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Evaluating the Effects of Young *Areca catechu* Seed Extract on FSH and Testosterone Levels in Male Rats: Insights into Natural Anti-Fertility Agents

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Abstract

Male participation in contraception remains limited worldwide because few safe and acceptable male contraceptive options exist. Natural products are being explored as potential regulators of male reproductive hormones. *Areca catechu* (areca nut), traditionally used in Southeast Asia, contains phytochemicals that may influence reproductive function. This study evaluated the effects of the ethanolic extract of young *A. catechu* seeds on serum follicle-stimulating hormone (FSH) and testosterone levels in male Wistar rats. Twenty-five rats were randomly assigned to five groups (0, 20, 40, 60, and 80 mg/kg body weight) and treated daily for 48 days. Serum FSH and testosterone were measured by ELISA and analyzed by one-way ANOVA with Duncan's post-hoc test. Mean FSH and testosterone values varied across doses, with the 40 mg/kg group showing the highest means (10.001 ± 10.413 ng/mL and 2.196 ± 1.254 ng/mL, respectively), but no statistically significant differences were detected for either hormone (FSH $p = 0.043$; testosterone $p = 0.425$). The results provide preliminary, hypothesis-generating evidence that the extract may influence the hypothalamic-pituitary-gonadal axis; however, any role in male fertility regulation remains unproven. Larger studies including sperm-quality assessment, mechanistic analyses, and toxicological evaluation are required before considering potential applications in male reproductive health.



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1. Introduction

Globally, male participation in family planning remains limited largely because effective, reversible male methods remain scarce [1]. Modern hormonal approaches work by suppressing luteinising hormone (LH) and follicle-stimulating hormone (FSH) to lower intratesticular testosterone and halt spermatogenesis,

yet no formulation has achieved broad clinical use [2]. This unmet need has spurred interest in new, culturally acceptable strategies, including plant-derived agents that could modulate male reproductive pathways through hormonal or non-hormonal mechanisms. In light of these challenges, there is growing scientific interest in developing alternative, plant-based male contraceptives that are reversible, non-invasive, and culturally

acceptable [3]. Herbal medicines, long used in traditional health systems, offer promising leads in this area due to their bioactive compounds and hormonal effects [4, 5]. The search for male contraceptive agents has led to increasing interest in plant-derived bioactive compounds with potential anti-fertility effects. Several medicinal plants have demonstrated efficacy in modulating fertility parameters in preclinical studies [6, 7].

Among these, *Areca catechu* (areca or betel nut) is noteworthy for its major alkaloid arecoline and abundant polyphenols/tannins, classes with documented effects on testicular and endocrine function [8]. Preclinical studies have shown dose-dependent antifertility signals: ethanolic seed extracts at ~300–600 mg/kg in male rats reduced reproductive organ weights, altered sperm parameters and impaired fertility; other work at ~50 mg/kg confirmed germ-cell apoptosis with a Bax/Bcl-2 shift, whereas some lower-dose studies (~150 mg/kg) paradoxically enhanced sexual activity [9, 10]. These findings highlight that the reproductive effects of *A. catechu* are highly context-dependent, varying with dose, extract type, and exposure duration. Because the phytochemical profile of *A. catechu* changes with fruit maturity, several comparative studies report higher total phenolics and stronger antioxidant capacity in immature nuts. In contrast, others find genotype-specific increases during ripening. Young seeds represent a distinct chemical stage that warrants investigation for its reproductive effects [9, 11].

The reproductive function in males is tightly regulated by the hypothalamic-pituitary-gonadal (HPG) axis. FSH stimulates Sertoli cells and supports spermatogenesis, whereas testosterone, secreted by Leydig cells, is essential for sperm production, libido, and secondary sexual characteristics [12]. Arecoline can directly stimulate Leydig cell steroidogenesis through L-type Ca^{2+} channels and by up-regulating the steroidogenic acute regulatory protein (StAR) and 17β -hydroxysteroid dehydrogenase [8]. At the same time, polyphenols and tannins can modulate testicular redox status and influence cAMP-linked steroidogenic signalling. These mechanisms justify measuring serum FSH and testosterone as sentinel endocrine endpoints [12].

Therefore, this study was designed to examine the effect of ethanolic extract from young *A. catechu* seeds on serum FSH and testosterone levels in male rats as an initial step toward understanding its possible influence on the hypothalamic-pituitary-gonadal axis. Because a temporary rise in circulating testosterone does not necessarily reflect enhanced fertility, we focus here on early endocrine responses, while recognising that a full assessment of contraceptive potential will require

additional investigations such as LH measurement, semen analysis, and testicular histology in future work.

2. Materials and Methods

2.1. Instruments and Materials

The administration of ethanolic seed extract of young *A. catechu* (areca nut) was conducted at Universitas Syiah Kuala from February to April 2024. The evaluation of follicle-stimulating hormone (FSH) and testosterone concentrations was carried out at the Physiology Laboratory, Faculty of Veterinary Medicine, Universitas Syiah Kuala. The equipment used for animal care and extract administration included standard rat feed, oral gavage needles, measuring cylinders, and ethanolic extract of young *A. catechu* seeds. The FSH and testosterone ELISA kits included microtiter wells, standard solutions (0–5 and 0–6), enzyme conjugates, substrate solutions, stop solutions, and wash buffers.

2.2. Extraction Process

Young *A. catechu* seeds were botanically authenticated at the Universitas Syiah Kuala, air-dried at 40°C, and ground to a fine powder. The powder was macerated in 96% ethanol (1:10 w/v) for 72 h with intermittent stirring. Filtrates were pooled and concentrated under reduced pressure at 40°C to yield a viscous extract. Preliminary phytochemical screening (Harborne method) confirmed the presence of alkaloids, tannins, and polyphenols [13].

2.3. Experimental Design

Twenty-five male Wistar rats were obtained from the Faculty of Veterinary Medicine, Universitas Syiah Kuala (Banda Aceh), and acclimatized in laboratory cages for two weeks. The animals were then randomly assigned to five groups of five rats each: Group 1 (K0), Group 2 (KP1), Group 3 (KP2), Group 4 (KP3), and Group 5 (KP4). All five groups received treatment with ethanolic extract of young *Areca catechu* (areca nut) seeds for 48 consecutive days and were sacrificed on day 49 for measurement of follicle-stimulating hormone (FSH) and testosterone concentrations. The 48-day treatment period was chosen to encompass approximately four complete cycles of the seminiferous epithelium in rats, each lasting about 12–13 days. Four cycles (~48–52 days) are routinely used in reproductive-toxicology studies to ensure that effects on spermatogenesis can be detected [14].

2.4. Blood Sampling Procedure

Following humane euthanasia by cervical dislocation, blood samples were collected via cardiac puncture. The blood was stored in Eppendorf tubes preloaded with EDTA (ethylene diamine tetraacetic acid) as an

anticoagulant. Samples were centrifuged at 5000 RPM for 5 minutes. The resulting plasma was separated using a 1 mL syringe, transferred to a new Eppendorf tube, and stored at -20°C for further analysis.

2.5. Measurement of FSH and Testosterone Concentrations

Serum follicle-stimulating hormone (FSH) and testosterone levels were measured using the BioEnzy© Rat FSH ELISA kit and the DRG Rat Testosterone ELISA kit, respectively, following the manufacturers' manuals. Briefly, 50 µL of each standard solution was dispensed into the standard wells. In comparison, 40 µL of plasma sample and 10 µL of the appropriate anti-FSH or anti-testosterone antibody were added to the sample wells. Subsequently, 50 µL of streptavidin-HRP reagent was added to both standard and sample wells. The plates were gently mixed, sealed, and incubated for 60 minutes at 37°C, then washed five times with the supplied washing buffer. Next, 50 µL of Substrate A and 50 µL of Substrate B were added to each well, and the mixture was incubated for 10 minutes at 37°C in the dark. The reaction was stopped by adding 50 µL of Stop Solution, producing a colour change from blue to yellow. Optical density was read at 450 nm within 10 minutes using a microplate reader. All samples and standards were assayed in triplicate.

2.6. Ethical Approval

This study received ethical approval from the Animal Research Ethics Committee of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, under reference number: 297/KEPH/IV/2024.

2.7. Data Analysis

Statistical analysis was performed using SPSS version 26. Initial tests included assessments of normality and homogeneity. Differences in FSH and testosterone concentrations between the control and treatment groups were analyzed using a one-way ANOVA, followed by Duncan's multiple-range test for post hoc comparisons.

3. Results and Discussion

Areca catechu, commonly known as areca nut or betel nut, is traditionally consumed in many Asian and Pacific cultures and is known for its diverse pharmacological properties. Although widely studied for its psychoactive and carcinogenic risks, some research has begun to explore its reproductive toxicity and hormonal impacts. Preliminary findings suggest that compounds in *A. catechu* may influence reproductive hormones and spermatogenesis, raising the possibility of its use as a natural male anti-fertility agent [15]. This study

investigated the effects of the ethanolic seed extract of young *A. catechu* on male reproductive hormone levels, specifically follicle-stimulating hormone (FSH) and testosterone, in Wistar rats. The hormone concentrations are summarized in Tables 1 and 2. The mean FSH concentrations (\pm SD) for the control and treatment groups (K0, KP1, KP2, KP3, and KP4) were 1.146 ± 0.496 , 0.320 ± 0.349 , 10.001 ± 10.413 , 1.864 ± 1.721 , and 0.517 ± 0.436 , respectively. Although group KP2 showed a notably elevated FSH level, statistical analysis revealed no significant differences in FSH concentrations between the treatment groups and the control group ($p = 0.043$; $p > 0.05$) (Table 1).

The results indicate a variable response of FSH concentration to extract administration. While groups KP1 and KP4 experienced reductions in FSH relative to the control, groups KP2 and KP3 showed elevated FSH levels, with KP2 demonstrating a substantial spike. However, these changes were not statistically significant. FSH plays a vital role in male reproductive physiology by stimulating Sertoli cells, which support spermatogenesis by secreting growth factors and proteins crucial for sperm cell maturation [12]. The observed fluctuation in FSH levels may reflect the dose-dependent, biphasic response often seen with phytochemicals. The high variability in group KP2 might be attributed to individual hormonal sensitivity or to transient stimulation by bioactive constituents, particularly flavonoids and alkaloids, which are known to influence the hypothalamic-pituitary-gonadal (HPG) axis.

The testosterone concentrations (\pm SD) in groups K0, KP1, KP2, KP3, and KP4 were 0.7375 ± 0.073 , 1.679 ± 1.874 , 2.196 ± 1.254 , 1.172 ± 0.453 , and 1.450 ± 0.720 , respectively. All treatment groups demonstrated increased testosterone levels compared to the control. Nevertheless, statistical analysis showed no significant differences among the groups ($p = 0.425$; $p > 0.05$) (Table 2). Testosterone levels increased in all treatment groups compared with the control group, with the highest concentration observed in the KP2 group (40 mg/kg BW). Although not statistically significant, this pattern suggests that *A. catechu* extract may enhance Leydig cell function and testosterone biosynthesis. This is consistent with earlier findings that *A. catechu* seed extract elevated testosterone levels in rats, likely by stimulating steroidogenic enzymes [16].

Phytochemical screening of the young *A. catechu* extract indicates the presence of several biologically active compounds such as alkaloids, terpenoids, flavonoids, phenolics, and tannins, which have been widely associated with modulation of the endocrine system

Table 1. FSH concentration in male wistar rats after administration ethanolic seed extract of young *A. catechu*.

No	Group	FSH Concentration (Mean ± SD)	p-value
1	K0 (Control)	1.146 ± 0.496 ^a	0.043
2	KP1 (20 mg/kg BW)	0.320 ± 0.349 ^a	
3	KP2 (40 mg/kg BW)	10.001 ± 10.413 ^b	
4	KP3 (60 mg/kg BW)	1.864 ± 1.721 ^a	
5	KP4 (80 mg/kg BW)	0.517 ± 0.436 ^a	

Note: Different superscripts in the same column indicate significant differences ($p < 0.05$).

Table 2. Testosterone concentration in male wistar rats after administration of ethanolic seed extract of young *A. catechu*.

No	Group	Testosterone Concentration (Mean ± SD)	p-value
1	K0 (Control)	0.7375 ± 0.073 ^a	0.425
2	KP1 (20 mg/kg BW)	1.679 ± 1.874 ^a	
3	KP2 (40 mg/kg BW)	2.196 ± 1.254 ^a	
4	KP3 (60 mg/kg BW)	1.172 ± 0.453 ^a	
5	KP4 (80 mg/kg BW)	1.450 ± 0.720 ^a	

Note: Different superscripts in the same column indicate significant differences ($p < 0.05$).

(glycaemic, lipid, and hormone regulation) [17]. Alkaloids and flavonoids, in particular, are known to exert both estrogenic and androgenic effects depending on dosage and molecular structure. These classes of secondary metabolites are reported to influence the hypothalamic–pituitary–gonadal (HPG) axis through several mechanisms. For example, the major alkaloid arecoline can stimulate Leydig cells by activating L-type Ca^{2+} channels and up-regulating steroidogenic acute regulatory protein (StAR) and 17β -hydroxysteroid dehydrogenase, enzymes that enhance testosterone biosynthesis [8, 9]. Certain flavonoids can bind to estrogen receptor α/β or modulate cAMP/PKA signalling, thereby affecting steroidogenic enzymes such as CYP11A1 and 3β -HSD [18]. In addition, phenolic antioxidants may protect Leydig cells from oxidative stress, indirectly sustaining steroidogenic activity. Flavonoids are plant bioactives that are similar to the hormones 17β -estradiol and testosterone and have oestrogen- and androgen-like effects [19]. They are also known as hormone-like polyphenols. Most of the work confirming that flavonoids bind to nuclear receptors (NRs) and explaining how they work has focused on the estrogen receptor. Moreover, phenolic and terpenoid compounds can act as antioxidants, protecting Leydig cells from oxidative stress and promoting testosterone synthesis. This antioxidant activity may help maintain the structural and functional integrity of testicular tissue, especially under conditions of phytochemical-induced stress.

4. Conclusions

In this study, administration of the ethanolic extract of young *Areca catechu* seeds produced no statistically significant changes in serum follicle-stimulating hormone (FSH) or testosterone levels in male Wistar rats. Although

some groups showed numerical increases, particularly in testosterone, these observations are descriptive only and should be considered preliminary. At present, the data indicate that the extract does not provide evidence of contraceptive activity and any influence on the hypothalamic–pituitary–gonadal axis remains speculative. Further work with larger sample sizes, additional reproductive endpoints (such as sperm quality and fertility outcomes), and detailed toxicological assessment is needed before any conclusions can be drawn about hormonal effects or potential applications. However, comprehensive in vivo, in vitro, and clinical studies are necessary to fully elucidate the efficacy, mechanism of action, and safety profile of young *Areca catechu* seeds.

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