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Alleviation of alloxan-induced diabetes and its complications in rats by *Actinodaphne hookeri* leaf extract

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Abstract

Leaves of *Actinodaphne hookeri* Meissn (Family Lauraceae; local name: Pisa) has been in use traditionally for the treatment of diabetes and disorders of the urinary tract which are more common in Chattisgarh and eastern part of India. In the present study, leaves of *A. hookeri* were subjected to phytochemical investigation and evaluated for anti-diabetic activity. The ethanol and the chloroform extract were found to have significant ($p<0.01$) blood glucose lowering effect. The extracts also significantly ($p<0.01$) lowered the increased serum cholesterol and low density lipoprotein levels. Preliminary phytochemical investigation revealed the presence of alkaloids, flavonoids, triterpenoids and glycosides as the major constituents in the ethanol extract. The chloroform extract also showed significant ($p<0.01$) antihyperglycemic activity and contained alkaloids and triterpenes. It is concluded that the anti-diabetic activity of *A. hookeri* may be due to the presence of alkaloids and triterpenes, and might be promising for the development of phytomedicine for diabetes mellitus along with its associated complications.

Introduction

Diabetes mellitus leads to metabolic disorder and is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both (Atkinson and Maclaren, 1994). It is also estimated that there are 30 to 33 million diabetic patients in India now and every fourth diabetes patient in the world today is an Indian. Indians are genetically more susceptible to diabetes and the World Health Organization predicts the number of diabetic persons in India would go up to 40 million by 2010 and to 74 million by 2025 (Pillai, 2006). However, insulin administration is effective to some extent in alleviating diabetes mellitus and increasing the life expectancy of diabetic patients, but there is limitations as well as draw backs for this therapy. Some oral hypoglycemic agents are also employed in this regard, but they are also not without adverse effects.

Actinodaphne hookeri Meissn. (Family Lauraceae; local name: Pisa) is a moderate evergreen tree found in many parts of India, more common in

the Chhattisgarh state and in the eastern part of India. Traditionally the leaves are used in the treatment of diabetes mellitus and in urinary disorders (Anonymous, 2003, Kirtikar and Basu, 1995). The phytoconstituents reported are quercetin-3-rhamnoside, amorphous alkaloid, hentriacontanone and hentriacontanol (Chopra et al., 1956; Yoganarasimhan, 2000). The present study aimed at investigating into the effect of ethanol, petroleum ether, chloroform and methanol extracts of *A. hookeri* leaves on blood glucose levels in normoglycemic and alloxan-induced diabetic rats.

Materials and Methods

Collection of plant material

A. hookeri leaves were collected in the month of June 2006, from Jog falls near a town called Sirsi of Karnataka State, India. The plant was authenticated by Dr. B.D. Huddar, Department of Botany, H.S. Kotambri Science Institute, Hubli, Karnataka State, India. The voucher specimen (No: 05 PG 358) was deposited in herbarium of Department of Pharmacognosy and Phytochemistry, KLES' College of Pharmacy, Hubli, India.

Preparation of extracts

Shade-dried and powdered leaves of *A. hookeri* was soxhlet extracted with ethanol (95%). Another batch was also soxhlet extracted successively with petroleum ether (40-60^{o}C), chloroform, and with methanol in increasing order of polarity. The extracts were concentrated under reduced pressure using rotary evaporator and the residue was dried in desiccators over anhydrous calcium chloride.

Qualitative chemical tests

All the extracts were tested to know the different constituents present in them by the standard procedures. The extracts were tested for sterols (Finar, 1975), alkaloids, triterpenes, saponins (Cromwell et al., 1955; Kokate et al., 1996), flavonoids (Geinssman et al., 1955), tannins (Trease and Evans, 1989), and carbohydrates (Hawks, 1971).

Chemicals

Tween 80 of analytical grade was purchased from Hi-Media Labs, India, Glucometer (Accuchek-Sensor) was purchased from Roche Diagnostics, Mumbai, India. Alloxan monohydrate was purchased from Spectrchem Pvt. Ltd Company, Mumbai. Glibenclamide from Aventis Pharma Ltd., India. All the solvents used for extraction were purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India.

Animals

All the experiments on animal were conducted according to protocols that were approved by the Institutional Animal Ethics Committee/ IAEC.Clear/2005-06/I). Anti-diabetic activity was carried out using 3 months old healthy albino Wister rats of either sex weighing between 150-200 g. Animals were allowed free access to tap water and pellet diet throughout the study. Acute toxicity studies were carried out using albino mice.

Acute toxicity study

Albino mice of either sex weighing between 20-30 g were used. The animals were fasted over night. Acute toxicity study was performed according to OECD guidelines; method followed is according to number 420. It was found that tolerated dose was higher than 5,000 mg/kg body weight (OECD, 2001).

Glucose tolerance test

Glucose tolerance test was carried out using the method described elsewhere (Babu et al, 2002). Four groups of 6 rats each were used for the study. Group I served as normal control (Vehicle: 1% Tween 80 emulsion), Group II animals were administered with alcohol extract, Group III animals were administered with petroleum ether extract, Group IV animals were administered with chloroform extract, Group V animals were administered with methanol extract. All the extracts were administered through oral route at a dose of 500 mg/kg. The rats of all the groups were administered with 60% glucose (3 g/kg orally) 30 min after the administration of extract. Blood samples were collected from the tail prior to drug administration and at 30, 90 and 150 min after glucose administration. Blood glucose levels were measured using Glucometer (Accuchek-Sensor).

Alloxan-induced diabetes

The albino rats weighing 150-200 g of either sex

were allowed to fast for 24 hours prior to experimentation and rendered diabetic by a single dose of intraperitoneal injection of alloxan 150 mg/kg body weight (Vogel and Gang, 2002) After 18 hours of injection of alloxan, diabetes was confirmed by testing urine and blood sugar level more than 200 mg/dL were selected for the further study. Animals were maintained for four days in diabetic condition for well establishment of diabetes. They were divided into seven groups. Group I: Healthy normal animals received only the vehicle (1% Tween 80); Group II: Untreated but diabetes-induced animals served as a negative control; Group III: Diabetes-induced animals and treated with standard drug glibenclamide 10 mg/kg body wt./day orally; Group IV: Diabetic animals and treated with ethanol extract; Group V: Diabetic animals and treated with petroleum ether extract; Group VI: Diabetic animals and treated with chloroform extract; Group VII: Diabetic animals and treated with methanol extract. All the extracts were given orally at the dose of 500 mg/kg body weight. After 15 days animals were sacrificed under ether anesthesia and blood was collected from the carotid artery for the determination of blood glucose level, serum total cholesterol, total triglyceride, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels (Kannur et al., 2006).

Statistical analysis

Comparison between control and drug treated groups were analyzed by software Prism 4.03 with one-way ANOVA. $p<0.05$ and $p<0.01$ were considered to be significant.

Results and Discussion

As shown in Table I, all the groups showed significantly ($p<0.05$) lowered the blood glucose level compared to those of Group I (treated with glucose only) at 150 min interval. But as the difference between the blood glucose levels in Group I from 30 min interval to 150 min interval was not significant. The values at 150 min were not of much importance. The reduction in blood glucose level which was due to normal metabolism of glucose and feed back mechanism (Rang et al., 2001). So, the increase in blood glucose had automatically reduced at 150 min time interval in case of all the extracts. But the Group II (treated with ethanol extract) maintained the blood glucose level significantly ($p<0.01$) lower than that of normal control (Group I), i.e. from 30 to 150 min whereas the other groups did not. Group III (petroleum ether) lowered the blood glucose level significantly ($p<0.05$) at 30 min but not at 90 min. Group IV (chloroform) lowered the blood glucose level significantly ($p<0.05$) at both 30 and 90 min. Group V (methanol) lowered the blood glucose level significantly ($p<0.01$) at 30 min and ($p<0.05$) 90 min. These results showed that all the extracts have hypoglycemic effect at specific time interval. Among the four extracts hypoglycemic effect was observed more consistently in case of the alcohol extract. This would suggests the presence of hypoglycemic components in ethanol extract. Chloroform extract contains triterpenes and alkaloids, methanol extract contains mainly phenolic (flavonoids and tannins) and carbohydrates (data not shown). Petroleum ether contains triterpenes

Table I

Effect of various extracts of *A. hookeri* on blood glucose tolerance in normal rats

Groups	Blood glucose level (mg/dL)			
	0 min	30 min	90 min	150 min
Glucose only	87.3 ± 3.7	120.0 ± 10.1	105.8 ± 5.2	99.6 ± 4.3
Glucose + Ethanol extract	78.7 ± 2.4	83.5 ± 0.3	70.8 ± 5.3[a]	66.0 ± 8.0
Glucose + Petroleum ether extract	85.7 ± 7.4	111.3 ± 10.4	95.3 ± 7.3[a]	75.8 ± 3.6
Glucose + Chloroform extract	87.2 ± 4.3	116.5 ± 16.4	85.0 ± 6.3[a]	70.0 ± 5.1
Glucose + Methanol extract	80.3 ± 7.1	90.3 ± 5.8	75.2 ± 4.3[b]	70.2 ± 8.7

The values are expressed as mean ± SEM; [a]$p<0.01$ when compared to control; [b]$p>0.05$ when compared to control

Table II

Effect of various extracts of *Actinodaphne hookeri* on blood glucose level in rats

Group	Blood glucose level (mg/dL)
I	72.3 ± 2.4
II	487.3 ± 6.9
III	175.3 ± 5.9[a]
IV	208.7 ± 6.9[a]
V	374.5 ± 16.5[a]
VI	222.7 ± 13.7[a]
VII	466 ± 7.4[b]

The values are expressed as mean ± SEM; [a]p<0.01 when compared to diabetic control; [b]p>0.05 when compared to diabetic control

present within it was identified by qualitative chemical test and thin layer chromatography.

As shown in Table II, all the treated groups except Group VII significantly (p<0.01) lowered the blood glucose level, compared to Group II diabetic control. Group VII failed to lower the increased blood glucose level significantly. Methanol extract contained only carbohydrates and phenolics where as petroleum extract contained triterpenes and chloroform extract contained both alkaloids and triterpenes. The ethanol extract contained all the constituents. This indicates that the lowering of the blood glucose levels were due to the presence of triterpenes and alkaloids.

As shown in the Table III, in the Group II (untreated but diabetic-induced) total cholesterol, triglyceride, LDL and VLDL level were increased significantly (p<0.01) when compared to Group I. But HDL level was decreased significantly (p<0.01) when compared to Group I. Group III and IV significantly (p<0.01) reduced the total cholesterol, triglyceride, LDL and VLDL, and significantly (p<0.01) increased the HDL level when compared to diabetic control (Group II). But these values (total cholesterol, triglyceride, LDL, VLDL and HDL) were not significant (p>0.05) when compared to Group I. Group V decreased the VLDL (p<0.01) and LDL (p<0.05) level significantly, Group V also decreased the total cholesterol and triglyceride not significantly, increased the HDL significantly. Group VI significantly reduced the total cholesterol, triglyceride and VLDL but not LDL. But the HDL level was increased close to the normal control. Group VII reduced the total cholesterol and VLDL significantly when compared to Group II. But not reduced the triglyceride and LDL value and also failed to increase the HDL value close to normal.

The effect of ethanol extract of A. *hookeri* (containing triterpenes, alkaloids, flavonoids, tannins and carbohydrates) on lipid parameters appeared better than the other extracts followed by chloroform (containing triterpenes and alkaloids) and petroleum ether (containing triterpenes). The alleviating effect upon lipid parameters appeared minimum in case of methanol extract (containing carbohydrates and tannins). This appeared similar to those as observed in case of blood glucose level. So, it indicates that the anti-diabetic effect of *A. hookeri* was perhaps due to triterpenes and alkaloids.

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Table III

Effect of *Actinodaphne hookeri* leaves extracts on lipid profile in diabetic rats

Parameters	Blood glucose level (mg/dL)						
	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
Total cholesterol	47.3 ± 3.1	76.3 ± 2.9	51.8 ± 2.5[a]	53.2 ± 4.1[a]	71.3 ± 3.9[b]	52.3 ± 2.9[a]	56.7 ± 4.7[a]
Triglyceride	81.3 ± 2.9	137.3 ± 4.9	93.5 ± 6.3[a]	108.8 ± 4.8[a]	123.2 ± 7.0[b]	106.5 ± 5.1[a]	120.3 ± 5.1[b]
HDL	46.0 ± 2.0	29.7 ± 2.2	47.7 ± 2.5[a]	46.7 ± 2.3[a]	39.2 ± 1.9[a]	46.5 ± 2.7[a]	31 ± 2.3[b]
LDL	8.5 ± 0.4	18.7 ± 1.3	10.0 ± 1.3[a]	10.0 ± 1.5[a]	11.8 ± 1.2[c]	20.0 ± 2.8[b]	23.0 ± 2.1[b]
VLDL	8.8 ± 0.8	22.3 ± 1.5	13.3 ± 1.5[a]	10.5 ± 0.9[a]	9.7 ± 1.2[a]	8.4 ± 0.8[a]	10.3 ± 1.3[a]

The values are expressed as mean ± SEM; [a]p<0.01 when compared to diabetic control; [b]p>0.05 when compared to diabetic control; [c]p<0.05 when compared to diabetic control

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