The Effect of Administration of *Avicennia Marina* Bark Extract on the Blood Testosterone Content of *Mus Musculus* Mice Jakarta Albino Strain

Prof. Dr. Nana Suhana, Drs*; Evi Setiadi, dr**; Cut Fauziah, Dra***

Abstrak

Avicennia marina, semacam tanaman bakau, di daerah Jawa dikenal dengan nama "api-api". Getah kayu tumbuhan ini di daerah Jawa sering digunakan sebagai obat antifertilitas tradisional. Penelitian terdahulu menunjukkan bahwa ekstrak kulit batang Avicennia marina mempunyai efek antifertilitas pada Mus musculus betina. Pada penelitian ini diselidiki efek pemberian ekstrak kulit batang Avicennia marina terhadap kadar testosteron darah mencit jantan strain albino Jakarta. Dasar pemikiran untuk penelitian ini ialah bahwa testosteron mempunyai peran penting pada spermato-genesis dan maturasi spermatosit di epididimis. Penelitian ini menggunakan rancangan acak kelompok dengan 5 kelompok perlakuan, yaitu kelompok kontrol tanpa perlakuan, kelompok kontrol dengan pemberian pelarut tween-80 dan kelompok-kelompok dengan pemberian ekstrak berturut-turut 0,3 g, 0,6 g dan 1,2 g/kg berat badan.Penentuan testosteron darah dilakukan dengan metoda Radioimmunoassay (RIA) menggunakan kit RIA Coat-a-count Diagnostic Products Corporation U.S.A. Hasil penelitian menunjukkan bahwa pemberian ekstrak kulit batang Avicennia marina dengan dosis 0,6 dan 1,2 g/kg berat badan dapat menurunkan kadar testosteron darah. Khasiat antifertilitas ekstrak kulit batang Avicennia marina mungkin disebabkan karena efek penurunan testosteron terhadap spermatogenesis dan maturasi spermatosit.

Abstract

Avicennia marina is a mangrove species, known by the name "api- api" in Java. The tree sap is often used in Java as a traditional anti-fertility drug. Earlier research has indicated that the extract of the Avicenna marina bark has an anti-fertility effect in the female mus musculus. We have studied the effect of the administration of Avicennia marina bark extract on the blood testosterone level of male mice of the Jakarta albino strain. The basic consideration for this reasearch was, that testosterone plays an important role in spermatogenesis and in the maturation of spermatocytes in the epididymis. A randomized design was used with five groups, i.e. a control group without any treatment, a control group given the tween-80 solvent and 3 groups given 0.3 grams, 0.6 grams and 1.2 grams of the extract per kilogram body weight respectively. Testosterone determination was performed by Radioimmunoassay (RIA), using the Diagnostic Products Corporation, U.S.A. Coat-a-count RIA Kit. The results of the study showed that daily administration of Avicennia marina bark extract with doses of 0.6 grams and 1.2 grams per kilogram body weight during 2 spermatogenic cycles did reduce the blood testosterone level. The anti-fertility effect of the Avicennia marina bark extract is probably due to the effect of reduced testosterone levels on spermatogenesis and maturation of spermatocytes.

***Keywords** : Avicennia marina, blood testosterone.*

INTRODUCTION

To control population growth, several family planning methods have been developed, mainly directed to women, while less attention have been given to male contraception. Methods of male contraception, commonly practiced at the present are vasectomy, coitus interruptus and the use of condoms.

Developing male contraceptive methods is indeed far more difficult than female contraception, as production of sperms is continuous, while ovulation only occurs once in a month. Steroid hormones used in contraceptive pills for women are not suitable for male contraception, because of the side-effects. Moreover azoospermia and oligospermia will only result after several months use of contraception. On the other hand

* *Department of Biology, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia*

** *Department of Biochemistry, Faculty of Medicine, University of Indonesia, Jakarta, Indonesia.*

*** *Faculty of Biology, National University, Jakarta, Indonesia*

spermatogenesis is restored only after 3 months to several years of discontinuation of contraception.[2]

Recently the possibilities of the use of natural or synthetic chemical compounds as male oral contraceptives are being investigated.[3]

Avicennia marina is of the genus *Verbenaceae*, in Java known by the name "Api-Api". It is a mangrove plant found in abundance in the Indonesian archipelago. The sap of this tree is often used in Java as a traditional anti-fertility drug for women.[4]

Earlier research by Gde Nyoman Astika has indicated that the *Avicennia marina* extract has an anti-fertility effect in female Mus musculus mice. The *Avicennia marina* plant contains active lupeol, a sapogenic triterpene. This chemical compound causes failure of maturation of the ovarian cells in treated female mice, causing interruption of the first and second division of meiosis. Lupeol is also known to depress the central nervous system and to cause irregularity of the estrus cycle and failure to produce pregnancy.[5]

It would be interesting to find out, whether the *Avicennia marina* bark extract also affects testosterone secretion in male mice. Several studies have indicated that reduced testosterone not only suppresses spermatogenesis in the seminiferous tubules, but also interferes with the maturation of spermatocytes in the epididymis. The testosterone level necessary for the maturation of spermatocytes in the epididymis is higher than that for spermatogenesis.[6]

The aim of this work was to study the effect of *Avicennia marina* bark extract on the blood testosterone level of male mice.

MATERIAL AND METHODS

The *Avicennia marina* bark was obtained from Tanjung Pasir Tangerang. The bark was weighed, aired, and then dried at 40°C for circa 24 hours or until the bark became brittle and easy to grind. The substance was then ground in a blender to a fine powder. The powder was poured in an Erlenmeyer flask and chloroform was added until the powder was totally submerged. The flask was then sealed and rotated, to get the powder evenly submerged. The solution was then sieved using filter paper. To separate the extract from the chloroform a rotavapor R 110 was used at 59°C. The thick extract was poured out of the rotavapor flask, weighed and then dissolved in 20% tween-80. Working solutions were prepared, containing the required amounts of the extract for the different groups of experimental animals, in 0.1 ml of sulution. The required amounts for 3 experimental groups were 0.3 grams, 0.6 grams and 1.2 grams of extract per kg of body weight respectively.

Twenty five male mice of the Jakarta albino strain, were divided into five groups, each consisting of five mice. Group A was a control group not given any treatment, group B a second control group, treated with the 20% tween-80 solvent. Groups C, D, and E, were given respectively 0.3 grams, 0.6 grams and 1.2 grams of extract per kilogram body weight. Prior to the experiment the male mice were mated with fertile female mice to test the fertility of the male mice. When the female mice showed symptoms of pregnancy, the male mice were separated from the females, and the treatment was started. The extract was administered daily through forced feeding, for the period of two spermatogenic cycles. The mice were then killed and blood was taken directly from the heart and the serum stored at -20°C until the time of analysis.

Determination of the serum testosterone was done by radioimmunoassay (RIA) using the Coat-a-Count Diagnostic Product Corporation USA Kit. Counting was done in the Gamma Chem 9612 Serono Gamma-Counter.

The data obtained were then tested on normality and homogeneity and if the distribution was found to be normal and homogeneous, the data were analysed by parametric statistics. If the data obtained proved not to be normal or homogeneous, the Kruskal- Wallis test was applied.

RESULTS AND DISCUSSION

The blood testosterone concentration of the mice following treatment are shown in Table 1.

Table 1. Blood testosterone concentration (ng/ml) in the control and experimental mice, following treatment with *Avicennia marina* bark extract during 2 spermatogenic cycles.

Repetition	Blood testosterone (ng/ml)					Total	Average
	A	B	C	D	E		
1.	0.89	2.35	0.49	2.12	0.47	6.34	1.26
2.	3.63	1.56	3.57	0.97	0.67	10.4	2.08
3.	3.50	3.74	0.97	2.24	1.75	12.2	2.44
4.	0.58	2.96	2.47	0.58	0.63	7.22	1.44
5.	2.81	1.85	2.06	0.37	0.63	7.70	1.54
Total	11.41	12.44	9.56	6.28	4.15	43.84	8.76
Average	2.28	2.49	1.91	1.26	0.83		
SD	2.94	2.05	2.19	0.95	0.41		

NOTE:
Group A = control group without any treatment
B = control group, given tween-80 solvent
C = treated with 0.3 grams *Avicennia marina* bark extract per kilogram body weight
D = treated with 0.6 grams *Avicennia marina* bark extract per kilogram body weight
E = treated with 1.2 grams *Avicennia marina* bark extract per kilogram body weight

The Liliefors normality test and the Bartlett test showed that the blood testosterone concentrations had normal and homogenous distribution, and consequently parametric statistical analysis could be applied. Analysis of varians showed significant difference of the blood testosterone concentrations of Group A, B, C, D, and E. Honest significance test showed significant difference between the blood testosterone levels of Group B (tween-80 solvent administered) and Group D (0.6 gram doses per kilogram body weight administered) and between Group B and Group E (1.2 grams doses per kilogram body weight).

Very significant difference was also found between Group A (Control Group without any treatment) and Group E.

It was therefore concluded that administration of *Avicennia marina* bark extract with doses of 0.6 and 1.2 gram per kilogram body weight could reduce the blood testosterone concentration. A most significant reduction was shown when 1.2 gram extract per kilogram body weight was administered, while 0.3 gram extract per kilogram body weight (group C) did not show a significant reduction of the blood testosterone concentration, possibly because with this small dose the quantity of lupeol is not sufficient to produce any effect. (Fig. 1).

Lupeol, the active substance in the *Avicennia* extract, may affect the hypothalamus-pituitary axis, causing interference in the secretion of GnRH (Gonadotropin Releasing Hormone) and subsequently the secretion of FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone).[5] These two hormones play an important role in spermatogenesis and in the maturation of spermatocytes in the epididymis. FSH stimulates spermatogenesis in the tubuli seminiferi and the secretion of the androgen-binding protein by the Sertoli cells. The androgen-binding protein transports testosterone synthesized in the interstitial Leydig cells under stimulation by LH to the seminiferi tubuli, where the testosterone is required for spermatogenesis. Lupeol may also, interfere directly with the production

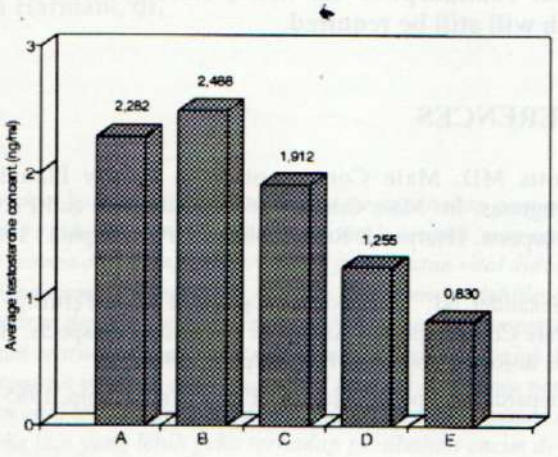

Figure 1. Average blood testosterone concentration in group A-E, after treatment with Avicennia marina bark extract.

Note :
Group A : control without treatment
B : administered tween-80 solvent
C : administered 0.3 g extract/kg body weight
D : administered 0.6 g extract/kg body weight
E : administered 1.2 g extract/kg body weight

of testosterone in the Leydig cells. If testosterone production is impaired, the meiotic cell division can be inhibited.[6] Several investigators have concluded that the maturation of the spermatozoa in the epididymis may be impaired if the testosterone concentration is low.[7] Du-ring maturation, the spermatozoa experience physio-logical and biochemical changes. At low testosterone levels these processes may be impaired and the spermatozoa produced, may not be normal.[8]

CONCLUSIONS

From the results of this study we concluded that the administration of *Avicennia marina* bark extract in doses of 0.6 gram per kilogram body weight to male mice of the Jakarta albino strain will cause a significant decrease in the blood testosterone concentration. A very significant decrease will result from administration of 1.2 gram extract per kilogram body weight, while 0.3 gram per kilogram body weight will not cause any significant decrease of the blood testosterone concentration.

The results of this study justifies the expectation that the *Avicennia marina* bark extract can be used as an oral contraceptive for male, although further research will still be required.

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