

The Effect of Neem Leaf (*Azadirachta indica* A. Juss) Extract on Tubules Seminiferous of Rat (*Rattus norvegicus*)

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ABSTRACT

This study aimed to determine the effect of neem leaf extract on the seminiferous tubules of white rats. This study used 15 adult white rats which divided into 5 treatment groups. The control group was only given water and each treatment group was given neem leaf extract with the dose of 50 (P1), 100 (P2), 150 (P3), and 200 (P4) mg/kg BW orally for 30 days. The rats were then euthanized with chloroform and the testicular organs were collected for histopathological preparations using Haematoxylin and Eosin staining method. The data were analyzed descriptively. The results showed that there were no histological changes of seminiferous tubules in control group as well as in P1 group. Spermatogenic cells, Sertoli cells, lamina basalis, and interstitial connective tissue started to lyse and thin out were observed in P2. In P3 group, the Sertoli cells, basal laminae, and interstitial connective tissue showed partial lysis. Meanwhile, in P4 showed lysis of the interstitial connective tissue in each tubule. This study showed that seminiferous tubule damage is dose-dependent with the administration of neem leaf extract. In conclusion, neem leaf extract could affect the spermatogenesis process at the dose of 150 mg/kg BW and 200 mg/kg BW.

Keywords: *Azadirachta indica* A.Juss, seminiferous tubules, spermatogenesis

1. INTRODUCTION

Neem tree (*Azadirachta indica* A. Juss) is a plant that grows in many parts of Indonesia. Neem tree can grow in dry climates to humid climates area. Neem tree was firstly discovered in the Hindustan region, India. The distribution of neem tree in South and Southeast Asia includes Pakistan, Sri Lanka, Thailand, Malaysia and Indonesia [1]. Neem tree can also be used as a contraception using in family planning (KB) programs.

Generally, fertility regulation is mostly aimed at women, while research is still being carried out for men to obtain an effective and safe fertility regulating method. The correct contraceptive method for men has not obtained convincing results compared to its side effects and the various obstacles that accompany it [2].

According to Ekasari (2011), from the results of research conducted by scientists on experimental animals and in humans, it was found that spermatozoa from

animals and humans became inactive after 30 seconds of contact with neem oil. The results obtained indicate that the oil used intravaginally before sex can prevent pregnancy. Histopathologically, it shows no disturbance in female organs, including vaginal, cervical, and uterine tissues [3]. According to Kardiman and Dhalimi (2003), neem leaves consists of azadirachtin, meliantriol, salannin, nimbin, nimbidine, and paraicin (an alkaloid and component of essential oils containing sulphide compounds) [4].

Singh *et al.* stated that the components that have an antifertility effect are nimbin, nimbidine, and NIM-76 found in neem leaves [1]. A study conducted by Arief (2014) proved that the administration of neem extract to white mice could reduce spermatozoa's quantity and quality at different doses [5]. However, the effect of neem leaf extract on seminiferous tubules in males has not been previously studied. Therefore, it is necessary to conduct a study to observe the histopathological image of the

seminiferous tubules in white rats after administration of neem extract.

2. MATERIALS AND METHODS

2.1. Research Design

This research is an experimental research implemented completely randomized design consisting of 5 groups: 1 control group and 4 treatment groups with 3 replications.

2.2. Research Procedure

2.2.1. Preparations of Neem Leaf Extract

The dried neem leaves are mashed and extracted using the maceration method with 96% ethanol solvent, then immersed in ethanol for 3-4 days. The extracted mixture is filtered using filter paper to separate the residue and the filtrate. The filtrate which consist of the solvent and the active ingredient of neem leaves is heated with a rotary evaporator to evaporate the solvent. The extract obtained is then put in a closed plastic bottle [5].

2.2.2. Administration of Neem Leaf Extract

This study used 15 white rats aged 60-120 days weighed 120-200 grams which divided into 5 groups. The experimental animals were acclimatized for 2 weeks and fed with pellets and water *ad libitum*. The administration of neem leaf extract was as follow: the control group was given distilled water; group P1 was given 50 mg/kg BW neem leaf extract with a volume of 0.5 ml; group P2 was given 100 mg/kg BW neem leaf

extract with a volume of 1 ml; group P3 was given 150 mg/kg BW neem leaf extract with a volume of 1.5 ml; and group P4 was given 200 mg/kg BW neem leaf extract with a volume of 2 ml. Neem leaf extract will be administered orally by using a gastric swab once per day for 30 days.

2.2.3. Sample collection and histopathological preparation

After 30 days, the experimental animals were euthanized using chloroform and necropsied to collect testicular organs, then fixed by 10% Neutral Buffered Formalin (NBF), followed by histological preparations using Hematoxylin Eosin (HE) staining method. Histological preparations was carried out in several stages, starting from tissue fixation, tissue processing (dehydration, clearing, infiltration, and embedding with paraffin), tissue cutting, and HE staining [6].

2.3. Data analysis

The histopathological features of the seminiferous tubules were analyzed descriptively by scoring and comparing the changes in reproductive cells observed in the five treatment groups.

3. RESULTS AND DISCUSSION

The histopathological image of the seminiferous tubules in the test group showed mild to severe damage through observation using a microscope with a magnification of 400x. The results of lesion scoring of the seminiferous tubules in all treatment groups are presented in Table 1.

Table 1. Histopathological features of seminiferous tubules

	Control group	Mild damage		Severe damage	
		P1	P2	P3	P4
Spermatogenic cells	+++	+++	++	++	+
Sertoli cells	+++	+++	++	+	+
Basal lamina	+++	+++	++	+	+
Interstitial tissue	+++	+++	++	+	-
Leydig cell	+++	+++	+++	+++	++

Notes:

- +++ : normal, intact, and available in large number
- ++ : lysis, began to thin out and the number decreased
- + : partial lysis
- : lysis

Table 1 showed that the seminiferous tubules of the control group is still normal, including spermatogenic cells, Sertoli cells, Leydig cells, basal lamina and interstitial tissue. Furthermore, P1 group showed similar feature to the control group. In P2 group, spermatogenic cells, Sertoli cells, lamina basalis, and interstitial connective tissue started to lyse and thin out. In P3 group, the Sertoli cells, basal laminae, and interstitial connective tissue showed partial lysis. Meanwhile, P4 group showed lysis of the interstitial connective tissue in each tubule.

No damage or any histopathological changes in each tubule of the control group which was given only

distilled water. P1 showed similar results with control group, indicating the administration of 50 mg/kg BW neem leaf extract did not affect spermatogenesis proven by the presence of reproductive cells. P2 showed slight changes in the seminiferous tubules; the number started to decrease; the spermatogenic cells begin to lyse; the basal lamina and the interstitial tissues started to thin out. P3 showed severe damage and a decrease in the number of spermatogenic cells. This is very clearly seen in the presence of lysed spermatozoa in the lumen of the seminiferous tubules. Whereas in group P4, spermatogenic cells were still found, although in severe damage condition and some cells experienced lysis (Figure 1).

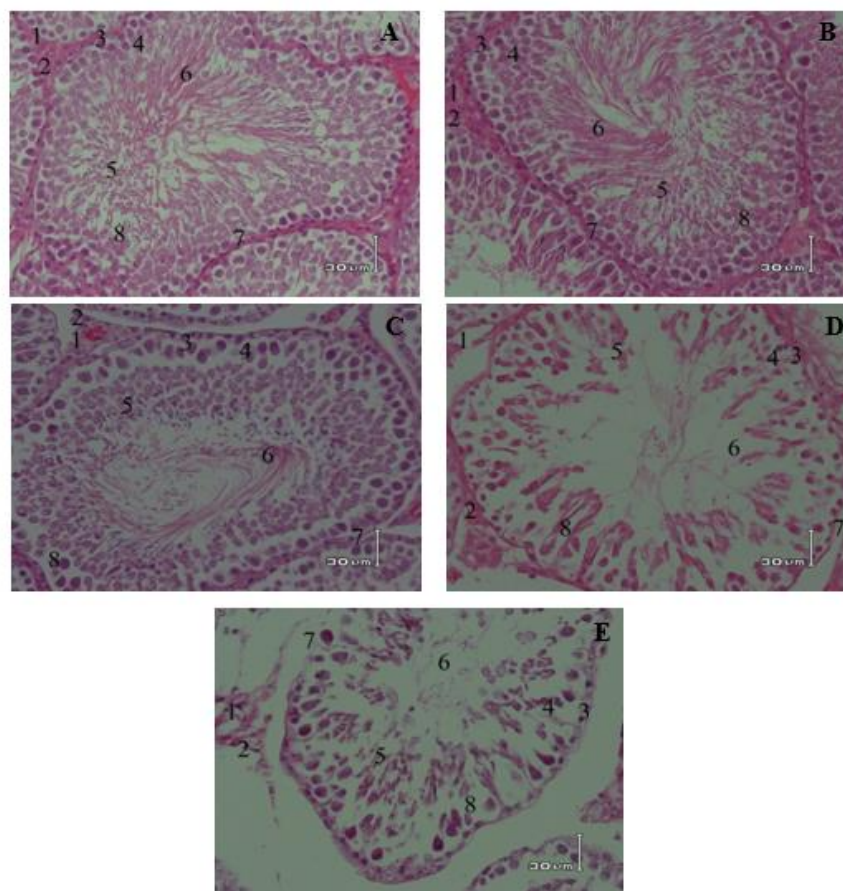


Figure 1 Histopathological findings of seminiferous tubules A) control group. B) given neem leaf extract 50 mg/kgBW. C) given neem leaf extract 100 mg/kgBW. D) given neem leaf extract 150 mg/kgBW. E) given 200 mg/kgBW of neem leaf extract. 1. Interstitial connective tissue. 2. Leydig cells. 3. Spermatogonia cells. 4. Spermatoocyte cells. 5. Spermatid cells. 6. Spermatozoa in the lumen. 7. Lamina basalis. 8. Sertoli cells. HE 400x.

The histopathology of the left testis showed similar feature with the right testis. This result showed that the administration of neem leaf extract did not affect in the structure and the presence of reproductive cells of the right and left testes. Furthermore, some of the Sertoli cells, basal laminae and interstitial tissue undergo lysis. This study also proven that the administration of neem leaf extract did not affect the histology of Leydig cells.

According to Dewi (2011), the seminiferous tubules of control group that was given 1 mL of distilled water did not experience any damage or changes in each tubule [7]. In the tubule, there are intact reproductive cells. Research conducted by Wahyuni *et al.*, showed slightly different results that the greater the dose of neem leaf extract given, the less the number of spermatogenic and spermatid cells, but with a dose of 300mg/Kg BW/day, the number of spermatogenic and spermatid cells increased compared to the previous dose [8].

The anti-androgenic and anti-spermatogenic properties of neem leaves have been reported to reduce spermatozoa's ability [9]. Zemjanis [10] suggested that the decreased of spermatozoa output is accompanied by an increase in the proportion of abnormal spermatozoa. This indicates that the administration of neem leaf extract affects spermatogenic cells' structure during the process of spermatogenesis or ejaculation.

Omarkar *et al.* stated that neem leaf extract has been reported to impair spermatogenesis, increase the number of headless spermatozoa, and significantly decrease spermatozoa tail motility, as well as causing a decrease of the fertility index [11]. This is consistent with Khan *et al.* that reported a significant increase in abnormal spermatozoa morphology in rats fed with neem leaf extract [12]. Wang *et al.* concluded that the pathological damage on mice after administration of neem oil is reversible, which can be gradually restored after long-term discontinuation of the neem leaf extract [13].

No decline on the number of Leydig cells on P1, P2, P3 and P4, however, a decrease of Leydig cells was observed on P4. Akpantah *et al.* stated that neem leaf extract can reduce serum LH levels. This causes a decrease in the function of Leydig cells in secreting testosterone [14]. Biswas *et al.*, adding that nimbidine also has spermicidal activity [15].

Young *et al.* reported that the damage in Sertoli cells causes a disturbance in the production of androgen binding protein (ABP), which is a product of Sertoli cells [16]. Furthermore, Grover *et al.* explained that although the production of androgens is normal in this study, these hormones cannot be utilized in the spermatogenesis process due to the absence of ABP which carries intratubular androgens [17].

4. CONCLUSION

Administration of neem leaf extract (*Azadirachta indica* A.Juss) orally to white rats (*Rattus norvegicus*) for 30 days at various doses showed damage to the seminiferous tubules and affected the spermatogenesis process at doses of 150 mg/kg BW and 200 mg/kg BW.

AUTHORS' CONTRIBUTION

All authors equally contributed to the manuscript preparation and editing.

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