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THE EFFECT OF *ARECA CATECHU. L* EXTRACT ON STREPTOZOTOCIN INDUCED HYPERGLYCAEMIA IN WISTAR RATS

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ABSTRACT

To evaluate the antidiabetic property of aqueous *Areca catechu* extract, experimentation was studied in an animal model. The levels of blood glucose, plasma insulin and serum lipid profile were estimated and studied in streptozotocin diabetic rats. The findings were compared between normal, diabetic and *Areca catechu* extract treated diabetic rats. The findings indicated that the administration of the aqueous *Areca catechu* extract tended to bring the parameters significantly towards the normal. The effect of the aqueous *Areca catechu* extract at a dose of 500 mg/kg body weight yielded a higher level of significance than the doses of 250 mg/kg body weight. Experiment suggested that *Areca catechu* extract had excellent effects on controlling the hyperglycemia and hyperlipidemia in diabetic rats.
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KEY WORDS: **Areca nut, Streptozotocin, Plasma insulin, Fasting glucose level**

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INTRODUCTION

Diabetes mellitus is a serious complex chronic condition that is a major source of ill health worldwide. This metabolic disorder is characterized by hyperglycemia and disturbances of carbohydrate, protein, and fat metabolisms, secondary to an absolute or relative lack of the hormone insulin. Besides hyperglycemia, several other factors including dislipidemia or hyperlipidemia are involved in the development of micro and macrovascular complications of diabetes that are the major causes of morbidity and death (Kameswararao, 2003). According to WHO projections, the prevalence of diabetes is likely to increase by 35%. Currently, there are over 150 million diabetic patients worldwide and this is likely to increase to 300 million or more by the year 2025. Statistical projection about India suggests that the number of diabetics will rise from 15 million in 1995 to 57 million in the year 2025, the highest number of diabetics in the world (Satyanarayana, 2006). Reasons for this rise include an increase in sedentary lifestyle, consumption of energy-rich diet, obesity, higher life span, etc. Evaluation of plant products to treat diabetes mellitus is of growing interest as they contain many bioactive substances with therapeutic potential. In recent years, several authors evaluated and identified the antidiabetic potential of traditionally used Indian medicinal plants using experimental animals. Previous studies have confirmed the efficacy of several medicinal plants in diabetes mellitus. Many indigenous Indian medicinal plants have been found to be useful to successfully manage diabetes(Hundekari et al., 2013). These effects remain to be investigated in several other Indian medicinal plants (Sharma, 1994)..

Areca catechu L.

(Palmaceae), commonly known as areca nut or betel nut in English, Supari in Bengali is a tall perennial palm occurring in sandy-clay land throughout the Indian subcontinent and other South East Asian countries. It is commercially cultivated across different parts of India for its seeds which are consumed as a masticatory. Its seeds have sialogogue properties and used as anthelmintic in veterinary practice. (Kokate et al., 2006). Previous researchers have reported some pharmacological properties of its seed. (Pithayanukul et al., 2009; Shrestha et al., 2010; Khan et al., 2011). However, there are no reports of the antidiabetic activity on *Areca catechu* nut. The present study was therefore aimed to investigate the possible antidiabetic effects of *A. catechu* nut aqueous extracts (AAE) against streptozotocin (STZ)-induced diabetic Wister albino rats.

METERIALS AND METHODS

Plant materials

Preparation of extracts

Tender *A. catechu seeds* were powdered and about100g was extracted with 100mL of boiled distilled water. The filtrate was concentrated under reduced pressure at 40°C and the extract was stored in refrigerator at 4°C for use in the subsequent experiments. *Animals,* healthy adult Wistar male albino rats having body weight around 170 ±5g at 60- 70 days from birth were procured from Entomology Research Institute Loyola College, Chennai, Tamilnadu. Housed individually at poly propylene cages, Maintained under standard conditions (12 h light and 12 h dark cycle, 25±3. °C); the rats were fed with standard rat pellet diet (pranav agro industry Ltd, Maharastra) and water ad libitum.The study was approved by the Animal Ethics Committee of the Institute (IAEC-ERI-LC-10).

Oral glucose tolerance test (OGTT)

The oral glucose tolerance test (Bonner-Weir, 1988) was performed in overnight fasted (18 h) rats. Rats were divided into 4 groups each group consists of six animals (n = 6). Group 1 served as normal control; group 2 was treated with AAE 250mg /kg b.wt; group 3 was treated with AAE 500mg /kg b.wt of; group 4 was treated with glibenclamide 600µg/kg bwt (Boukhris, et al., 2012). Glucose (2 g/kg) was fed 30 min after the administration of extracts. Blood was withdrawn from the retro orbital sinus under ether inhalation at 30, 60 and 120 min of glucose administration and glucose levels

were estimated using a GOD- POD method (Trinder, 1969). *Induction of Non – Insulin dependent diabetes mellitus (NIDDM)* NIDDM was induced in overnight fasted adult wistar strain albino rats weighing 170 ± 5g by single intraperitoneal injection of freshly prepared streptozotocin(STZ), (Sigma – Aldrich, Bangalore) (40 mg/ Kg bwt) in 0.1 M citrate buffer (pH = 4.5). After seven days of STZ administration, blood glucose level of each rat was determined. Rats with a blood glucose level above 200mg/dl were considered diabetic and included in the study.

Experimental design

In the experiment totally 30 rats (6 normal; 24 STZ diabetic surviving rats) were used. The rats were divided into 5 groups of six rats each. The extract was dissolved in 2% tween 80 solutions and administered orally for 21 days. Group I: Normal control rats, Group II: Diabetic control rats, Group III: Diabetic rats treated with AAE 250mg/kg bw, Group IV: Diabetic rats treated with AAE 500mg/kg bw, Group VI: Diabetic rats treated with Glibenclamide (600 µg/Kgbw).At end of the 21 day study, the animals were euthanized between 0900 - 1100 h to minimize diurnal variation. Fasting glucose level by glucose oxidase - peroxidase method (Triender, 1969).,Plasma insulin was estimated by ELISA method and Lipid profile (total cholesterol, high-density lipoprotein (HDL) cholesterol, and triglyceride) levels in serum were determined according to the instructions of the manufacturer (Merck, Mumbai, India).

Statistical Analysis

One- way ANOVA and Student's t-test (SPSS program; version 11.5) were carried out to compare the data with the level of significance set at $p \leq 0.05$. *A.catechu* aqueous extract (AAE), result when administered prior to glucose loading produced a significant reduction ($P < 0.05$) in the rise in blood glucose levels at 60 min after glucose administration. AAE at doses of 250 and 500 mg/kg produced 13.00%, and 20.02% reduction in blood glucose respectively when compared to vehicle treated group at 60 min (Table 1).Table 2 shows the effect of long term study of AAE on STZ induced diabetic rats. STZ-treated diabetic rats showed a significant increase in the levels of blood glucose when compared to normal rats. After treatment with AAE at 250 and 500mg/kg the blood glucose was significantly ($P<0.05$) reduced compared to the diabetic rats. STZ caused a significant decrease in plasma insulin. Administration of AAE (250 and 500 mg/kg) caused significant ($P < 0.05$) increase in insulin levels when compared with diabetic control (Table 3).Table 4 represents the serum lipid profile of normal and diabetic control and diabetic treated rats. Over fivefold increases were observed in total cholesterol, triglycerides and two fold decreases in HDL cholesterol level in diabetic control compared to normal control. AAE 250 and 500mg/kgbw treated diabetic rats significantly reduced total cholesterol, triglycerides and increased HDL cholesterol compared to diabetic control.

Table 1

Glucose tolerance test of AAE in normal rats –induced diabetic rats

Treatment	Serum glucose level			
	0 Hr	30 min	60 min	120 min
Normal control	68.93±2.21	136.36±1.24	133.02±1.44	123.00±1.39
AAE 250 mg/kgbw	66.98±1.02	128.71±1.35**	108.19±1.65**	97.12±0.83**
AAE 500mg/kgbw	63.92±1.63	122.57±2.29**	98.90±.872**	75.13±2.01**
Glibenclamide 600µg/kgbw	64.55±1.30	124.35±0.98**	113.43±1.02**	78.80±1.01**

All the values are (g) mean ± SEM for six rats ** values deviate very significantly from diabetic control group (p≤ 0.05).

Table 2
Effect of long term study of AAE on fasting serum glucose level in STZ-induced diabetic rats.

Treatment	Fasting serum glucose level			
	0 day	7th Day	14th Day	21st Day
Normal control	72.46±29.28	98.13±11.41	84.42±10.00	87.86±11.06
Diabetic Control	346.62±13.2	643.62±11.60	419.42±14.95	418.59+24.59
Diabetic+ AAE 250 mg/kgbw	451.69±17.85	428.68±18.74	402.10±11.55	569.06±62.53
Diabetic + AAE 500mg/kgbw	494.4±21.37	595.25±23.35	391.5±19.40	328.48+12.96
Diabetic +glibenclamide 600µg/kgb.wt	354.21±10.54	312.45±14.25	269.15±14.21	213.21±5.24

All values are in mg/dl; Mean ± SEM; n=6

Table 3
Effect of oral administration AAE on plasma insulin levels in STZ induced diabetic rats.

Treatment	Plasma Insulin (µU/ml)
Normal control	126.97±1.59
Diabetic control	47.50±5.60
Diabetic+ AAE 250 mg/kgbw	84.43±1.54*
Diabetic + AAE 500mg/kgbw	102.59±2.89**
Glibenclamide 600µg/kgbw	116.68±1.95*

All the values are (mg/dl) mean ± SEM for six rats,* values deviate significantly from diabetic control ** values deviate very significantly from diabetic control group (p≤ 0.05).

Table 4
Effect of the AAE on total cholesterol, triglycerides and HDL-cholesterol in STZ induced diabetic rats after 21 day treatment.

groups	Serum Lipid profile(mg/dl)		
	Total cholesterol	HDL- cholesterol	Triglycerides
Normal control	49.59±1.40	70.51±1.29	50.72±1.75
Diabetic control	205.06±10.99	24.51±1.41	204.45±1.80
Diabetic + AAE (250 mg/kgbw)	148.85±2.98*	46.89±2.20*	137.20±1.80
Diabetic + AAE (500 mg/kgbw)	95.18±1.42*	75.84±1.28**	73.07±1.19**
Diabetic + glibenclamide (600 mg/kgbw)	96.82±3.60*	72.86±1.12**	76.04±1.52**

All the values are (mg/dl) mean ± SEM for six rats,* values deviate significantly from diabetic control ** values deviate very significantly from diabetic control group (p≤ 0.05).

DISCUSSION

The present paper discussed about the antidiabetic effect of *A. catechu* aqueous extract in streptozotocin induced diabetic rats. In our study STZ was selected for induction of diabetes in rats rather than alloxan. STZ is well known for its selective pancreatic β-cell cytotoxicity and has been extensively used to induce diabetes mellitus in animals (Raju and Balaraman, 2008) and it is less toxic than alloxan and allows a consistent maintenance of diabetes mellitus. The experimental diabetic model used in this study was type 2 diabetic since low dose of STZ (40 mg/kg.bw) destroyed half of the population of pancreatic beta cells and there were residual beta cells which secreted insufficient insulin causing type 2 diabetes (Gomes et al., 2001). Over-production (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissues are the fundamental basis of hyperglycemia in diabetes mellitus (Latner, 1958). From GTT it could be concluded that AAE at the dose of 250 and 500mg/kg showed the maximum improvement in glucose tolerance. When AAE was administered to glucose loaded normal rats (GTT), reduction in the blood glucose levels was observed after 60 min. The decline reached its maximum at 2 h. Oral administration of AAE extract for 21 days

caused a significant decrease in blood glucose levels. The promising mechanism by which FAB mediated its antidiabetic effect could be by potentiation of pancreatic secretion of insulin from existing β-cells of islets ,as was evident by the significant increase in the level of insulin in the extract treated animals. The hypoglycemic activity of AAE was compared with glibenclamide, the standard drug used for many years to treat diabetes, to stimulate insulin secretion from pancreatic β-cells (Tian et al., 1998).Hypercholesteremia and hypertriglyceridemia are primary factors involved in the development of atherosclerosis and coronary heart disease which are the secondary complications of diabetes (Ananthan et al., 2003). FAB significantly reduced serum triglycerides and total cholesterol and significantly increased HDL-cholesterol in STZ-diabetic rats. Thus, it is reasonable to conclude that *Areca catechu* aqueous extract could modulate blood lipid abnormalities.

CONCLUSION

From this study, we can conclude that *Areca catechu* aqueous extract has beneficial effects on blood glucose level. It has the potential to impart the therapeutic effect in diabetes.

Conflict of interest
None

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