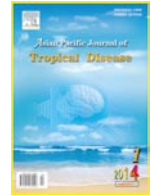




Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd



Document heading

doi: 10.1016/S2222-1808(14)60430-8

© 2014 by the Asian Pacific Journal of Tropical Disease. All rights reserved.

Antihyperglycemic activity of *Areca Catechu* flowersRupesh Ghate¹, Virupanagouda Police Patil^{1*}, Shivakumar Hugar¹, Nanjappaiah Hanakunte Matha¹, Navanath Vishwanathappa Kalyane²¹P.G. Dept. of Pharmacology, BLDEA's College of Pharmacy, BLDE University campus, Bijapur-586103, Karnataka, India²P.G. Dept. of Pharmaceutical chemistry, BLDEA's College of Pharmacy, BLDE University campus, Bijapur-586103, Karnataka, India

PEER REVIEW

Peer reviewer

Dr. Vishwanathswamy AHM, Professor of Pharmacology, KLE University's College of Pharmacy, Vidyanagar, Hubli, Karnataka, India.
Tel: +919448667355
Fax: +91-836-2371694
E-mail: vmhiremath2004@yahoo.com

Comments

In the present study, authors evaluated antidiabetic activity of different extracts of *A. catechu* flowers. The extracts were also subjected to preliminary phytochemical screening and acute toxicity test. The results indicate that ethanolic extract of plant possesses more significant antihyperglycemic activity compared to aqueous extract. The observed potential antidiabetic effect of this extract could be due to the presence of flavonoids.

Details on Page S151

ABSTRACT

Objective: To evaluate the antidiabetic effect of *Areca catechu* (*A. catechu*) flower extracts in alloxan induced diabetic rats.

Methods: Petroleum ether, ethanol and aqueous extracts of *A. catechu* flowers were administrated orally at the dose of 500 mg/kg for hypoglycemic effect in alloxan induced diabetic rats for 21 d. The anti-diabetic potential was validated through various biochemical parameters and body weight.

Results: The results revealed that, the ethanol and aqueous extracts of *A. catechu* flowers have shown a significant antidiabetic efficacy in alloxan induced diabetic rats. Further, this is confirmed by significantly restoring the levels of biochemical parameters and improvement in body weight. Preliminary phytochemical investigation reveals high phenolic constituents in both extracts.

Conclusions: The significant antihyperglycemic effect of ethanol and aqueous extracts in alloxan induced diabetic rats could be due to high phenolic constituents by effectively reversing the levels of biochemical parameters and also improvement in body weight. So it might be of value in the treatment of diabetes.

KEYWORDS

Alloxan, Antidiabetic, *Areca catechu*

1. Introduction

Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia, altered metabolism of carbohydrates, lipids and protein results from insulin deficiency. It is a large public health problem in the world[1]. Towards these pathologies which may appear with

multiple and diversified causes and appearances, modern medicine does not find any curative treatments. Hence, searching the safe and potent remedies from the herbal origin for the treatment of hyperglycemic disorders has become the most fascinating and desired area of research for the pharmacologists.

Areca catechu Linn. (*A. catechu*) (family: Arecaceae) is

*Corresponding author: * Virupanagouda.P.Patil, P.G. Dept. of Pharmacology, B.L.D.E.A's College of Pharmacy, B.L.D.E. University campus, Bijapur-586103, Karnataka, India.

Tel: 09844164121

Fax: 08352-262643

E-mail id: patilk183@gmail.com

Article history:

Received 20 Nov 2013

Received in revised form 25 Nov, 2nd revised form 28 Nov, 3rd revised form 3 Dec 2013

Accepted 20 Dec 2013

Available online 28 Jan 2014

prominently used for treatment of different ailments and it is one of the crude materials of Indian system of medicine. Literature survey of *A. catechu* reveals its various medicinal properties like antibacterial and antiviral^[2], antioxidant^[3], anticholinergic^[4], anti-inflammatory/anti-melanogenesis^[5], hypolipidemic^[6], antiovarulatory^[7], hepatoprotective activities^[8]. Areca nut (*A. catechu*) is commonly used as an ingredient of betel quid, which also includes leaf of the creeping vine *Piper betel* and lime with or without tobacco. Betel quid chewing has been popular, especially in many Southeastern Asian countries. The use of areca nut as a masticator by humans has been known since the 4th century AD in different parts of the world and in India it is at least 2000 years old. It is cultivated through tropical India and flourishes in dry plateau of Mysore, Canara, Malabar, Southern India and Assam^[9].

In vivo hypoglycemic activity of *A. catechu* seeds have been already documented in the literature^[10]. However, no scientific reports are available on antidiabetic potential of *A. catechu* flowers. Hence, this study was conducted to evaluate the antidiabetic effect of *A. catechu* flowers on alloxan induced diabetes in rats and also to modulate abnormal biochemical parameters associated with alloxan induced diabetic model.

2. Materials and methods

2.1. Collection of plant material and preparation of extracts

The flowers of *A. catechu* were collected in the month of July from the garden of the Bijapur (Karnataka), and the sample was authenticated by Dr. MB Mulimani, Professor of Botany, SB Arts and KCP Science College, Bijapur.

The flowers were dried in shade at room temperature. The dried flowers were powdered by using grinder into coarse powder, packed into Soxhlet column and subjected to successive extraction using petroleum ether, 70% ethanol and distilled water. Excess of solvents from the extracts were recovered using rotary flash evaporator to obtain semisolid crude extracts. The obtained crude extracts were stored in airtight container in refrigerator below 10 °C for further studies.

2.2. Qualitative phytochemical screening

The extracts of *A. catechu* flowers were subjected to various qualitative tests for the presence or absence of different phytochemical constituents.

2.3. Experimental animals

Male albino rats (150–180 g) and albino mice (20–25

g) of either sex were procured from Sri. Venkateshwara Enterprises, Bangalore. The animals were acclimatized for a period of 10 d under standard environmental conditions such as temperature [(26±2) °C], relative humidity (45%–55%) and 12 h dark/light cycles maintained in the quarantine. All the animals were fed with rodent pellet diet and water *ad libitum* under strict hygienic conditions. Ethical clearance for performing the experiments on animals was obtained from Institutional Animal Ethics Committee (Reg. No. 1076/c/07 CPCSEA).

2.4. Determination of acute toxicity (LD₅₀)

The acute toxicity of *A. catechu* flowers extracts were evaluated in mice. The mice weighing between 20–25 g were fasted for 24 h prior to the acute toxicity study. Drug treated and control groups were placed in polypropylene cages with free access of food and water. Mortality and general behavior of the animals were observed continuously for the initial 4 h, and intermittently for the next 6 h and then again at 24 h and 48 h after dosing. Animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 h, with special attention during the first 4 h, and daily thereafter, for a total of 21 d. The acute toxicity (LD₅₀) of extracts was determined by fixed dose method (OECD guide line No. 423) of CPCSEA. The 1/5th of LD₅₀ cut off value of the extracts were selected as screening doses for the study^[11].

2.5. Evaluation of anti-diabetic activity

Fasting blood glucose of animal was determined after depriving food for 16 h with free access of drinking water. Hyperglycemia was induced by a single *i.p.* injection of 160 mg/kg of alloxan monohydrate in sterile saline. After 3 d of alloxan injection, the hyperglycemic rats (glucose level between 250–300 mg/dL) were separated and divided into different groups comprising of 6 rats each for the anti-diabetic study. Group A: served as normal control and received vehicle; Group B: served as diabetic control and received alloxan monohydrate; Group C: served as standard and received alloxan monohydrate and glibenclamide; Group D: served as treated and received alloxan monohydrate and petroleum ether extract of flowers of *A. catechu* (500 mg/kg, *p.o.*); Group E: served as treated and received alloxan monohydrate and 70% ethanolic extract of flowers of *A. catechu* (500 mg/kg, *p.o.*) and Group F: served as treated and received alloxan monohydrate and aqueous extract of flowers of *A. catechu* (500 mg/kg, *p.o.*).

The treatment was started from the same day except normal control and diabetic control groups for a period of 21 d. During this period, animals in all groups had free access to standard diet and water. Blood glucose levels were

estimated on the first and last day of the treatment. On the last day, blood samples were collected from overnight fasted rats by carotid bleeding under mild ether anesthesia for biochemical estimations.

The biochemical parameters studied were fasting blood glucose, serum urea, serum cholesterol and serum triglycerides. Morphological parameter was body weight^[12,13].

2.6. Statistical analysis

The data obtained from the above findings will be subjected to statistical analysis using One-way ANOVA followed by Turkey Kramer multiple comparison test to assess the statistical significance of the results. Values of $P < 0.01$ were considered significant.

3. Results

3.1. Determination of acute toxicity (LD_{50})

The petroleum ether, 70% ethanolic and aqueous extracts were studied for acute toxicity at a dose of 2000 mg/kg *i.p.* in albino mice. The extracts were found devoid of mortality for the animals. Hence 2500 mg/kg was considered as LD_{50} cut off value.

So the screening doses were selected for the evaluation of antidiabetic activity as per OECD guidelines No. 423 (Annexure – 2D) and the fixed dose method are mentioned below:

1. 500 mg/kg petroleum ether extract (1/5th of 2500 mg/kg).
2. 500 mg/kg 70% ethanolic extract (1/5th of 2500 mg/kg).

3. 500 mg/kg aqueous extract (1/5th of 2500 mg/kg).

3.2. Antidiabetic study

3.2.1. Effect of *A. catechu* flowers extracts on fasting blood glucose level in diabetic rats

A marked rise in fasting blood glucose level was observed in diabetic control compared to normal control rats. Ethanol and aqueous extracts exhibited significant anti-hyperglycemic activity in 21 d treatment. Whereas the petroleum ether extract also caused reduction in blood glucose level but the results were found statistically not significant. Glibenclamide produced a significant reduction in blood glucose compared to diabetic control. The results are shown in the Table 1.

3.2.2. Effect of *A. catechu* flowers extracts on body weight in diabetic rat

Normal control animals were found to be stable in their body weight but diabetic rats showed significant reduction in body weight during 21 d treatment. Alloxan mediated body weight reduction was significantly reversed by the ethanol and aqueous extracts. However, the effect of petroleum ether extract on body weight of the animals was found statistically not significant. Results are shown in Table 1.

3.2.3. Effect of *A. catechu* flowers extracts on biochemical parameters

Serum cholesterol, serum triglycerides and serum urea levels were decreased significantly by ethanol and aqueous extracts due to 21 d of treatment, when compared to diabetic control group. However, the petroleum ether extract

Table 1

Effect of *A. catechu* flower extracts on fasting blood glucose level in alloxan induced diabetic rats.

Groups	Treatment	Fasting blood glucose level (mg/dL)		Body weight of the animal (g)	
		Initial	After 21 d	Initial	After 21 d
A	Normal control	125.02±2.54	123.50±2.44	154.50±1.25	159.05±1.74
B	Diabetic control (vehicle)	288.24±2.86	252.30±4.65	164.30±0.76	122.27±2.53
C	Alloxan + glibenclamide (0.5 mg/kg)	275.44±3.53	124.30±4.63 ^a	155.70±0.43	149.32±2.56 ^a
D	Alloxan + petroleum ether extract (500 mg/kg)	278.70±3.10	233.20±2.56 ^b	162.60±1.45	132.43±1.83 ^b
E	Alloxan + ethanolic extract (500 mg/kg)	263.05±0.33	132.50±3.06 ^a	161.40±1.35	142.32±1.58 ^a
F	Alloxan + aqueous extract (500 mg/kg)	269.07±3.01	133.50±4.54 ^a	152.50±1.24	136.39±0.94 ^a

Values are Mean±SEM; $n=6$.

P value: ^b < 0.05 and ^a < 0.001 *v.s.* diabetic control.

Table 2

Effect of *A. catechu* flower extract on biochemical parameters in alloxan induced diabetic rats.

Groups	Treatment	Serum Triglycerides (mg/dL)	Serum Cholesterol (mg/dL)	Serum Urea (mg/dL)
A	Normal control	85.36±1.73	98.40±0.63	27.05±0.98
B	Diabetic control (vehicle)	165.20±2.43	172.40±2.23	78.43±0.62
C	Standard (alloxan+ glibenclamide 0.5mg/kg)	102.30±1.64 ^a	113.20±1.26 ^a	32.46±2.40 ^a
D	Alloxan + petroleum ether extract (500 mg/kg)	155.30±1.49 ^b	163.46±2.32 ^b	71.33±1.42 ^b
E	Alloxan + ethanolic extract (500 mg/kg)	127.80±2.17 ^a	124.80±1.07 ^a	46.49±1.54 ^a
F	Alloxan + aqueous extract (500 mg/kg)	125.20±2.51 ^a	135.20±1.68 ^a	40.03±1.32 ^a

Values are Mean±SEM; $n=6$.

P value: ^b < 0.05 and ^a < 0.001 *v.s.* diabetic control.

decreases the serum cholesterol, serum triglycerides and urea levels but results are less significant. Results are shown in Table 2.

4. Discussion

Diabetes mellitus is chronic disorder caused by partial or complete insulin deficiency. This causes abnormal metabolism of glucose, protein and lipid, leading to acute and chronic complications. The major complications are the premature and extensive atherosclerosis involving renal, peripheral and cardiovascular vessels. Disorder is more likely to increase risk for coronary heart disease because of alteration in serum lipid profile.

Alloxan, a β cytotoxin induces chemical diabetes in wide variety of animal species by damaging the insulin secreting cells of pancreas. Literature survey indicates that alloxan treated rats are hyperglycemic. Alloxan produces partial destruction of pancreatic β cells and these animals may have surviving β cells, and regeneration of β cells is possible[14].

In the present study, treatment with ethanol and aqueous extracts of *A. catechu* flowers exhibited significant activity to decrease the blood glucose level in alloxan treated rats. The hypoglycemic effect may be due to increased secretion of insulin from β cells of pancreas *i.e.* pancreatotrophic action or due to regeneration of pancreatic cells that were partially destroyed by alloxan.

The preliminary phytochemical screening of the plant showed the presence of flavonoids, steroids, tannins, saponins in ethanol and aqueous extracts. It is well known that flavonoids[15]and sterols possess antidiabetic property[16].

Weight loss is prominent symptoms of diabetes and it has been attributed to the gluconeogenesis *i.e.* catabolism of protein and fats, which is associated with characteristic loss of body weight due to increased muscle wasting and loss of tissue protein[17]. Results on body weight reveals that, the normal control rats have not gained weight throughout the study. However, diabetic control rats steadily lost body weight. Ethanol and aqueous extracts have protected the rats from losing their body weight may be due to their interference with catabolism of proteins and fats.

Diabetic rats were observed to have increased plasma lipids like cholesterol and triglycerides which are responsible for several cardiovascular disorders[18]. The higher lipid levels observed in diabetic rats may be due to increased mobilization of free fatty acids from peripheral depots and also due to lipolysis caused by hormones[19]. Ethanol and aqueous extracts significantly reversed the levels of serum cholesterol and triglycerides and thereby offering protection from cardiovascular diseases.

The ethanol and aqueous extracts may lead to regeneration

of β cells of pancreas and potentiate insulin secretion from surviving β cells. Increase in insulin secretion and consequent decrease in blood glucose levels may lead to inhibition of lipid peroxidation and control of lipolytic hormones.

Diabetes mellitus is one of the major disorders which affect the kidneys; urea, uric acid and creatinine are markers of renal function. In the present study, increase in serum urea was observed in diabetic rats and this level was significantly decreased by the ethanol and aqueous extracts and glibenclamide compared to diabetic control rats.

In conclusion, the results of the present study demonstrated the antihyperglycemic effect of *A. catechu* flowers ethanol and aqueous extracts in alloxan induced hyperglycemic rats possibly by restoring the biochemical parameters and improvement in body weight. The potential hypoglycemic effect of these extracts could be due to presence of active constituents, which is evident by the preliminary phytochemical screening.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We would like to thank the Principal, BLDEA's College of Pharmacy, for providing necessary facilities to perform this research.

Comments

Background

A. catechu is prominently used for treatment of different ailments and it is one of the crude materials of Indian system of medicine. Literature survey of *A. catechu* reveals its various pharmacological properties like antibacterial and antiviral, antioxidant, antimicrobial, anticholinergic, anti-inflammatory/anti-melanogenesis, hypolipidemic, antiovolatory, hepatoprotective activities. *In vivo* hypoglycemic activity of the *A. catechu* seeds has been already documented in the literature. However, no scientific reports are available on antidiabetic potential of *A. catechu* flowers. Hence, this study was conducted to evaluate the antidiabetic effect of *A. catechu* flowers on alloxan induced diabetes in rats.

Research frontiers

The present study is performed to assess antidiabetic activity of different extracts of *A. catechu* flowers in alloxan

induced diabetic rats. Fasting blood glucose level and other biochemical estimations were determined after a treatment period of 21 d with *A. catechu* flower extracts.

Related reports

The present study demonstrated the hypoglycemic effect of ethanol and aqueous extracts of *A. catechu* flowers in alloxan induced hyperglycemic rats. The results of the present study are in agreement with previous research report on antidiabetic activity of seeds extract of same plant (Chempakam B, 1993). The potential hypoglycemic effect of these extracts could be due to presence of active constituents.

Innovations & breakthroughs

Modern medicine does not offer any curative treatments and exhibits higher incidence of adverse effects on long-term use. Hence, searching the safe and potent remedies from the herbal origin for the treatment of diabetes mellitus has become the most desired area of research. More studies on evaluation of antidiabetic property of various parts of title plant is lacking in the literature. The results of present investigation demonstrated that the *A. catechu* ethanolic flower extract showed more potent antidiabetic activity compared to aqueous extract.

Applications

It is important to notice that *A. catechu* is commonly used to chew along with betel leaves by most of the people in India after meal. The results of the present study suggest that ethanolic and aqueous extracts of *A. catechu* exhibited antidiabetic property. Thus, the present study proves the scientific reason behind chewing *A. catechu* with betel leaves.

Peer review

In the present study, authors evaluated antidiabetic activity of different extracts of *A. catechu* flowers. The extracts were also subjected to preliminary phytochemical screening and acute toxicity test. The results indicate that ethanolic extract of plant possesses more significant antihyperglycemic activity compared to aqueous extract. The observed potential antidiabetic effect of this extract could be due to the presence of flavonoids.

References

- [1] Baquer NZ, Gupta D, Raju J. Regulation of metabolic pathways in liver and kidney during experimental diabetes: effects of antidiabetic compounds. *Indian J Clin Biochem* 1998; **13**: 63–80.
- [2] Anthikat RR, Michael A. Study on the areca nut for its antimicrobial properties. *Pharmacognosy* 2009; **1**(1): 42–45.
- [3] Lin ES, Li CC. Evaluation of superoxide radical scavenging capacity and reducing power of areca flower extracts. *J Med Plants Res* 2010; **4**(10): 975–981.
- [4] Gilani AH, Ghayur N, Saify ZS, Ahmed SP, Choudhary MI, Khalid A. Presence of cholinomimetic and acetylcholinesterase inhibitory constituents in betel nut. *Life Sci* 2004; **75**(20): 2377–2389.
- [5] Lee KK, Choi JD. The effects of *Areca Catechu* L extract on anti-inflammation and anti-melanogenesis. *Int J Cosmet Sci* 1999; **21**(4): 275–284.
- [6] Byun SJ, Kim HS, Jeon SM, Park YB, Choi MS. Supplementation of *Areca catechu* L. extract alters triglyceride absorption and cholesterol metabolism in rats. *Ann Nutr Metab* 2001; **45**(6): 279–284.
- [7] Shrestha J, Shanbhag T, Shenoy S, Amuthan A, Prabhu K, Sharma S, et al. Antioviulatory and abortifacient effects of *Areca catechu* (betel nut) in female rats. *Indian J Pharmacol* 2010; **42**(5): 306–311.
- [8] Pithayanukul P, Nithitanakool S, Bavovada R. Hepatoprotective potential of extracts from seeds of *Areca catechu* and nutgalls of *Quercus infectoria*. *Molecules* 2009; **14**(12): 4987–5000.
- [9] Nelson BS, Heischober B. Betel nut: a common drug used by naturalized citizens from India, Far East Asia, and the South Pacific Islands. *Ann Emerg Med* 1999; **34**(2): 238–243.
- [10] Chempakam B. Hypoglycemic activity of arecoline in betel nut *A. catechu* Linn. *Indian J Exp Biol* 1993; **31**(5): 474–475.
- [11] Raghavan PV. Expert consultant, CPCSEA, OECD, guideline No. 423; 2001.
- [12] Tailang M, Gupta BK, Sharma A. Antidiabetic activity of alcoholic extract of *Cinnamomum zeylanicum* leaves in alloxan induced diabetic rats. *PJSR* 2008; **1**: 9–11.
- [13] Ghule AE, Jadhav SS, Bodhankar SL. Effect of ethanolic extract of seeds of *Linum usitatissimum* (Linn.) in hyperglycaemia associated ROS production in PBMNCs and pancreatic tissue of alloxan induced diabetic rats. *Asian Pac J Trop Dis* 2012; **2**(5): 405–410.
- [14] Sharma N, Garg V. Antihyperglycemic and antioxidative attribute of hydroethanolic extract of *Butea monosperma* (Lam.) seeds and its active constituents. *Indian J Exp Biol* 2011; **49**: 756–766.
- [15] Havsteen BH. The biochemistry and medical significance of the flavonoids. *Pharmacol Ther* 2002; **96**(2–3): 67–202.
- [16] Koffi N, Ernest AK, Marie-Solange T, Beugré N, Noël ZG. Effect of aqueous extract of *Chrysophyllum cainito* leaves on the glycaemia of diabetic rabbits. *Afr J Pharm Pharmacol* 2009; **3**(10): 501–506.
- [17] Jain S, Bhatia G, Barik R, Kumar P, Jain A, Dixit VK. Antidiabetic activity of *Paspalum scrobiculatum* Linn. in alloxan induced diabetic rats. *J Ethnopharmacol* 2010; **127**(2): 325–328.
- [18] Alarcon-Aguilar FJ, Campos-Sepulveda AE, Xolalpa-Molina S, Hernandez-Galicia E, Roman-Ramos R. Hypoglycemic activity of *Ibervillea sonora* roots in healthy and diabetic mice and rats. *Pharm Biol* 2002; **40**(8): 570–575.
- [19] Qi XY, Chen WJ, Zhang LQ, Xie BJ. Mogrosides extract from *Siraitia grosvenori* scavenges free radicals *in vitro* and lowers oxidative stress, serum glucose, and lipid levels in alloxan-induced diabetic mice. *Nutr Res* 2008; **28**: 278–284.