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The effects of *Areca cathecu* and *Ficus deltoidea* methanolic extracts on glycemic and reproductive parameters in alloxan-induced diabetic rats

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ABSTRACT: Background: Diabetes mellitus is a metabolic diseases characterized by disordered metabolism and abnormally high levels of blood glucose. **Aim:** This study was conducted to study the effects of *Areca catechu* seeds and *Ficus deltoidea* fruits methanolic extracts on glycemic and reproductive parameters of alloxan-induced diabetic rats. **Methodology:** Diabetes mellitus was induced by a single intraperitoneal injection of 100 mg/kg bwt alloxan monohydrate. Blood glucose level was estimated using electronic glucometer and sperm parameters were measured using Makler chamber. Insulin and testosterone were estimated by ELISA kits. **Results:** *A. catechu* and *F. deltoidea* methanolic extracts significantly reduced blood glucose levels and increased insulin secretion. Both extracts also significantly increased sperm quality and testosterone secretion in diabetic rats. **Conclusion:** Present study demonstrated anti-hyperglycemic and pro-fertility effects of *A. catechu* and *F. deltoidea* methanolic extracts in diabetic rats. Further studies are currently undergoing to determine their mechanisms and mode of action that decreases the blood glucose levels and increases the sperm quality in diabetic rats.

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INTRODUCTIONS:

Approximately, 642 million of the world population will suffer from Diabetes mellitus by the year of 2040 [1]. Currently, type 2 diabetes has affected millions of people regardless of age, sex, race and economic status. Type 2 diabetes is caused by insulin resistance, which is defined as defective insulin signalling and a decreased insulin efficiency to induce glucose transport from the blood into key target cells such as a muscle and adipocyte cells [2]. The consequences of untreated insulin resistance are hyperinsulinemia, reduced skeletal

muscle metabolic flexibility, accumulation of fat in the liver and infertility [3-5]. Factors contributing to diabetes and infertility are overweight, lack of physical activities, high fat diets, smoking, genetics and even aging [6]. Although the use of synthetic drugs has been recognized to be effective in treating diabetes as well as infertility, the use of plant-based treatments remains a major choice especially in an underdeveloped and developing countries [7]. There are many hypoglycaemic plants and plants that have the potential to treat fertility known through the folklore but less commercialized and less exposed to the general public.

Areca catechu (Fig 1) commonly known as betel nut in English or 'pinang' in Malay is a tall perennial palm occurring in sandy clay land throughout South East Asia and Indian subcontinent [8]. Traditionally, *A. catechu* seeds are consumed as masticatory, especially in India subcontinent and Malaysia. Scientifically, a few papers have been published describing medical value of *A. catechu* such as the wound healing, hepatoprotective, anti-inflammatory and anti-oxidant [9-12]. However, *A. catechu* also may have an adverse effect on the reproductive system by reducing sperm count in mouse [13]. Toxicology study also demonstrated that this species might be carcinogenic [14].

Fig 1. *Areca catechu* ripe fruits.

Meanwhile, *Ficus deltoidea* (Fig 2) or 'Mas cotek' in Malay is a large shrub native to Southeast Asia. The rural population in Malaysia use this plant to treat wounds, rheumatism, sores, diabetic and as after-birth tonic [15]. Previous researchers published varied results on the effect of *F. deltoidea* on male fertility. Naghdi and co-workers reported that hydroalcoholic extract of *F. deltoidea* leaves improved sperm quality of mice [16]. In contrast, Norrizah and colleague demonstrated that methanolic extract of *F. deltoidea* leaves decreased sperm quality of rats [17]. Toxicity study revealed that LD_{50} of the methanolic extract of *F. deltoidea* was greater than 5000 mg/kg [18].

Fig 2. *Ficus deltoidea* plant.

The present investigation was undertaken to reveal the potential of two Malaysia local plants, *A. catechu* and *F. deltoidea* in controlling blood glucose levels and its effect on the fertility of alloxan-induced diabetic male rats.

MATERIALS AND METHODS:

The methanol was procured from HmbG Chemicals, Germany (Supplied by local agent; AmanSemesta Enterprise, Malaysia). The alloxan monohydrate was procured from Sigma Aldrich, USA. All other chemicals and reagents used in this Research study were of analytical grade and procured from authorized dealer.

Plants materials and methanolic extraction:

Areca catechu seeds and *Ficus deltoidea* fruits were collected from State of Selangor, Malaysia. The identification and authentication was conducted at the Universiti Teknologi MARA Animal Physiology Research Laboratory, where a voucher specimen (AI-1015 and AI-1016) was deposited. Fresh *A. catechu* seeds and *F. deltoidea* fruits were washed, air dried at room temperature and then grounded into a powder form before maceration with absolute methanol for three cycles. Each cycle involved three days soaking at room temperature. The extracts were filtered and concentrated using a rotary evaporator (BuchiRotavapor R-210, Switzerland) under reduced pressure at 40^0C to yield a concentrated methanolic extracts.

Animals and experimental designs:

All the procedures for animal studies have been supervised and approved by the Faculty of Applied Sciences Research Ethics Committee, Universiti Teknologi MARA and Faculty of Science and Mathematics, Universiti Pendidikan Sultan Idris (AREC

3/669/27). A total of 30 male Sprague Dawley rats were obtained from Universiti Malaya Animal Facilities. They were housed in polycarbonate cages at a room temperature of 22 ^{0}C, with a 12 h light and 12 h dark cycles. Prior to the experimental protocols, all rats were quarantined for one week and fed with a standard rodent pellets and water *ad libitum*. Then the rats were divided into five groups with six animals each. The Group A healthy rats were treated with distilled water (2 ml/kg bwt), Group B Diabetic rats were treated with distilled water (2 ml/kg bwt), Group C Diabetic rats were treated with standard drug Glibenclamide (0.1 mg/kg bwt), Group D Diabetic rats were treated with tested drug that is *A. catechu* fruits extract (100 mg/kg bwt) and Group E Diabetic rats were treated with another tested drug that is *F. deltoidea* fruits extract (100 mg/kg bwt).

Diabetes mellitus was induced by a single intraperitoneal injection of a freshly prepared, 100 mg/kg bwt alloxan monohydrate [19]. After alloxan injection (within 24 h post-alloxan), all rats received a solution of water and 15% of glucose, in addition to standard rodent pellets. The diabetes was allowed to develop within five to six days after alloxan administration. Rats with fasting blood glucose concentration less than 10 mmol/l were excluded from the study. Then all rats were treated with the respective treatments/drugs for four weeks.

Blood glucose measurements:

Blood was sampled from the tail vein, on a weekly basis for four weeks. A drop of fresh blood was immediately assayed for glucose content using an Accutrend Advantage II Clinical Glucometer (Roche, Swiss).

Insulin and testosterone assays:

All blood samples were collected in the morning in order to minimize the diurnal variation of hormone levels [20]. Blood was sampled at the end of the experiment by cardiac puncture, collected in EDTA tube and centrifuged to separate the plasma. Plasma samples were stored at -20°C (ECONAVI Top Freezer Refrigerator NR-BL308VSMY, Panasonic, Malaysia) until hormones analysis. The concentration of insulin in blood plasma was determined by solid-phase enzyme-immunoassay kits obtained from Medgenic Diagnostic, USA. The concentration of testosterone in the blood plasma was measured using solid-phase enzyme-immunoassay kits obtained from Diatech Diagnostic Inc., Boston, USA.

Sperm quality evaluation:

Sperm was collected from Cauda epididymis. Sperm quality including concentration, motility and morphology was assessed. Sperm were counted using a Makler chamber according to manufacturer's instructions (Sefi Medical Instruments, USA). Sperm motility was assessed by visual estimation under the light microscope with 20X objective lense (Olympus CX21, Japan) [21]. For sperm morphology, the number and percentage of normal sperm was determined by examining air dried slide after staining with giemsa [22].

Statistical analysis:

The data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS). Level of significance was accepted at $p<0.05$.

RESULTS AND DISCUSSION:

The effects of *Areca catechu* and *Ficus deltoidea* on fasting blood glucose and plasma insulin levels in alloxan-induced diabetic rats:

The results of fasting blood glucose and plasma insulin levels in each group are shown in Table 1 and 2. Fasting blood glucose level significantly ($p<0.05$) higher in alloxan-induced diabetic rats compared to healthy rats. Alloxan selectively destroys pancreas β-cells, causing the fluctuation of fasting blood glucose levels in alloxan-induced diabetic rats [23]. Fasting blood glucose levels decreased by 88.48 % ($p<0.05$) in alloxan-induced diabetic rats treated with glibenclamide (Group C, served as positive control), compared to alloxan-induced diabetic rats treated with distilled water (Group B, served as negative control). Glibenclamide is an oral hypoglycemic drug that stimulates the pancreas β-cells to secrete more insulin [24].

The study clearly demonstrated that both plants extracts successfully reduced the fasting blood glucose levels in alloxan-induced diabetic rats. Fasting blood glucose levels decreased significantly ($p<0.05$) by 76.91 % in Alloxan-induced diabetic rats treated with *A. catechu* methanolic extract (Group D) compared to alloxan-induced diabetic rats treated with distilled water (Group B, served as negative control). Meanwhile, *F. deltoidea* methanolic extract demonstrated capability in reducing fasting blood glucose levels of alloxan-induced diabetic rats by 82.58 %, compared to negative control group (Group B). This result shows that *F. deltoidea* methanolic extract has a better potential as an oral hypoglycemic drug, compared to *A. catechu* methanolic extract.

In addition to the adjustment of the fasting blood glucose levels, both plants extracts demonstrated capabilities to stimulate the pancreas β-cells to secrete more insulin in alloxan-induced diabetic rats (Table 2). Insulin secretion increased by 52.9% in diabetic rats treated with *A. catechu* and 61.8% in diabetic rats treated with *F. deltoidea*, compared to negative control. Anti-hyperglycemic potential of *A. catechu* and *F. deltoidea* methanolic extracts in alloxan-induced diabetic rats could be due to high phenolic contents [25-26]. Various phenolic compounds were able to attenuate hyperglycemia by inhibit α-glucosidase and α-amylase activities, stimulate insulin secretion by β-cell and improve adipose tissue metabolism [27-28].

The effects of *Areca catechu* and *Ficus deltoidea* on sperm parameters and plasma testosterone levels in alloxan-induced diabetic rats:

The results of sperm parameters and plasma testosterone levels in each group are shown in Table 3.The present study showed that sperm quality and testosterone secretion were affected significantly by diabetes mellitus. Sperm count, sperm motility and the percentage of normal sperm decreased in diabetic rats (Group B) by 55.96, 41.86 and 63.3%, respectively. Meanwhile, blood plasma testosterone decreased by 54.97 % in diabetic rats (Group B) compared to healthy rats (Group A). Animal studies have proved that diabetes mellitus induce a significant decrease in male fertility and sexual functions [29-30]. This impairment occurred due to hormonal changes, neuropathy and increased oxidative stress [31].

A.catechu and *F. deltoidea* appears to have improved and provided positive impact on male fertility. In diabetic rats treated with *A. catechu* (Group D), sperm count, sperm motility and the percentage of normal sperm increased by 48.35, 30.22 and 52.19 %, respectively. Blood insulin also increased by 46.27% in diabetic rats treated with *A. catechu* (Group D) compared to negative control rats (Group B). Meanwhile, in diabetic rats treated with *F. deltoidea* (Group E), sperm count, sperm motility, the percentage of normal sperm and testosterone level increased by 53.94, 36.04, 58.68 and 48.53%, respectively, compared to negative control (Group B). This positive effect may be due to the ability of *A. catechu* and *F. deltoidea* in reducing oxidative stress and lipid peroxidation, and restore the normal levels of pituitary-gonadal hormones. Previously, Lee, *et al*. reported that *A. catechu* methanolic extract showed strong scavenging activity against super-oxide anion radical [32]. In contrast to what was reported by Yuan, *et al*., there was no negative impact on sperm motility in diabetic rats treated with *A. catechu* methanolic extract [33]. This discrepancy may be due to different extract used in both experiments. The current study utilised *A. catechu* crude extract, meanwhile Yuan, *et al*., used isolated compound (alkaloids) from *A. catechu*. In addition, Aris, *et al*., documented that *F. deltoidea* fruits is a good source of antioxidant and antioxidant play a key role in improving glycemic and fertility of diabetic male (animal and human) [34].

CONCLUSION:

As a conclusion, *A. catechu* and *F. deltoidea* methanolic extracts demonstrated capabilities in reducing blood glucose and restoring fertility in diabetic rats, and that are consistent with previously published data. Only sperm motility of rats treated with *A. catechu* reported differently in other studies, which could be due to different extracts or models, different age of animals or time dependent alterations.

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Table 1. Fasting blood glucose levels of alloxan-induced diabetic rats treated with *Areca catechu* and *Ficus deltoidea* methanolic extracts.

Groups	Initial	Week 1	Week 2	Week 3	Week 4
A	4.57±0.13*	4.38±0.11*	4.63±0.27*	4.58±0.25*	4.41±0.18*
B	18.37±3.69	23.74±2.83	27.17±3.74	25.73±3.91	27.16±2.67
C	17.43±2.75	10.59±1.12*	6.48±1.75*	3.68±0.25*	3.13±0.49*
D	18.18±2.43	20.42±3.97	10.16±1.85*	9.88±1.75*	6.27±1.18*
E	17.74±1.47	18.24±4.75	7.23±1.24*	6.08±2.82*	4.73±1.93*

Values (mmol/l) are presented as mean ± standard error of means (n=6). *Statistically significant at p<0.05 V/s. diabetic control (Group B). A: healthy rats + dH2O, B: diabetic + dH2O, C: diabetic + glibenclamide, D: diabetic + *Areca catechu*, E: diabetic + *Ficus deltoidea*.

Table 2. Plasma insulin levels of alloxan-induced diabetic rats treated with *Areca catechu* and *Ficus deltoidea* methanolic extracts.

Groups	Insulin (U/L)
A (healthy rats + dH_2O)	12.15±0.38*
B (diabetic rats + dH_2O)	3.48±0.12
C (diabetic rats + glibenclamide)	10.87±0.29*
D (diabetic rats + *Areca catechu*)	7.39±0.15*
E (diabetic rats + *Ficus deltoidea*)	9.11±0.17*

Values are presented as mean ± standard error of means (n=6). *Statistically significant at p<0.05 V/s. diabetic control (Group B).

Table 3. Reproductive parameters of alloxan-induced diabetic rats treated with *Areca catechu* and *Ficus deltoidea* methanolic extracts.

Parameters	A	B	C	D	E
Sperm count (10^6 sperm/ml)	48.46±1.92*	21.34±2.23	39.17±3.33*	41.32±2.92*	45.72±1.17*
Sperm motility (%)	83.47±3.91*	48.53±4.49	63.38±2.16*	69.55±3.62*	75.88±1.11*
Normal sperm (%)	95.84±7.93*	35.17±8.14	59.23±4.33*	73.57±3.83*	85.12±5.97*
Blood testosterone (ng/ml)	8.15±0.18*	3.67±0.25	5.11±0.37*	6.83±0.16*	7.13±0.49*

Values are presented as mean ± standard error of means (n=6). *Statistically significant at p<0.05 V/s. diabetic control (group B). A: healthy rats + dH_2O, B: diabetic + dH_2O, C: diabetic + glibenclamide, D: diabetic + *Areca catechu*, E: diabetic + *Ficus deltoidea*.

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