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Reversibility Time in Testicular Damage on Male Wistar Rat after Treatment of *Averrhoa blimbi* L. Fruits Extract as Antifertility

Kartiawati Alipin*, Indra A. Rochman, Desak M. Malini, Madihah Madihah

Department of Biology, Faculty of Mathematics and Natural Sciences,
Universitas Padjadjaran, Raya Bandung Sumedang Km 21 Jatinangor, Indonesia

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*Corresponding author: kartiawati@unpad.ac.id

Abstract

Belimbing wuluh (*Averrhoa blimbi*) fruit is commonly used traditionally as antifertility. The bioactive compounds of *A. blimbi* have been known could inhibit spermatogenesis and development of testes, as well as decrease the quality and quantity of spermatozoa, thus, caused seminiferous tubular atrophy. This study aimed to determine the reversibility time in testicular damage after the treatment of *A. blimbi* fruit extract on male Wistar rat. The method employed a completely randomized design (CRD) consists of nine treatments with three replications each. The treatment of the extract at a dose 1140 mg/kg BW and control group was given the solvent. After 14 days of treatment, the reversibility time was examined in testis histological section by interval of seven days each until 49 days. The results showed that the treatment of *A. blimbi* fruit extract caused disruptions in seminiferous tubules, i.e. decreased the number of spermatogonium and spermatid, as well as the diameter of lumen and seminiferous tubules. The reversibility time was observing at 21 days after the end extract treatments, by increasing the number of of spermatogonium and spermatid, as well as the diameter of lumen and seminiferous tubules that significantly different with infertile rat ($p<0.05$). In conclusion, the reversibility time was 21 days after the treatment of *A. blimbi* fruit extract as antifertility.
Key words: *Averrhoa blimbi*, rats, reversible time, tubulus seminiferous

Waktu Reversibilitas Kerusakan Testis pada Tikus Wistar Jantan setelah Pemberian Ekstrak Buah *Averrhoa blimbi* L. Sebagai Antifertilitas

Abstrak

Buah Belimbing wuluh (*Averrhoa blimbi*) secara tradisional telah digunakan sebagai antifertilitas, kandungan senyawa kimia yang terkandung dalam *A. blimbi* diketahui dapat menghambat spermatogenesis, menghambat perkembangan testis, menurunkan kualitas dan kuantitas spermatozoa, menyebabkan artropi tubulus seminiferus. Penelitian ini bertujuan untuk mengetahui waktu reversibilitas pada kerusakan testis tikus Wistar jantan setelah pemberian ekstrak buah *A. blimbi*. Metode penelitian menggunakan Rancangan Acak Lengkap (RAL) dengan 9 perlakuan dan 3 ulangan. Perlakuan ekstrak dengan dosis 1140 mg/kg bb dan perlakuan kontrol hanya diberi pelarut. Hasil penelitian menunjukkan bahwa perlakuan ekstrak buah *A. blimbi* menyebabkan gangguan pada tubulus seminiferus dengan terjadinya penurunan jumlah spermatogonium dan spermatid, demikian juga diameter lumen dan tubulus seminiferus. Waktu reversibilitas terjadi pada hari ke 21 setelah pemberian perlakuan dilihat dengan terdapatnya peningkatan jumlah spermatogonium dan spermatid serta diameter lumen dan tubulus seminiferus yang berbeda nyata secara signifikan dibandingkan dengan tikus infertil ($p<0.05$). Kesimpulan, waktu reversibilitas terjadi pada hari ke 21 setelah perlakuan ekstrak buah *A. blimbi* sebagai antifertilitas.
Kata Kunci: *Averrhoa blimbi*, tikus, tubulus seminiferus, waktu reversibilitas.

1. Introduction

Medicinal plants in Indonesia are very abundant and commonly used traditionally as a material in herbal medicine. One of them is used as a contraceptive drug, bioactive compounds contained in medicinal plants can be used as an antifertility agent. These compounds generally include classes of steroids, alkaloids, oxalic acid, isoflavonoid, triterpenoid and xanthone.[1]

Currently men's involvement in family planning programs is expected. Therefore, contrast medication required for men. The use of medicinal plants for male contraceptives is still being sought because it has to meet several conditions such as can lead to azoospermia, easy to use, not causing side effects and toxic effects, does not interfere with libido or sexual behavior and is reversible.[2]

Reversible is a process that can come back as usual after a significant change.[3] The measurement of spermatogenesis reversibility can be based on the number of spermatozoa[4], spermatogenic cells,[5] and total serum proteins in the testes and epididymis.[6]

Belimbing wuluh (*Averrhoa bilimbi*) is one of the plants whose fruit is used as a medicine to reduce male fertility which contains alkaloids, flavonoids, saponins, steroids and oxalic acids, which are known to inhibit spermatogenesis, inhibit the development of the testes, decrease the quality and quantity of spermatozoa, causing seminiferous tubular artrophy, and pituitary hypertrophy.

Based on several studies on rat, was known that giving 14.4 mL of fruit juice of belimbing wuluh from 20 gram of belimbing wuluh fruit could decrease the percentage of Strong A strain A female birth is 63.3% and 58.3% for Wistar female rats with controls given only drinking water.[2]

Flavonoid compounds could decrease the number of rat spermatozoa (*Rattus norvegicus*).[7] Extract of ethanol belimbing wuluh (*A. bilimbi*) fruit dose 1140 mg / kg bb was the best dose in increasing infertility of male rats.[8] Evidence of reversibility spermatogenesis has been done by Djannah et al. (2000)[9], inhibition of spermatogenesis after plant extract from Saga seed (*Abrus precatorius* L.) was reversible after 27 days of cessation of extract.

According to the results of Nurdiansyah (2013)[8] study, this study was conducted to test whether the ethanol extract of belimbing wuluh (*A. bilimbi*) fruit caused reversibility in spermatogenesis of male Wistar rats. The reversibility time was examined by histological observation of testis.

2. Method

2.1. Extraction of plant materials

Belimbing wuluh (*A. bilimbi*) fruits were air-dried to a constant weight and blended to a coarse powder. The dried powder was soaked and macerated on 95% ethanol for 72 hours and every 24 hours the macerate was collected. The macerate was then evaporated using a rotary evaporator, resulting in a paste extract.

2.2. Experimental animals

Twenty-seven male Wistar rat (160-200 g) were obtained from the animal house of the Laboratory of Biosystematics, Department of Biology, University of Padjadjaran, Indonesia. The rats were weighed and sorted into nine groups of three animals each so that their average weight approximately equal. They were housed in a standardized environmental condition and fed with piglet standard diets (CP-551, PT. Charoen Pokphand) and water which was given *ad libitum*.

Animal care and handling conformed to accepted guidelines. The treatment of the extract at a dose 1140 mg/kg BW and control group was given the solvent. After 14 days of treatment, the reversibility time was examined in testis histological section by interval of seven days each until 49 days (Table 1).

2.3. Parameters observed (Histological observation of testis)

Histological observations of testis were performed under a microscope by measuring the number of spermatogonium and spermatid, diameter of lumen and seminiferous tubules in 10 different fields using 400 times magnification with 1 replication.[10]

Table 1 Experimental design of the treatment

ID	Treatment	Reversible time (days)
Negative control (NC)	Citrate buffer and CMC 0.5%	0
P 1	*A. blimbi* fruit extract	0
P 2	*A. blimbi* fruit extract	7
P 3	*A. blimbi* fruit extract	14
P 4	*A. blimbi* fruit extract	21
P 5	*A. blimbi* fruit extract	28
P 6	*A. blimbi* fruit extract	35
P 7	*A. blimbi* fruit extract	42
P 8	*A. blimbi* fruit extract	49

2.4. Data analysis

Results were expressed as mean ± standard deviation (SD). Statistical significance was analyzed using one-way ANOVA followed by Duncan multiple range test. P values less than 0.05 were considered significant.

3. Result

The histological structure of testis after 14 days treatment of *Averrhoa blimbi* fruit extract showed at Figure 1. Based on the results of observations of testicular histological incisions the average result of the observed parameters is found in Table 2.

4. Discussion

The results of the variance analysis (ANAVA) on spermatogonia cell counts were not significantly different, this showed that the treatment did not affect the number of spermatogonia. The average of seprmatogonia obtained was different from that of Ganiswarna (1995)[11] which states that saponin content in belimbing wuluh (*A.bilimbi*) could suppress the production of testosterone so that spermatogenesis became obstructed. The inhibition of spermatogenesis could be seen from the growth of spermatogenic cells, one of which is spermatogonium cells.

Based on the parameters of the average number of spermatids it was known that in P1 (Table 2) had the lowest spermatid count compared with other treatments, it was suspected the effect of ethanol extract of belimbing wuluh fruit berries against the average number of spermatids while P4 had spermatid amount which is not significantly different with control treatment (C), it is suspected as a result of discontinuing the use

Table 2 Average Calculation of Testis Histological Parameters

NO.	ID	Number of Spermatogonia	Number of Spermatids	Area of Lumen (µm2)	Diameter of Seminiferous tubules (µm)
1	C	49,73 ± 6,64(a)	127,37 ± 7,04 (bc)	1.734 ± 3,77 (a)	190,98 ± 8,67 (c)
2	P1	51 ± 4,03(a)	91,27 ± 6,44 (a)	6.728 ± 7,72 (d)	105,2 ± 47,01 (a)
3	P2	61,77 ± 5,89(a)	103,83 ± 5,59 (ab)	3.383 ± 5,34 (bc)	139,49 ± 3,45 (ab)
4	P3	52,6 ± 4,31(a)	107,90 ± 3,66 (ab)	2.093 ± 4,25 (ab)	133,96 ± 9,94 (ab)
5	P4	46,97 ± 2,8(a)	137,27 ± 5,47 (c)	2.277 ± 8,13 (ab)	184,4 ± 5,78 (c)
6	P5	58,57 ± 7,94(a)	131,77 ± 8,59 (c)	2.502 ± 6,22 (ab)	159,9 ± 9,34 (bc)
7	P6	51,11 ± 4,12(a)	142,91 ± 6,13 (c)	2.321 ± 7,94 (ab)	179,86 ± 4,23 (c)
8	P7	47,66 ± 3,19(a)	135,23 ± 5,4 (c)	1.996 ± 9,41(a)	161,21 ± 5,72 (bc)
9	P8	50,07 ± 4,89(a)	129,37 ± 2,04 (bc)	1.554 ± 3,88(a)	165,5 ± 3,11(bc)

Note: The value is expressed as the mean ± standard deviation (n=3). Data were analyzed using one-way ANOVA followed by Duncan multiple range test. Difference alphabet in the same column showed P values less than 0.05 and considered significant

of extract belimbing wuluh fruit after 21 days then spermatogenesis process back to normal.

The observed spermatid cell was a rounded spermatid which was a cell resulting from secondary spermatocyte division. Early stage spermatids could be distinguished from their small size, nuclei with dense chromatin areas, and are located near the centre of the seminiferous tubule, with the formation of the early spermatids spermatocytogenesis ends. After those spermatids underent a complex differentiation process called spermiogenesis so that it changed spermatid to spermatozoa.[12]

Saponins contained in belimbing wuluh fruit is a type of saponin acid. The similarity of the structure between neutral saponins which is a steroid derivative and an acid saponin having a triterpene structure led to competition of receptor binding between the two. In addition to saponin compounds, other bioactive compounds that also affected the decrease in the average number of spermatid cells was alkaloids. Alkaloid compounds are cytotoxic so that the cytotoxic effect would cause metabolic disorders of spermatogenic cells.[13]

Alkaloids also caused an interruption of meiotic cleavage when spermatogenesis, ie when the primary spermatocyte meiotic division first forms a secondary spermatocytes and when the secondary spermatocytes perform the second meiotic into spermatid.[14]

Based on the average of the lumens it was known that P1 (Table 2) has the largest lumen area compared with other treatments. This was caused by P1 is the ethanol extract treatment of belimbing wuluh fruit was the largest because the observation time on the 0 th day after the extraction or the last day of the extract of ethanol belimbing wuluh fruit. So it is still affected by the effects of bioactive compounds of belimbing wuluh fruit, one of which is oxalic acid compound. Oxalic acid compounds contained in belimbing wuluh fruit might affect the process of spermatogenesis. This compound is known to have the ability as adstringensia was a compound that could cause Leydig cell atrophy.[15]

Based on the parameters of the diameter of the seminiferous tubules, it was known that P1 has the smallest diameter of the seminiferous tubule compared with other

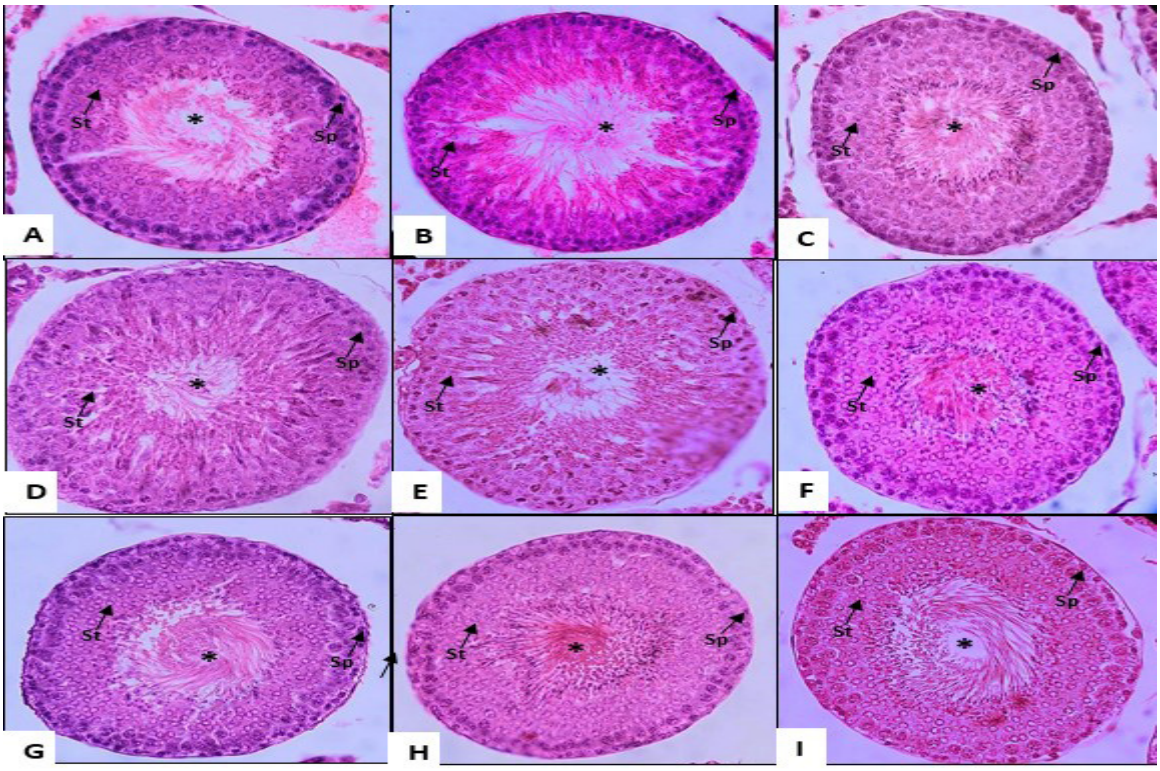

Figure 1 Photomicrograph of seminiferous tubules from rats testis. hematoxylin_eosin stain. M. 100×.
Note: Sp: Spermatogonia cell; Sp: Spermatid cell; *: lumen of seminiferous tubules A=C, B=P1, C=P2, D=P3, E=P4, F=P5, G=P6, H=P7, I=P8

treatments. The decrease in diameter of the seminiferous tubules might be caused by bioactive compounds contained in the extract of ethanol of belimbing wuluh such as saponins that could cause the growth of spermatogenic cells and diameter of seminiferous tubules.[16]

In addition, according to Tjay and Rahardja (2002)[15] oxalic acid was a chemical compound that was also contained in belimbing wuluh fruit and could affect spermatogenesis. This chemical compound had the ability as adstringensia that could wrinkle tissues and cells that were suspected to affect the shrinking seminiferous tubules. While P4 had a diameter of seminiferous tubule was is not significantly different from control treatment (C), was is suspected after termination for 21 days then diameter of seminiferous tubule had the same diameter as control in line with spermatogenesis process which returned to normal.

5. Conclusion

Based on the measurements of the parameters above it can be seen that P4 was the optimal time required for the reversibility of spermatogenesis of male Wistar rat post-administration ethanol extract of belimbing wuluh fruit. Based on the reversible effect caused by post-administration ethanol extract of belimbing wuluh fruit then the extract had fulfilled one of the requirements as a good antifertility agent that was reversible.

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