

Administrations of young betelnut fruit extract (*Areca catechu L.*) increased testosterone concentration in old male wistar white rat (*Rattus norvegicus*)

Dessy Indriana Hatta^{1*}, Wimpie Pangkahila^{1,2}, I Gede Made Aman^{1,3}

ABSTRACT

Background: With increasing age, testosterone level is tend to and cause sexual dysfunction disorders such as erectile dysfunction, sexual arousal disorders, ejaculatory disorders, orgasm disorders to infertility. Young betel nut extract (*Areca catechu L.*) is an herbal plant that can increase sexual arousal (libido) which has many nutrients including antioxidants. The purpose of this study was to prove that administration of young areca fruit extract could increase testosterone levels in old male wistar white rats (*Rattus norvegicus*).

Method: This study was an experimental study using a pretest-posttest control group design that used 16 wistar male rats (*Rattus norvegicus*) aged 18 months old, weighing 225-250

grams divided into 2 (two) groups, namely the control group (Placebo) and the treatment group who were given young betel nut extract 100 mg / kgBB / day (P1). The study was conducted for 28 days followed by blood sampling for testosterone levels.

Results: The results showed significant increase in testosterone levels in treatment group compared to the control group, ($p < 0.05$). The testosterone level increased from 14.813 ± 1.343 ng/ml before treatment to 19.602 ± 1.345 ng/ml after treatment.

Conclusion: Based on the results of the analysis it can be concluded that the administration of *Areca catechu L.* extract can increase Testosterone levels in old male wistar white rats (*Rattus norvegicus*).

Keywords: Testosterone, Sexual Dysfunction, Young betelnut extract

¹Master Program in Biomedical Sciences Study Program Specificity of Anti-Aging Postgraduate Program, Universitas Udayana

²Department of Andrology and Sexology, Faculty of Medicine, Universitas Udayana

³Department of Pharmacology, Faculty of Medicine, Universitas Udayana

*Corresponding Author:
Dessy Indriana Hatta;
Master Program in Biomedical Sciences Specificity of Anti-Aging; Postgraduate Program Udayana University Denpasar;
drcanthique@gmail.com

INTRODUCTION

Sexual drive in men and women is influenced by four factors namely testosterone level, psychological factors, health condition, and previous sexual experience. Decreased testosterone levels in men occur at around 30 years of age. Decreased testosterone levels in men due to increasing age are caused by functional disorders and often associated with reduced numbers of Leydig cells, impaired feedback mechanisms in the hypothalamic-pituitary-testicular process, and reduced bioavailability of sex steroid hormones. The number of Leydig cells decreases with age. At the age of 20, the number of Leydig cells was 700 million. Furthermore, it is reduced by 6-7 million every year during the aging process¹.

Indonesia has a lot of biodiversity in the forest, sea and other natural products, such as plants that can be processed into medicines. Many plant species can be used as alternative treatments to overcome reproductive problems. Herbal medicine uses natural products and considered relatively low risk. However, proper study is needed to assess the validity of the effect of such plant extracts to be used widely.

Betelnut (*Areca catechu L.*) is a plant that has an aphrodisiac effect that can increase sexual arousal which is closely related to libido. The effect of stimulants that affect sexual activity in male rats has been studied and the results showed an increase in sexual activity (mating behavior test), and also enlargement of the size of sex organs after treated by with areca nut extract for 28 days². The extract also found to enhance endocrine effect of testosterone by increasing the expression of *steroidogenic acute regulatory* (StAR) and *L-type calcium channels* that enhance the activity of *17-hydroxysteroid dehydrogenase*³.

Phytochemical results of young areca extract from previous studies obtained alkaloids, saponins (steroidal), phytosterols and tannins using TLC (Thin Layer Chromatography)². On January 30, 2018 phytochemical tests of young areca fruit extracts were analyzed from the extract of young betel nut (*Areca catechu L.*) in a laboratory at Udayana University Bali-Indonesia and showed that young betelnut extract contain following substances : Flavonoid 1324.60 mg / 100 gr OE (Quarctetin Equivalent), Phenol 7645.82 mg / 100

gr GAE (Gallic Acid Equivalent), Tanin 1391847.83 mg / 100gr TAE (Tannic Acid Equivalent), Antioxidant Capacity 153691.5 mg / L / GAEAC (Gallic Acid Equivalent) Antioxidant Capacity), and Vitamin C 31428, 57 mg / 100 gr. Meanwhile, fitotestosterone, testosterone, and growth hormone were detected. LC-MS and GC-MS analysis showed that the extract also contain arecoline 7.98%, arecaidine 1.87%, guvacoline 1.46%, catechin 1.03% and leucocyanidin 1.43%. Other compounds presented in the extract including alkaloids (+), Flavonoids (+), Triterpenoids (+), Saponins (+), and Phenolates (tannins) (+). Therefore, this study was conducted to prove whether the administration of young betelnut fruit extract can increase testosterone levels.

METHODS

This research is experimental research by using a pretest-posttest control group design that uses

16 wistar male rats (*Rattus norvegicus*) aged 18 months old, weighing 225-250 grams divided into 2 (two) groups, each of which is 8, one group as a control group who were given aquedest placebo (P0), and one treatment group who were given young betel nut extract 100 mg / kgBB / day (P1). The study was conducted for 28 days where for 7 days adaptation was then followed by 21 days of treatment, after which a blood sample was taken to test testosterone levels.

RESULTS

The results showed that young betelnut extract was potent enough to significantly increase the testosterone levels compared to the control group p-value = 0.961 (0.961 < 0.05). Table 1 shows the average testosterone level of the control group (P0) after being given treatment for 21 days (posttest) was 14.81288 ng / ml, while in the treatment group (P1), it was 19.60225 ng / ml. Analysis with independent sample T-test showed t-value = -7.128 and p-value = 0.000. This means that there is a significant difference in average testosterone levels between the treatment group and the control group.

DISCUSSION

Comparative tests or comparisons between the control group with only aquabidest (placebo) and the treatment group treated by young betelnut extract 100 mg / KgBB / day were analyzed using the Independent T-Test. Based on the results of the analysis, the mean of the control group was 14.81288 ng / ml and the mean treatment group that was 19.60225 ng / ml. Then an analysis to compare using the T-Independent Test shows t value = -7.128 and p-value = 0.000. This means that the difference in average testosterone level between the control group (P0) and the treatment group was significantly different (p < 0.05). It can be said that the young areca extract of the treatment group in old male wistar rats can significantly increase testosterone levels compared to the control group given a placebo. Phytochemical test also found that the extract contain alkaloids (+), Flavonoids (+), Triterpenoids (+), Saponins (+), Phenolics (tannins) (+). Flavonoids, alkaloids, and catechin from areca fruit has been known as natural antioxidants that can protect leydig cells from damage caused by free radicals and help regenerate leydig cells. Catechin is a polyphenol compound that enhance the function of the superoxide dismutase (SOD) enzyme that act as internal antioxidant. High levels of antioxidants can be used to neutralize free radicals which are one of the causes of disease.

Table 1. The t-test result of mean testosterone level comparison between control and treatment group

Sample Group	n	Mean Tes	t	P-value
Control (P0)	8	14.81288	-7.128	0.000
Treatment (P1)	8	19.60225		

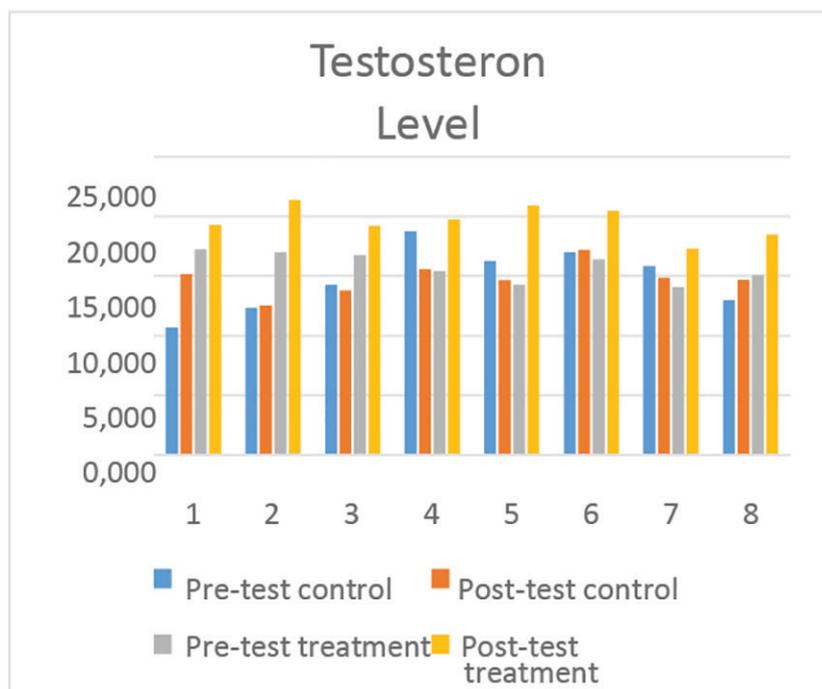


Figure 1. The change in testosterone level in both group prior and after treatment

Antioxidants are substances that can slow down the oxidation process. Flavonoids are one of the phenolic compounds which have potential as antioxidants and have bioactivity as a drug and as an antidote to free radicals because they can transfer hydrogen atoms to free radical compounds by stopping the initial stages of the reaction⁴. Alkaloids are produced by various organisms including bacteria, fungi, and plants as secondary metabolites which show beneficial bioactive effects. Alkaloids work to inhibit replication, transcription of viral RNA and viral polymerase activity. Piperin is an alkaloid group that functions to increase libido, spermatogenesis, increase sperm maturation, increase excretion of fructose by seminal vesica as the main nutrient of spermatozoa⁵.

Tanin is a phenolic group compound that has anti-proliferation and apoptosis properties. Phenolic compounds directly have an anti-proliferative effect because they directly interact with aryl hydrocarbon receptors and inhibiting the enzyme nitric oxide synthase (NOS). Inhibition of NOS induces apoptosis via the p53 pathway. p53 is a protein that regulates cell cycles and apoptosis⁶. High levels of antioxidants can be used to bind free radicals which are one of the causes of disease. Oxidation is a type of chemical reaction that involves increasing oxygen, releasing hydrogen, or releasing electrons. The oxidation process is a natural event that occurs in nature and can occur everywhere including our body⁴.

Vitamin C in betel nut also considered as good antioxidant. Vitamin C can directly capture oxygen free radicals, either with or without an enzyme catalyst. Its reaction with reactive oxygen compounds is faster than other components⁴. This vitamin deficiency in the testis could increase oxidative stress and cause testicular cell damage as well as decreased testosterone production. Studies have shown that vitamin C works in the hypothalamic-pituitary-testicular axis by increasing testosterone levels significantly⁷.

According to Dasgupta et al. (2010) Arecoline is the most commonly found alkaloid from betelnut⁸. The effect of arecoline on the pineal-testicular axis in mice between day and night is different and the study was carried out in both photophase and scotophase. Arecoline inhibits the pineal activity, but stimulates testicular function which can increase testosterone and its target organs perhaps through cholinergic muscarinic receptors. Arecoline also induces the formation of early stages of rat spermatids while stimulation of leydig cells

causes an increase in testosterone synthesis in vivo in mice by regulating the hormone testosterone. The mechanism of action of arecoline to increase testosterone production is likely by stimulation of a non-c-AMP dependent steroidogenesis pathway that is found within leydig interstitial cells. Direct arecoline activity can also stimulate testicular leydig cells through L-type calcium channels by increasing the activity of 17-hydroxy-steroid dehydrogenase (HSD).

The steroid hormone biosynthesis in the testis begins with the transfer of cholesterol from the outside into the mitochondrial membrane that is facilitated by StAR. The expression of StAR protein is enhanced by arecoline, although the production of this protein is not responsible for the immediate increase in testosterone release from Leydig cells. Until now, the conversion of cholesterol to pregnolone was catalyzed by the P450 enzyme located in the mitochondrial membrane. The results of this study report that administration of arecoline can increase pregnenolone production⁷.

Toxicity is an important aspect of plant pharmacology and drugs that must have safety standards before being used as medicines. Safety studies on experimental animals were carried out through toxicity tests. Areca toxicity studies showed that the LD50 found in doses >15,000 mg / kg can be used as a drug formulation⁹. In another study about the toxicity test of areca nut ethanol extract showed that the dose can be increased to 3000 mg / kb without any sign of toxicity¹⁰.

CONCLUSION

According to the results of this study, it can be concluded that the administration of young betelnut (*Areca cathecu L.*) at a dose of 100 mg / KgBB / day for 21 days can increase testosterone levels in old male wistar white rats (*Rattus norvegicus*).

REFERENCES

1. Pangkahila, W. *Anti Aging Tetap Muda dan Sehat*. Jakarta: PT. Kompas Media Nusantara: 13:20-21,33-34,27-29,54-55, 77-78,58,63-64,74-75. 2017.
2. Anthikat, R. R. N., Micheal, A, and Ignacimuthu, S. Aphrodisiac effect of *Areca catechu L.* and *Pedalium murex* in rats, *Journal of Men's Health* 2013; 10(2), pp. 65– 70. doi: 10.1016/j.jomh.2012.07.003.
3. Saha, I, and Chatterji, U. *Ultrastructural and Hormonal Changes in the Pineal – Testicular Axis Following Arecoline Administration in Rats*.

2017. 198(February):187–198. doi: 10.1002/jez.a.
4. Lim, T. K. Edible Medicinal and non- Medicinal Plants: Volume 4, Fruits, Edible Medicinal and Non-Medicinal Plants. *Fruits* 2012;1(4): 1–1022. doi: 10.1007/978-94-007-4053-2.
 5. Peng, W., Liu, W., Wu, J.Y., Sun, N., He, T., Gao, Y.X., Wu, Y.X., and Ji, C. *Areca catechu* L. (Arecaceae): A Review of its Traditional Uses, Botany, Phytochemistry, Pharmacology and Toxicology. *Journal of Ethnopharmacology* 2015; 164:340–356. doi: 10.1016/j.jep.2015.02.010.
 6. Duval A and Avérous L. Characterization and Physicochemical Properties of Condensed Tannins from *Acacia catechu*. *Journal of Agricultural and Food Chemistry* 2016; 64(8):1751–1760. doi: 10.1021/acs.jafc.5b05671.
 7. Akmal M, Mahdi C, dan Am A. Peningkatan Konsentrasi Testosteron pada Tikus Akibat Paparan Ekstrak Air Biji Pinang. *Jurnal Veteriner* 2010; 11(4): 244-250.
 8. Dasgupta R, Chatterji R, Nag U.T.C., Sengupta C., Nag S, and Maiti D.B. Ultrastructural and Hormonal Modulations of the Thyroid Gland Following Arecoline Treatment in Albino Mice, *Molecular and Cellular Endocrinology* 2010;319(1–2): 1–7. doi: 10.1016/j.mce.2010.01.005.
 9. Sari LM, Suyatna FD, Utami S, Chairul C, Subita GP, Whulandhary YS and Auerkauri EI. Acute Oral Toxicity Study of *Areca Catechu* Linn Aqueous Extract in Sprague -Dawley Rats. *Molecular and Cellular Endocrinology* 2014;7(5):2013–2015.
 10. Kafle SS, Shenoy T, Amuthan S, Prabhu A, Mohan K, Sheethal S, Shrestha S, and Jyoti. Antifertility effect of *Areca catechu* in male albino rats. *International Journal of Pharmaceutical Sciences Review and Research* 2011;10(1):79–82.