ANTI-HYPERGLYCAEMIC ACTIVITY OF CASSIA KLEINI LEAF EXTRACT IN GLUCOSE FED NORMAL RATS AND ALLOXAN-INDUCED DIABETIC RATS

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ABSTRACT

Objective: To study the effect of Cassia kleinii on serum glucose levels in both normal and diabetic rats.

Methods: The extracts of dried root (suspension in water) and leaf (water, alcohol and n-hexane) were screened for their effects on serum glucose levels in glucose overloaded rats. The most active extract (alcohol extract of leaf) was tested for antidiabetes activity in alloxan-induced diabetic rats and for hypoglycaemic activity in normal fasted rats.

Results: The plant leaf as well as its alcohol extract (but not the other extracts) exhibited concentration dependent antihyperglycaemic effect in glucose loaded rats. However, the extract did not show hypoglycaemic effect in fasted normal rats. In alloxan-induced diabetic rats the extract (200 mg/kg) showed remarkable efficacy.

Conclusion: The study reveals for the first time the antihyperglycaemic activity of Cassia kleinii leaf (alcohol extract) in both glucose fed hyperglycaemic and alloxan-induced diabetic rats. The extract seems promising for the development of a phytomedicine for diabetes mellitus.

KEYWORDS Diabetes mellitus antidiabetic glucose overload herbal extracts hypoglycaemia

INTRODUCTION

In folk/tribal medical practice many plants are used to treat diabetes mellitus in South India. Most of these medicinal plants are not scientifically validated for their therapeutic efficacy and safety. Scientific studies on these plants are likely to provide invaluable antidiabetes drugs.

Although, there are numerous traditional medicinal plants reported to have hypoglycaemic properties1-10 many of them proved to be not very effective in lowering glucose levels in severe diabetes11. Further, most of the hypoglycaemic agents used in allopathic medicine are reported to have side effects in the long run12. Therefore, there is a need to search for effective and safe drugs for diabetes.

Earlier studies from this laboratory have shown that the aerial parts of Artemisia pallens wall. (endemic to South India) have antidiabetes properties in alloxan-induced diabetic rats13. The search for effective herbal drugs for the treatment of diabetes, based on ethnomedical clues continues in this laboratory. An ethnomedical search conducted by the authors revealed that a herb, Cassia kleinii W.&A. (Caesalpiniaceae) is used as a remedy for diabetes in folk medical practice, in certain remote villages of Kanyakumari Dist., Tamil Nadu, India. This plant is not in use in traditional systems of medicine such as Ayurveda and Siddha. Further, there is no scientific study on the medicinal property of this plant. The present study was carried out to find the presence of any antidiabetes activity of this wild herb in rats.

MATERIALS AND METHODS

Plant material and preparation of extracts: Cassia kleinii was collected from Kanyakumari district of Tamil Nadu, India, in summer. A voucher specimen of the herb was identified by the taxonomists at...
Tropical Botanic Garden and Research Institute (TBGRI), Palode, Thiruvananthapuram Dist., Kerala, India and deposited in the herbarium of TBGRI, No. 47600.

The leaves and roots were dried in the laboratory at room temperature and powdered separately. The water suspensions of the plant parts were prepared by grinding the powder in 2% gum acacia (w/v). A 10% suspension of root or leaf was used for oral administration.

To prepare the water extract, 10 gm of the leaf powder in 100 ml distilled water was stirred magnetically for 4 h at room temperature. The residue was removed by filtration and the filtrate was freeze dried in a lyophiliser (the yield of the water extract was about 1.2 gm from 10 gm dried plant leaf powder).

The alcohol and n-hexane extracts of the plant leaf powder were prepared similarly using ethyl alcohol and n-hexane respectively instead of distilled water. However, in these cases the filtrates were evaporated to dryness at 40°C under reduced pressure in a rotary evaporator. Approximately 1.0 gm of dried alcohol extract or 0.5 gm of n-hexane extract was obtained from 10 gm plant leaf powder.

The extracts were suspended in 5% Tween 80 and used for oral administration. The extracts were suspended in 5% Tween 80 and used for oral administration (Tween 80 was added in water extract also to facilitate comparison with alcohol and n-hexane extracts).

In case of alcohol extract, the effect of heat on the extract was studied by keeping the extract in a boiling water bath for 15 min. Then it was brought to room temperature and used.

Animals: Male Wistar albino rats (150-200 gm) were used. They were fed a standard rat pellet (Amruth India Ltd.) and water ad libitum and maintained under standard laboratory conditions (temperature 24-28°C, relative humidity 60-70%). Animals described as fasted were deprived of food for 16 h but had free access to water.

Glucose tolerance tests: Rats were divided into several groups. One group was kept as vehicle control which received 1 ml of 2% gum acacia or 5% Tween 80, p.o. The experimental groups received water suspensions of the plant powder (leaf or root) (500 mg/kg) in 2% gum acacia or different doses of water, alcohol and n-hexane extracts (50, 100, 200 or 400 mg/kg) (in 5% Tween 80) in an identical manner. (In the screening study, a high dose of 500 mg [dry weight]/kg of the plant parts was taken to detect activity, if any).

The rats of all the groups were loaded with 60% glucose (3 gm/kg, p.o.) 30 min after drug (water suspension or extract) administration. Blood samples were collected from the tail just prior to drug administration and at 30, 90 and 150 min after glucose loading. Serum glucose levels were measured immediately. Six fasted animals were used in each group.

Hypoglycaemic study in normal fasted rats: To investigate the hypoglycaemic effect, if any, of the alcohol extract (active extract), the fasted rats were divided into 3 groups of 6 each. One group received 1 ml of 5% Tween 80 and served as control. The other groups received 2 different concentrations of alcohol extract (100 or 200 mg/kg) in an identical manner. Blood samples were collected at 60 and 120 min after drug administration and glucose levels were measured as described above.

Alloxan-induced diabetic rats: Rats were injected with alloxan (60 mg/kg) through the tail vein. 5 days later, blood samples were drawn and glucose levels determined to confirm the development of diabetes. The diabetic rats exhibiting blood glucose levels in the range of 400-450 mg/100 ml were selected to determine the efficacy of alcohol extract of the plant.

Determination of the efficacy of the plant drug (alcohol extract) in diabetic rats: The alloxan induced diabetic rats were divided into 4 groups of 6 each. Group I was given 1 ml of 5% Tween 80, p.o. daily and served as control. Group II was given alcohol extract daily (200 mg/kg, p.o.) and group III received the same dose of the extract twice a day (morning and evening). Group IV received insulin (5 U/kg, i.p., Knoll Pharmaceuticals Ltd., India) daily. The treatment was continued for 15 days. Blood samples were collected in morning 1 h after drug administration on day 1, 4, 7, 10 and 15. On day 15, animals were killed after blood collection and liver samples were removed for glycogen estimation.

Estimation of blood glucose: Blood glucose was estimated spectrophotometrically using a commercial assay kit (Monozyme, India, Ltd). In each case
blood sample was collected and serum was separated. Ten µl of serum was used for each assay.

**Determination of liver glycogen:** Liver glycogen was estimated by the method of Carroll *et al* 14.

**Statistical treatment:** Statistical evaluation was done using one-way analysis of variance (ANOVA). Post-hoc comparisons were done using Dunnett's test. P values <0.05 were considered significant.

**RESULTS**

The effect of the water suspension of root or leaf of *Cassia kleinii* on glucose tolerance in fasted rats is shown in Figure 1. Both root and leaf at a dose of 500 mg/kg significantly increased the tolerance for glucose.

When different extracts of the leaf were tested for their effect on glucose tolerance, the alcohol extract was found to be effective at a dose of 200 mg/kg. The water as well as n-hexane extracts were found to be inactive at the above dose level. (Figure 2). The antihyperglycaemic property of the alcohol extract was found to be resistant to heat. Keeping the extract in a boiling water bath for 15 min, did not result in any significant change in its activity (Figure 2).

The results presented in Figure 3 demonstrate that antihyperglycaemic activity of the drug (alcohol extract) was concentration dependent. Optimum activity was observed at 200 mg/kg level. An increase in the dose of the drug from 200 mg to 400 mg/kg did not result in any significant increase in the antihyperglycaemic activity of the drug. The drug was effective at a lower dose (100 mg/kg) in depressing the peak value of blood sugar at 30 or 90 min after glucose loading. At a dose of 200 mg/kg the efficacy of the drug was found to be more than that at 100 mg/kg. The drug was not very effective at 50 mg/kg level.

Interestingly, the alcohol extract did not exhibit hypoglycaemic effect in the fasted normal rats even at 200 mg/kg level. At this dose, the alcohol extract did not influence serum glucose levels both at 60 min (control 64.7±5.7; treated 68.8±5.1; mean±SD) and 120 min (control 65.4±5.8; treated 72.3±3.5) after drug administration.

In alloxan diabetic rats, the blood glucose levels were in the range of 400 to 500 mg/100 ml, which can be considered as severe diabetes. In the untreated control group, out of the 6 animals 2 died of diabetes on 6th day. In the survived animals blood glucose level was found to be more than 400 mg/100 ml (Figure 4). In the drug treated (200 mg/kg) groups the blood glucose level steadily decreased and it was below 200 mg/100 ml on the 7th day and thereafter. The drug treatment once a day was found to be marginally better than that twice a day. The plant drug was found to be almost equal to insulin (5 U/kg) in its efficacy in this alloxan-induced diabetic model in lowering blood glucose levels. (The dose [5 U/kg] was selected, based on a published report16).

The effect of the plant drug on body weight and liver glycogen content in the diabetic rats is given in Table 1. The body weight was slightly increased in the normal control rats whereas in the diabetic rats there was a significant decrease in body weight. Insulin as well as the herbal drug treatment significantly decreased this reduction in body weight. There was a significant reduction in liver glycogen levels (in 15 days) in alloxan-diabetic rats. Plant drug treatment remarkably attenuated this reduction in glycogen content. The drug treatment twice a day did not show significant differences in liver glycogen levels compared to that in once a day treated rats.

**DISCUSSION**

The study reports for the first time the antihyperglycaemic effects of *Cassia kleinii* in rats. The alcohol extract of this plant is an attractive material for the development of a potent phytomedicine for diabetes. Alternatively, identification of the active principle(s) can possibly lead to the development of allopathic type of drug.

It is interesting to note that the drug exhibited only antihyperglycaemic effects and not hypoglycaemic effect in fasted rats. Further, the drug showed optimum activity at 200 mg/kg and a further increase did not result in a significant decrease in glucose levels. Thus, it appears that unlike insulin and other common hypoglycaemic agents overdose of the drug may not result in hypoglycaemia. Interestingly, *Cassia kleinii* extract showed marked antihyperglycaemic effect in the alloxan-induced
Table 1. Effect of *Cassia kleinii* leaf alcohol extract on body weight and liver glycogen in diabetic rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial (0 day)</th>
<th>Final (15th day)</th>
<th>Liver glycogen (mg/gm wet tissue)</th>
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<tr>
<td>Normal control</td>
<td>184.3 ± 11.1</td>
<td>196.8 ± 10.1</td>
<td>47.8 ± 2.0</td>
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<td>Diabetic control (5% Tween 80, p.o.)</td>
<td>183.2 ± 10.3</td>
<td>138.7 ± 9.1</td>
<td>6.7 ± 0.7&lt;sup&gt;a&lt;/sup&gt;</td>
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<tr>
<td><em>Cassia kleinii</em> (200 mg/kg, p.o.) Once a day</td>
<td>185.3 ± 12.1</td>
<td>175.5 ± 10.1*</td>
<td>34.5 ± 1.7&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>(-5.2)</td>
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<tr>
<td><em>Cassia kleinii</em> (200 mg/kg, p.o.) Twice a day</td>
<td>188.0 ± 10.2</td>
<td>177.3 ± 9.7*</td>
<td>33.4 ± 2.4&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>(-5.6)</td>
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<tr>
<td>Insulin (5 U/kg, i.p.)</td>
<td>181.0 ± 8.5</td>
<td>171.1 ± 7.9*</td>
<td>40.3 ± 1.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
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<td>&lt;0.01</td>
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Values are mean±SD, n=6 in each group except diabetic control group where n=4. <sup>a</sup>P<0.001 (compared to normal control or drug treated rats); <sup>b</sup>P<0.001 (compared to diabetic control); *P<0.001 (compared to diabetic control). Values in the parentheses represent % change (loss or gain) in body weight.

Figure 1. Effect of leaf or root (water suspension) of *Cassia kleinii* on glucose tolerance in fasted rats.

Values are mean±SD, n=6 in each group; *P<0.05 (compared to control).
Figure 2. Effect of different extracts of *Cassia kleinii* leaf on glucose tolerance in fasted rats.

Values are mean±SD, n=6 in each group; *P<0.05 (compared to control).

Figure 3. Effect of different doses of *Cassia kleinii* leaf (alcohol extract) on glucose tolerance in fasted rats.

Values are mean±SD, n=6 in each group; *P<0.05; **P<0.001 (compared to control).
Figure 4. Effect of *Cassia kleinii* leaf (alcohol extract) on serum glucose in alloxan-induced diabetic rats.

Values are mean±SD; n=6 in each group except control (diabetic) group where n=4 on 7th, 10th and 15 day; two rats died on the sixth day. *Daily drug administration was started from day 1; blood was collected 1 h after drug administration. *P<0.05; **P<0.01 (compared to control).

Severe diabetic rats. This effect was almost comparable to that of insulin (5 U/kg). Alloxan induces diabetes by destroying β-cells and the destruction is almost complete. Therefore, unlike the clinically used oral sulphonylurea drugs, this herbal drug does not seem to work by stimulating β-cells to release insulin and/or potentiating the sensitivity of peripheral tissues to insulin. This suggests that its main mechanism of action may not be potentiation of insulin release from pancreatic β-cells and thus the drug could be effective in insulin independent diabetes also. However, this remains to be confirmed by measuring the levels of insulin in the control and drug treated rats. The methanol extract of *Artemisia pallens* also showed somewhat similar effect in alloxan diabetic rats. However, in this case drug was found to be unstable to heat (100°C) and the active n-hexane extract of the plant lost its biological activity on storage in refrigerator or at room temperature within a week. However, in the present study the extract was found to be heat stable. Thus it is an attractive extract for the development of an invaluable oral antihyperglycaemic drug.
The mechanism of action of the drug is not clear. It may be mimicking some or all of the actions of insulin on the metabolism of glucose.

Studies are in progress in this laboratory to elucidate in detail the mechanism of action of this drug at the cellular and molecular level. The compounds responsible for the antidiabetic property are being purified. Further, detailed toxicity studies are being carried out in rodents.

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