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Antidiabetic activity of *Areca catechu* leaf extracts against streptozotocin induced diabetic rats

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ABSTRACT

Areca catechu L. (Palmaceae), commonly known as Areca nut in English, is a perennial tree occurring throughout the Indian subcontinent and used traditionally for several medicinal purposes. The present study was expected to evaluate anti diabetic activity of petroleum ether, chloroform and methanol extracts of *A. catechu* leaf in Wister rats. Diabetes mellitus was induced in rats by single intraperitoneal injection of streptozotocin (STZ, 50 mg/kg body weight). After STZ induction, the hyperglycemic rats were treated with all three extracts orally at the dose 200 mg/kg body weight daily for 15 days. Glibenclamide (0.5 mg/kg b. w., p.o.) was used as reference drug. The fasting blood glucose levels were measured on every 5th day during the 15 day treatment. All the extracts at 200 mg/kg orally significantly ($p < 0.001$) exhibited anti diabetic activity in STZ-induced diabetic rats by reducing and normalizing the elevated fasting blood glucose levels as compared to those of STZ control group. The methanol extract was most active. The present study concludes that *A. catechu* leaf confirmed promising anti diabetic activity in STZ-induced diabetic Wister rats.

Key words: Antidiabetic, *Areca catechu*, streptozotocin, leaf

INTRODUCTION

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, glycosurea, and negative nitrogen balance and it is mainly due to absolute deficiency or diminished effectiveness of insulin. It is the most prevalent disease in the world affecting 25% of the population and afflicts 150 million people and is predicted to rise to 300 million by 2025. [1] Diabetes is still not completely curable by the present antidiabetic therapy.

Insulin therapy is the only satisfactory approach in diabetic mellitus, even though it has several drawbacks like insulin resistance, anorexia, brain atrophy, and fatty liver in chronic treatment. [2] There are several oral hypoglycemic agents used therapeutically but certain adverse effects and weak effectiveness of them has led to the search for more effective agents. Therefore, herbal drugs are gradually gaining popularity in the treatment of diabetes mellitus. The major qualities of herbal medicine seem to be their supposed efficacy, low incidence of serious adverse effects and low cost.

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 masticatory. Its seeds have sialogogue properties and used as anthelmintic in veterinary practice. [3] Previous researchers have reported some pharmacological properties of its seed. [4-6] However, there are no reports on the experimental pharmacological studies on this plant's leaf. The present study was therefore aimed to investigate the possible antidiabetic effects of *A. catechu* leaf extracts against streptozotocin (STZ)-induced diabetic Wister albino rats.

MATERIALS AND METHODS

Plant material: The leaves of *Areca catechu* L. (Palmaceae) were collected during August- September 2011 from Barrackpore, West Bengal, India. The plant material was taxonomically identified at the Central National Herbarium, Botanical Survey of India, Howrah, and West Bengal, India. The voucher specimen [CNH/91/2011/Tech.II/587] was maintained in our research laboratory for future reference. The leaves from the plant were collected and the leaves were shade-dried with occasional shifting and then coarse powder with mechanical grinder and stored in an airtight container for use in the study.

Drugs and chemicals: Streptozotocin (STZ) from SISCO Research Laboratory, Mumbai, India; Glibenclamide from Hoechst, Mumbai, India. All the other reagents used were of analytical reagent grade obtained commercially.

Preparation of extract: The powdered plant material (153.4 g) was extracted with petroleum ether (700 ml) for 2 days in a percolator, followed by extraction with the chloroform (650 ml) similarly for 2 days and again extraction similarly with methanol (700 ml). The extacts were separately taken, filtered and evaporated to dryness on hot

water bath. The dry extracts (pet. ether, yield: 1.85 % w/w, chloroform, yield: 1.32 % w/w, methanol, yield: 8.25 % w/w) were kept in a vacuum desiccators until use. Preliminary phytochemical analysis performed on pet. ether extract (pH 6.0) revealed the presence of triterpenoids and steroids; chloroform extract (pH 7.0) revealed the presence of alkaloids and triterpenoids; metahnol extract (pH 6.0) revealed the presence of alkaloids, steroids, saponins, tannins, glycosides and carbohydrates. [7]

Experimental animals: Adult male Wister albino rats weighing 170-200 g, procured from registered breeders (Rita Ghosh & Co., Kolkata, India) and were housed in a clean polypropylene cage with not more than four animals per cage and maintained under standard laboratory conditions (temperature 25 ± 2°C with dark/light cycle 12/12 h). They were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The animals were acclimatized to laboratory conditions for 10 days prior to experiment. All experimental procedures described were reviewed and approved by the Institutional Animal Ethics Committee.

Acute toxicity: The acute oral toxicity of pet ether extract, chloroform extract, methanol extract of *A. catechu* in male Swiss albino mice was studied as per reported method. [8] These extracts were given to four groups (n = 6) of animals at 500, 1000, 1500 and 2000 mg/kg body weight (b.w.) *per os* (p.o.). The treated animals were kept under observation for 2 days, for mortality and general behavior. No death was observed till the end of the study.

Induction of diabetes: Diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of streptozotocin (50 mg/kg body weight). After 3 days, fasting blood glucose levels were measured and the animals showing blood glucose level 225 mg/dl were used for the present investigation.

Experimental protocol: The rats were divided into six groups (n = 6). Except group I which served as normal non diabetic control all other groups were comprised of diabetic rats. Group II served as diabetic (STZ) control. Groups III, IV and V, received pet. ether, chloroform and methanol extracts at the dose of 200 mg/kg b.w., p.o., respectively; and group V received the reference drug glibenclamide (0.5 mg/kg b.w., p.o.) daily for 15 days. [9] Fasting blood glucose (FBG) level of each rat was measured on 0, 5^{th}, 10^{th} and 15 day by using a one touch glucometer (Accu-check).

Body weight: The body weights of rats of each group were measured before and after 14 days of the treatments.

Serum biochemical parameters: The blood was used for the estimation of serum biochemical parameters viz. serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), total cholesterol were estimated by using commercially available reagent kits (Span Diagnostic Ltd., Surat, India). Serum total protein was estimated according to the reported method. [10]

Statistical Analysis: The experimental data were expressed as mean ± standard error of mean (SEM). Statistical significance was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's *post hoc* test of significance. *P* values of < 0.001 were considered as statistically significant.

RESULTS AND DISCUSSION

The present work was aimed to study the anti diabetic activity of different solvents extracts from *A. catechu* leaf in STZ-induced diabetic rats. The results of this study revealed that pet ether, chloroform and methanol extract at the doses of each 200 mg/kg body weight orally, demonstrated effective anti hyperglycemic activity in STZ induced diabetic rats; and restored body weight towards normal.

Streptozotocin (STZ) is an antibiotic obtained from *Streptomyces achromogenes*. STZ enters the pancreatic cells via a glucose transporter-GLUT2 and causes alkylation of deoxyribonucleic acid (DNA) leading to pancreatic damage. Its toxicity depends upon the potent alkylation properties combined with the synergistic action of nitric oxide and reactive oxygen species that continue to DNA fragmentation. As a result of STZ action, streptozotocin pancreatic cells are destroyed by necrosis. STZ is not only damaging to the pancreatic cells but also to hepatocytes, nephrons and cardiomyocytes. [9, 11]

In the present study, hyperglycemia was observed in rats after 3 days of STZ-induction. Treatment with pet. ether, chloroform and methanol extracts in STZ-induced diabetic rats, started reducing fasting blood glucose levels after 5 days and made them completely normoglycemic after 15 days. The antidiabetic effect of pet. ether, chloroform and methanol extract each at 200 mg/kg dose was found to be comparable to that the effect exerted by the reference drug, glibenclamide at the dose of 0.5 mg/kg (Table 1).

However, the methanol extract was found to be most active followed by chloroform and pet. ether extracts respectively.

Induction of diabetes with STZ had been associated with a characteristic loss of body weight, which is due to increased muscle wasting and loss of tissue proteins. Diabetic rats treated with the pet. ether, chloroform and methanol extract showed significant improvement in body weight as compared to the STZ control animals; hence pet. ether, chloroform and methanol extract exhibited marked effect in controlling the loss of body weights of diabetic rats (Table 2). The results of the present study are in agreement with those of previous workers. [12]

It has been well established that elevated levels of SGOT, SGPT and SALP are indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes implying hepatocellular damage. [13] Serum total proteins on the other hand are related to the function of the hepatic cells revealing the functional status of the hepatic cell.

Elevated serum cholesterol and triglyceride levels in STZ challenged rats indicated impaired fat metabolism. Altered serum biochemical parameters in STZ-induced rats were reported elsewhere. [9] The test extracts decreased the elevated serum enzyme activities, cholesterol contents with elevation of total protein content in the STZ treated rats which are comparable to the normal control group (Table 3).

Table: 1. Effect of *A. catechu* leaf extracts on fasting blood glucose levels.

Group	Dose	Day 0	Day 5	Day 10	Day 15
I (Normal Saline)	5 ml/kg	76.85±2.1	75.32±4.2	74.92±3.6	74.72±2.9
II (STZ)	50 mg/kg	278.54±9.2*	280.95±10.5*	285.73±11.4*	292.72±9.8*
III (STZ + Pet ether extract)	200 mg/kg	261.36±11.9	110.65±7.8**	91.45±1.6**	87.73±1.8**
IV (STZ + Chloroform extract)	200 mg/kg	265.76±16.5	90.59±4.4**	83.58±3.2**	75.81±2.9**
V (STZ + Methanol extract)	200 mg/kg	271.53±10.3	93.45±3.7**	82.69±1.8**	74.92±2.6**
VI (STZ+ Glibenclamide)	0.5 mg/kg	279.53±13.3	95.45±2.8**	81.69±1.5**	72.91±2.4**

Values are expressed as mean ± SEM (n = 6); *p < 0.001 compared with saline control and **p < 0.001 compared with STZ control group

Table: 2. Effect of *A. catechu* leaf extracts on body weight, liver weight and kidney weights.

Group	Dose	Initial body wt (g)	Final body wt (g)
I (Normal Saline)	5 ml/kg	179.76±7.8	183.54±5.2
II (STZ)	50 mg/kg	174.68±7.2	149.54±4.5*
III (STZ+ Pet ether extract)	200 mg/kg	165.54±4.2	150.72±2.9**
IV (STZ+ Chloroform extract)	200 mg/kg	169.41±5.2	151.94±1.8**
V (STZ + Methanol extract)	200 mg/kg	176.23±5.5	165.76±3.3**
VI (STZ + Glibenclamide)	0.5 mg/kg	178.53±4.5	169.76±3.6**

Values are expressed as mean ± SEM (n = 6); *p < 0.001 compared with saline control and **p < 0.001 compared with STZ control group

Table 3: Effect of *A. catechu* leaf extracts on different serum biochemical parameters

Group	Dose	SGOT (IU/L)	SGPT (IU/L)	Total Protein (g/dl)	ALP (IU/L)	Cholesterol (mg/dl)
I (Normal saline)	5ml/kg	21.18±4.9	23.81±3.8	7.18±1.1	168.1±13.2	151.63±9.6
II (STZ)	50 mg/kg	38.4±5.5*	40.5±4.5*	4.72±0.5*	236.5±11.8*	212.6±13.8*
III (STZ+ Pet ether extract)	200 mg/kg	27.9±3.6**	29.6±3.7**	6.18±2.7**	219.6±13.5**	192.8±11.8**
IV (STZ+ Chloroform extract)	200 mg/kg	24.3±3.6**	27.7±6.3**	6.37±3.1**	196.7±13.6**	187.58±10.2**
V (STZ + Methanol extract)	200 mg/kg	23.64±2.7**	26.6±4.7**	6.91±3.6**	192.9±9.7**	179.29±10.8**
VI (STZ + Glibenclamide)	0.5 mg/kg	22.54±2.9**	24.9±3.7**	7.1±3.3**	191.7±9.8**	169.69±10.5**

Values are expressed as mean ± SEM (n = 6); *p < 0.001 compared with saline control and **p < 0.001 compared with STZ control group

Preliminary phytochemical analysis revealed the presence of triterpenoids and steroids in pet. ether extract; chloroform extract revealed the presence of alkaloids and triterpenoids; metahnol extract revealed the presence of alkaloids, steroids, saponins, tannins, glycosides and carbohydrates. The observed antidiabetic activity of all the extracts may be due to mainly the presence of triterpenoid compounds.

In the present investigation, oral administration of pet ether, chloroform and methanol extracts on 15 days continuous treatment to STZ-induced diabetic rats demonstrated prominent reduction and normalization of elevated blood sugar levels i.e. anti hyperglycemic or antidiabetic effect, comparing to respective control rats. The methanol extract was most active followed by chloroform and pet. ether extracts. Therefore, it can be concluded that the extracts of *Areca catechu* leaf possessed remarkably effective antidiabetic potential against streptozotocin induced diabetes in Wistar rats. The findings of the present study is encouraging enough to warrant further studies on this pant in pursuit of a new oral hypoglycaemic agent.

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