Acorus calamus 150mg/kg with distilled water in non diabetic rats (Phase II).

	Distilled Water	Acorus 150mg/kg	p value
Day 0	83.17±1.72	84.83±3.06	0.27
Day 3	84.33±2.59	53.17±4.02	<0.05
Day 7	83.33±1.21	56.83±2.31	<0.05
Day 14	83.00±1.55	64.67±3.08	<0.05
Day 21	84.83±1.72	73.83±3.19	<0.05
Day 28	85.17±2.86	82.33±3.20	0.13

Among the alloxan induced diabetic groups, it was observed that *Acorus calamus* 150mg/kg and Glibenclamide 0.5mg/kg reduced blood sugar levels from day 3 onwards and this trend continued till day 28. The hyoglycaemic action of *Acorus calamus* 150mg/kg and Glibenclamide was statistically significant compared to distilled water group on all days ($p < 0.05$) (Table 7). On days 3 and 7, hypoglycaemic action of *Acorus calamus* 150mg/kg was not as much as Glibenclamide and the difference between these two groups was statistically significant ($p < 0.05$). But on days 14, 21 and 28, the hypoglycaemic action of *Acorus calamus* 150mg/kg was comparable to Glibenclamide 0.5mg/kg. The difference of means between these two drugs on days 14, 21 and 28 was not statistically significant ($p = 0.066$, 0.093 and 0.103 respectively) (Table 8). Hypoglycaemic actions of distilled water, *Acorus calamus* 150mg/kg and Glibenclamide 0.5mg/kg can be compared in Table 8.

Table 7: Comparison of hypoglycemic action of Acorus calamus 150mg/kg and Glibenclamide 0.5mg/kg with distilled water in diabetic rats (Phase II).

	Distilled water	Glibenclamide 0.5mg/kg	Acorus 150mg/kg	p value of DW VS Glibenclamide	p value of DW VS Acorus 150
Day 0	295.17±5.85	297.50±3.45	295.83±4.62	0.407	0.811
Day 3	304.67±4.59	243.83±5.31	262.33±4.13	<0.05	<0.05
Day 7	314.67±6.19	225.67±5.99	241.83±5.95	<0.05	<0.05
Day 14	305.33±5.96	206.50±5.86	213.17±7.34	<0.05	<0.05
Day 21	295.67±5.12	186.83±5.12	192.17±5.60	<0.05	<0.05
Day 28	285.83±5.60	169.83±8.33	176.50±5.68	<0.05	<0.05

Table 8: Comparison of hypoglycemic action of Acorus calamus 150mg/kg with Glibenclamide 0.5mg/kg in diabetic rats (Phase II).

	Glibenclamide 0.5mg/kg	Acorus 150mg/kg	p value of glibenclamide VS acorus 150
Day 0	297.50±3.45	295.83±4.62	0.552
Day 3	243.83±5.31	262.33±4.13	<0.001
Day 7	225.67±5.99	241.83±5.95	<0.001
Day 14	206.50±5.86	213.17±7.34	0.066
Day 21	186.83±5.12	192.17±5.60	0.093
Day 28	169.83±8.33	176.50±5.68	0.103

DISCUSSION

Diabetes mellitus is a rapidly growing problem worldwide. This rapid growth has led to attraction of researchers to develop different modalities for its treatment. Currently various classes of anti-diabetic drugs are available in market along with insulin and insulin analogues. Unfortunately, none of the available drugs are free from adverse effects. There is need for a medication which can control diabetes without causing any adverse effect. Herbal medicines are gaining attention for the previous reason.[20] *Acorus calamus* is a plant which has been used in herbal medicine for treatment of diabetes mellitus.[10]

In phase I of our study, we compared blood sugar lowering action of 3 doses of *Acorus calamus* - 100, 150 and 200mg/kg. It was found that 150 and 200mg/kg reduced blood glucose levels at all recorded time intervals. We selected the lower dose 150mg/kg as its blood glucose lowering action was similar to 200mg/kg. This will also minimize the risk of adverse effects if any. 100mg/kg dose was excluded as it failed to produce significant blood glucose lowering action in comparison with distilled water.

In phase II, hypoglycemic action of *Acorus calamus* 150mg/kg was tested in non-diabetic rats against control. It was found that *Acorus calamus* 150mg/kg produced hypoglycemia over days 3 - 28 which was not seen in control group ($p < 0.05$). Because of this observation *Acorus calamus* can be considered as hypoglycemic drug rather than euglycaemic drug.

In this phase, we also compared hypoglycemic effect of *Acorus calamus* 150mg/kg against control and a standard oral hypoglycemic drug - Glibenclamide (0.5mg/kg) in alloxan induced diabetes rats. It was observed that *Acorus calamus* 150mg/kg produced hypoglycemia from day 3 - day 28. Such hypoglycemic action was also seen by glibenclamide but the magnitude of hypoglycemia on days 3 and 7 was quite less by *Acorus calamus* 150mg/kg compared to Glibenclamide ($p < 0.05$). However the magnitude of hypoglycemia on days 14, 21 and 28 was similar to Glibenclamide ($p = 0.066$, 0.093 and 0.103 respectively).

Our study results are similar to other studies on *Acorus calamus* done before. Prisilla et al, demonstrated that methanolic extract of *Acorus calamus* 200mg/kg has got significant anti hyperglycemic activity compared to 50mg/kg and 100mg/kg in streptozotocin induced diabetic rats. Action of *Acorus calamus* was also compared to Glibenclamide 0.5mg/kg and it was found to be similar. They proposed increased insulin secretion from beta cells to be the mechanism of hypoglycemia of *Acorus calamus.*[12]

Si et al, demonstrated anti hyperglycemic action of *Acorus calamus* in glucose/amylum challenged normal mice. Ethyl acetate fraction of *Acorus calamus* in doses of 400 and 800mg/kg significantly decreased fasting blood glucose, and suppressed hyperglycemia after oral glucose (2g/kg) administration in normal mice.[14]

Hartati et al, extracted 26 fractions from ethyl acetate extract of *Acorus calamus.* All fractions were subjected to hypoglycemic testing by in vitro α glucosidase inhibition test method and they reported that 7 of 26 fractions have hypoglycemic action.[13]

Our study results also correspond with in vitro studies. Si et al, have also demonstrated hypoglycemic action of *Acorus calamus* in vitro. They proposed insulin releasing and alpha-glucosidase inhibitory activity as mechanisms of causing hypoglycemia. This was demonstrated in vitro using HIT-T15 cell line and alpha-glucosidase enzyme. *Acorus calamus* increased insulin secretion in HIT-T15 cells. Similar action was seen with gliclazide too.[14]

Hence from our study results, we are in conclusion that methanolic extract of *Acorus calamus* in dose of 150mg/kg possesses significant hypoglycemic action. Further studies need to be done on different diabetic models in rats and also in different species to further establish the hypoglycemic action of *Acorus calamus.* We recommend that phytochemicals extracted from *Acorus calamus* should be tested in further studies instead of the entire extract to identify the active pharmaceutical ingredient. Potential adverse effects and toxicities also need to be evaluated. In future, *Acorus calamus* may find its place as an established anti diabetic or add on drug over the existing oral anti diabetic drugs.

CONCLUSION

Our study shows that *Acorus calamus* has hypoglycemic action and it is comparable with standard drug glibenclamide. Further studies are required to establish hypoglycemic action of *Acorus calamus* for its use as oral hypoglycemic agent in diabetes mellitus.

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