

EVALUATION OF ANTI-DIABETIC ACTIVITY OF *HYDNOCARPUS LAURIFOLIA* IN STREPTOZOTOCIN INDUCED DIABETIC RATSSAILAJA RAO P^{1*}, KRISHNA MOHAN G², PRATHIMA SRINIVAS³

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ABSTRACT

Objective: The objective of this study was to evaluate the anti-hyperglycemic activity of different extracts of *Hydnocarpus laurifolia* seeds in both normal and diabetic rats.

Methods: Male Wistar rats weighing about 180-250 g were taken and divided into eleven groups, with six rats in each group. Diabetes was induced by giving streptozotocin (30-50 mg/kg) intraperitoneally. Rats that showed blood glucose levels >250 mg/dl were selected for the study. Metformin (50 mg/kg) was given as a standard oral hypoglycemic agent. Oral glucose tolerance test was performed in all groups of rats.

Results: The petroleum ether and ethylacetate extracts of *H. laurifolia* seeds at different doses was prepared and administered orally. The blood glucose levels were estimated by glucose-oxidase method. Anti-hyperglycemic activity of the test drugs in diabetic rats showed a significant reduction in blood glucose levels ($p < 0.0001$) at 1 hr, 2 hr and 4 hrs respectively when compared to diabetic group.

Conclusion: The results suggested that *H. laurifolia* seed extract may have potent anti-diabetic activity, justifying the use of the drug for the treatment of diabetes mellitus.

Keywords: *Hydnocarpus laurifolia*, Achariaceae, Oral glucose tolerance test, Anti-hyperglycemic activity.

INTRODUCTION

Diabetes mellitus is a chronic disorder characterized by elevated blood sugar levels either due to defective insulin secretion or action, or both. It is associated with altered metabolism of carbohydrates, fats, and proteins [1].

Diabetes is a serious metabolic disorder with micro and macrovascular complications that results in significant morbidity and mortality. Elevated blood sugars cause changes in the blood vessels thus affecting the eyes, kidneys, nerves, heart, brain, and the feet [2]. The increasing number of ageing population, consumption of calorie-rich diet, sedentary lifestyle, obesity, stress have led to a tremendous increase in the number of diabetic patients worldwide. Although the current treatment provides good glycemic control, it presents various side-effects. Thus, it is necessary that we look for a new and more efficacious drug in the herbal world. Despite considerable progress in the management of diabetes mellitus, with insulin therapy, oral hypoglycemic agents, restricted diet, exercise either singly or in combination, the search for indigenous antidiabetic agent still continues, as there are remarkably good results have been reported with traditional medicine [2]. In the recent years, herbs are being effectively tried in a variety of pathophysiological states.

India is a country with rich natural resources and variety of medicinal plants. In contrast to synthetic drugs, herbal drugs enjoy the advantages of comparatively less toxic than synthetic drugs, more harmony with the biological system and affordable to all classes of people.

The plants that show significant pharmacological activity and low toxicity need extensive screening. *H. laurifolia* belongs to the family; Achariaceae (flacourtiaceae) is one of the ancient plants in the world, which is used in the traditional system for diabetes. It is a tree found

in tropical forests and Western Ghats of south India. It is useful in the treatment of intestinal worms, helminthiasis, infected wounds, skin diseases, fever, bloating, piles and wounds with inflammation [3,4]. The chemical constituents contain hydnocarpic acid and its homologues. It also contains oleic acid and palmitic acid. Chalmogra oil obtained from this plant is used in the treatment of skin diseases including leprosy [5]. The preliminary phytochemical screening of *H. laurifolia* was done and revealed the presence of alkaloids, phenolics, flavonoids, carbohydrates, saponins and glycosides.

METHODS**Collection of plant material**

H. laurifolia seeds (5 kg) were collected from Tirumala Hills, Tirupathi, Andhra Pradesh during July-August 2011. The Authentication was done by S. V. University, Botany Department, Tirupathi.

Preparation of extract

The seeds obtained were powdered in the electric grinder, placed in closed vessels. To the powder, required quantities of petroleum ether and ethylacetate were added and allowed to stand for 7 days occasionally shaking. The liquid was strained off; solid residue was pressed, clarified by filtration and then subjected to evaporation.

Animals

Animal protocol was approved by Institutional Animal Ethical Committee (IAEC) of Committee for Purpose of Control and Supervision of Experimentation on Animals through its reference no: IAEC/SVCP/2011/006, dated: 26/7/11.

Male Wistar rats, weighing (180-250 g) were obtained from National Institute of Nutrition, Hyderabad. The animals were housed with free access to food and water for at least 1 week in an air-conditioned room (25°C) under a 12 hrs light: Dark cycle prior to the experiment. They

were fed with standard diet (Hindustan Lever Pvt., Ltd.) and water ad libitum.

Antidiabetic activity [6]

Induction of experimental diabetes: Diabetes was induced by a single intraperitoneal injection of freshly prepared streptozotocin (STZ) solution (Sisco Research laboratories Pvt., Ltd. Mumbai-93, India. Batch No: T-835796) at a dose of 30-60 mg/kg in acetate buffer 0.1 M, pH 4.5 to overnight fasted rats. Control rats received only buffer solution. Diabetes was identified by polydipsia, polyuria and by measuring non-fasting blood glucose levels 48 hrs after injection of STZ. Animals with blood glucose <250 mg/dl were not considered for the study [6].

Experimental groups

The animals were divided into eleven groups of 6 animals each.

Group I: Normal untreated rats (control).

Group II: Normal rats administered with petroleum ether seed extract (PESE) (100 mg/kg) (po).

Group III: Normal rats administered with PESE (300 mg/kg) (po).

Group IV: Normal rats administered with ethylacetate seed extract (EASE) (100 mg/kg) (po).

Group V: Normal rats administered with EASE (300 mg/kg) (po).

Group VI: Diabetic rats.

Group VII: Diabetic rats administered with metformin 50 mg/kg (po).

Group VIII: Diabetic rats administered with PESE (100mg/kg) (po).

Group IX: Diabetic rats administered with PESE (300 mg/kg) (po).

Group X: Diabetic rats administered with EASE (100 mg/kg) (po).

Group XI: Diabetic rats administered with EASE (300 mg/kg) (po).

Animals of Group I were given with 0.9% saline and served as control and Groups II, III, IV and V were given with petroleum ether and ethyl acetate extract of doses of 100 and 300 mg/kg orally. Group VI were diabetic control rats. Metformin was given as standard oral hypoglycemic agent at 50 mg/kg. Group VII was given with standard oral hypoglycemic agent. Diabetes-induced Groups VIII, IX, X and XI were given with the petroleum ether and ethylacetate extracts at dose of 100 and 300 mg/kg orally. The rats were treated for about 10 days, and blood was withdrawn from orbital sinus puncture. Blood glucose levels were estimated by glucose-oxidase method [7].

Oral glucose tolerance test (OGTT) [8]

Diabetes was induced by an intra-peritoneal injection of freshly prepared STZ (30-50 mg/kg) in rats. 11 groups of six animals in each group were used. The OGTT was performed in overnight fasted (18 hrs) animals. After overnight fasting a 0 minutes blood sample (0.2 ml) was taken from each rat in the different groups viz., normal, normal + PESE (100 mg/kg), normal + PESE (300 mg/kg), normal + EASE (100 mg/kg), normal + EASE (300 mg/kg), diabetic, diabetic + metformin (50 mg/kg), diabetic + PESE (100 mg/kg), diabetic + PESE (300 mg/kg), diabetic + WASE (100 mg/kg), diabetic + EASE (300 mg/kg). Test drugs were administered orally in 0.25% carboxymethylcellulose, and standard drug metformin was also administered orally to diabetic rats. Glucose solution (2 g/kg) was administered orally 30 minutes after the administration of extracts. Blood samples were taken at 0 minutes, 30 minutes, 60 minutes, 90 minutes and 120 minutes after glucose administration. All the blood samples were collected with potassium and sodium fluoride solution for the estimation of blood glucose.

Effect of OGTT in rats

All the rats from different groups were subjected to OGTT. Student's t-test was applied when two groups among were compared, and the results were evaluated. The values were discussed in Table 1.

Statistical analysis

The results of the estimations were reported as mean \pm SEM. Student's t-test was applied when two groups among were compared. The values were considered as significant when $p < 0.05$, $p < 0.001$, and $p < 0.0001$. Statistical calculations were performed using GraphPad Prism (Table 2).

RESULTS AND DISCUSSION

Historical literatures reveal that knowledge regarding diabetes existed since brahmic period as this was mentioned in ayurvedic text books-Sushruta Samhita written in 4th and 5th centuries B.C. [9]. Phytochemical screening is an essential and very important part of medicinal plants research [10]. After the completion of the extraction process, the obtained petroleum ether extract and ethylacetate extract were identified for the presence of phytochemicals. Phytochemical screening of the extract revealed the presence of alkaloids, phenolics, flavonoids, carbohydrates, saponins and glycosides.

Several animal experimental models have been in use to evaluate hypoglycemic activity such as alloxan monohydrate, STZ, etc. STZ is a nitrosurea compound produced by *Streptomyces achromogens*, which specially induces DNA strand breakage in β -cells causing diabetes mellitus. As there is no incidence of the spontaneous revision with STZ and it is also observed that more than 90% of rats is becoming diabetic [11]. Therefore, the STZ induced diabetic model has been widely employed to induce diabetes in experimental animals. The searching for new anti-diabetics drugs from natural plants is still attractive because they contain substances that take alternative and safe effect on diabetes [12]. When there was a comparison of diabetic rats with the normal rats, there was an increase in blood glucose levels significantly. It showed that STZ produced the diabetogenic response in Wistar strain rats [13].

The results obtained were evaluated. In rats, diabetes was induced by using STZ at a dose of 30-60 mg/kg, where blood glucose levels were >250 mg/dl which indicated the induction of diabetes. OGTT was performed in all the rats from Group I to group XI (n=6), and the results were evaluated. All the drug-treated groups in diabetic rats showed a significant reduction in blood glucose values at 60, 90 and 120 minutes ($p < 0.0001$) respectively when compared to the diabetic control group.

At the same time, the hypoglycemic activity of the PESE and EASE extract of *H. laurifolia* were also studied in normal rats. A continuous treatment was given to the specified groups for a period of 10 days and the blood glucose concentrations were observed on the 11th day. Evaluation of hypoglycemic activity of different extracts of *H. laurifolia* seeds was done and a Group from II to V were compared with normal rats and was observed that there was no significant decrease in blood glucose levels and was also observed that there was no difference in glucose levels in all groups from Group II to V when compared with Group I, i.e., the values were almost to be more (or) less same.

Anti-hyperglycemic activity of different extracts of *H. laurifolia* seeds was evaluated, the extract treated diabetic groups were compared to diabetic control group. When PESE (100 mg/kg) extract treated group was compared to diabetic group, there was a significant reduction ($p < 0.0001$) in glucose levels at 1st, 2nd and 4th hrs. Similarly, PESE (300 mg/kg) extract treated group was compared with diabetic control; there was a reduction in blood glucose levels significantly ($p < 0.0001$). Then, EASE (100 mg/kg) extract treated group and EASE (300 mg/kg) were also compared with diabetic group and was found that there was a decrease in blood glucose levels significantly ($p < 0.0001$). It was observed that there was a significant decrease in blood glucose levels in EASE (100) and EASE (300mg/kg) extract treated groups at 1st, 2nd and 4th hrs, but PESE (100 mg/kg) and PESE (300 mg/kg) extract treated groups showed a significant reduction in glucose levels at 2nd and 4th hrs

Table 1: Effect of *Hydnocarpus laurifolia* seed extract on OGTT in normal and diabetic rats

Groups	Treatment	Blood glucose levels (mg/100 ml)				
		0 minutes	30 minutes	60 minutes	90 minutes	120 minutes
I	Normal (control)	80.17±1.48	114.17±5.23	101.67±4.59	92.5±3.59	85.83±1.54
II	Normal+PESE (100)	94.4±3.80	90.17±2.33	86.83±2.17	86.63±1.72	86.33±2.37
III	Normal+PESE (300)	86.58±3.21	84.83±2.48	83.17±2.75	83.67±1.69	83.83±2.06
IV	Normal+EASE (100)	82.5±2.60	88.17±1.58	88.17±1.58	84.5±1.59	84.33±1.43
V	Normal+EASE (300)	87.5±2.07	84.17±2.0	85±4.29	84.17±2.39	81.33±1.85
VI	Diabetic control	301.67±32.50***	279.17±7.79***	301.67±10.14***	302.5±10.31***	294.17±14.49***
VII	Diabetic+Metformin	291.67±15.37 NS	273.33±12.30 NS	240±13.41**	177.5±7.27****	135.83±6.63***
VIII	Diabetic+PESE (100)	280.83±5.54 NS	260.83±3.51 NS	190.08±1.29****	176.75±1.11*** NS	157.93±2.08***
IX	Diabetic+PESE (300)	270.83±4.36 NS	262.5±4.42 NS	240.83±8.89 ^a NS	185.83±5.68*** NS	149.32±1.52****
X	Diabetic+EASE (100)	276.67±2.48 NS	212.5±4.03***	198.33±2.11****	180.83±0.83*** NS	165±1.82****
XI	Diabetic+EASE (300)	275.83±3.51 NS	258.33±4.94 NS	200±4.77****	191.67±3.33*** NS	167.5±2.81****

Values were reported as mean±standard error of the mean (SEM), diabetic control compared with normal, ***p<0.0001; diabetic group compared with test drug treated groups, **p<0.001, NS: Non-significant; diabetic group compared to standard drug treated group, **p<0.01; diabetic standard drug treated group compared to diabetic test drug treated group, ***p<0.0001, **p<0.001. ***p<0.0001 extremely statistically significant; **p<0.001 highly statistically significant (values in parenthesis indicate the rate of significance). PESE: Petroleum ether seed extract, EASE: Ethylacetate seed extract, OGTT: Oral glucose tolerance test, SEM: Standard error of the mean

Table 2: Blood glucose levels in normal and diabetic rats treated with *Hydnocarpus laurifolia* seed extract

Groups	Treatment	Blood glucose levels (mg/100 ml)			
		0 hrs	1 hrs	2 hrs	4 hrs
I	Normal (control)	94.4±3.80	86.59±3.21	91.38±2.03	82.97±0.85
II	Normal+PESE (100)	87.5±3.095	92.167±3.13 NS	94±2.75 NS	102.33±4.45 NS
III	Normal+PESE (300)	91±2.22	93.67±4.36 NS	94.17±3.51 NS	98.33±4.60 NS
IV	Normal+EASE (100)	94±2.26	94.83±1.85 NS	94±1.91 NS	99.16±3.51 NS
V	Normal+EASE (300)	94.67±3.74	100.17±2.52 NS	99.83±2.24 NS	98.17±1.56 NS
VI	Diabetic control	268.75±5.5***	272.22±3.95***	280.95±3.26***	281±4.78***
VII	Diabetic+metformin	280.07±15.36	262.5±8.54	153.34±2.48***	138.33±1.82***
VIII	Diabetic+PESE (100)	280.83±13.57 NS	192.06±8.57***	152.75±2.16***	151±2.19****
IX	Diabetic+PESE (300)	270.83±4.36 NS	210.57±3.69***	148.95±1.38***	148.31±1.49***
X	Diabetic+EASE (100)	276.67±2.47 NS	221.28±10.01***	185.1±4.42***	157.5±6.29***
XI	Diabetic+EASE (300)	275.83±3.51 NS	228.6±5.04****	197.5±7.27****	155.83±6.54****

Values were reported as mean±SEM, diabetic control compared with normal, ***p<0.0001; diabetic control compared with diabetic test drugs treated groups, ***p<0.0001, NS: Non-significant; diabetic control compared to standard drug treated group, ***p<0.0001; diabetic standard drug treated group compared to diabetic test drugs treated group, ***p<0.0001, **p<0.001, *p<0.05. ***p<0.0001 extremely statistically significant; **p<0.001 highly statistically significant; *p<0.05 statistically significant (values in parenthesis indicate the rate of significance***). PESE: Petroleum ether seed extract, EASE: Ethylacetate seed extract, SEM: Standard error of the mean

only. The test drug treated groups in diabetic rats were also compared to standard drug treated group.

A significant reduction in the blood glucose level in diabetic rats treated with both petroleum ether and ethyl acetate extracts indicated that the seeds may be useful in the management of diabetes.

CONCLUSION

Our study had shown that different doses of *H. laurifolia* exhibited a significant anti-hyperglycemic activity in diabetic animals. Thus, consumption of this drug could be helpful in improving the hyperglycemia and preventing diabetic complications. To understand the mechanism of anti-diabetic activity of *H. laurifolia*, further studies like anti-hyperlipidemic and anti-oxidant activity and essential and being carried out.

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