Research Article

Evaluating the Antifertility Potential of Andrographolide Compounds on Male Rats Wistar

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ABSTRACT

We report here that andrographolide (AND), a natural compound which is mayor containt in *Andrographis paniculata* (*A. paniculata*). AND is responsible to the pharmacology activities such us antidiabetic, antidyslipidemic, antioxidant, antiinflammatory and anticancer. This study would to know the safety effect of AND on testicular organs that will affect sperm formation in male wistar rats. It began with separation of AND in *A. paniculata* herb by using recrystallization method. Test rats were then grouped into normal group, AND dose 2, 6 and 18 mg / kg BW. Administration of AND compound was given for 5 weeks, and its sperm morphology, testicular weight and seminiferous tubules histopathology were observed. Based on observations, administration of AND 2, 6, and 18 mg/kg BW for 5 weeks did not affect the testicular weight and the number of spermatozoa of male Wistar rats where there was no significant difference between each group. The histopathologic results also showed that AND compound did not cause destruction of the seminiferous tubular tissue in the testicular organ. So, AND is safely administered to male wistar rats. AND did not affect the testicular weight, number of spermatozoa and semimeniferus tubular tissue morphology.

Keywords: AND, recrystallization, bitter, safe, seminiferous tubules.

INTRODUCTION

Andrografolid (AND) was a diterpene lactone compound that is contained in *Andrographis paniculata* (Burm.f) Ness plant. AND compounds content in leaves was higher than in whole plants, besides, the environmental growth conditions affected the amount of its content[1, 2].

Based on the study results, AND compound had several pharmacological effects. AND compounds from *A. paniculata* were able to cause apoptosis in breast cancer cells at 0.35 mM, 0.70 mM and 1.40 mM concentration, 24, 48 and 72 hours after administration[3]. Ethanol extract of *A. paniculata* had hypoglycemia effect on rat test [4]. Water extract of *A. paniculata* leaf could decrease the action of HMGCo a reductase enzyme *in vitro*[5]. Nugroho et al. (2012) found that administration of purified extract of *A. paniculata* herb and AND on hyperlipidemia and hyperglycemia rats reduced their glucose, triglyseride, and LDL blood level, but not their cholesterol blood level[6]. The purified extract of *A. paniculata* herb 72 mg/kg BW was administered for 60 days in male wistar strain rats showed anti-hyperlipidemia activity and was able to inhibit atherosclerosis AND 18 mg / kg BW was given to male wistar rats and it was able to inhibit the occurrence of atherosclerosis by inhibiting ox-LDL formation and able to decrease total cholesterol, triglyceride and LDL levels in rats[7,8]. Some of the pharmacological activities possessed by *A. paniculata* were caused by the presence of its chemical compounds.

The chemical compounds contained in the *A. paniculata* water extract were AND terpenoid compounds, flavones and lactones[9]. AND was the major compound contained in *A. paniculata* herb. In the development of herbal medicine, it was important to know not only the pharmacological effects, but also its side effects. *A. paniculata* ethanol extract had side effects that caused damage to the seminiferous tubules which were the spermatozoid-forming tissues. The increase of *A. paniculata* extract dose would lead to the increase of seminiferous tubular tissues damage which made *A. paniculata* ethanol extract had an antifertility effect[10]. AND compounds were contained in *A. paniculata* ethanol extracts and this research was conducted to know the AND compound effect which could cause the seminiferous tubules damage to the male Wistar rats.

MATERIALS AND METHODS

Material

A. paniculata herbs were obtained from Kulonprogo, Yogyakarta. Technical ethanol solvent, methanol p.a, technical chloroform, ethyl acetate technical, n-hexane, NaCl solution 0.9%, formalin 10%, HE dye (hematosin-eosin). All of solvent and reagent was purchased from PT. Kurnia Jaya, Indonesia.

Animals for experiment

Wistar male albino rats, aged two months, 140±20 g of weight, maintained under controlled condition, were used in the experiment. The rats were fed with standard diet and given drink ad libitum. Ethical Clearance of the animal's handling was approved by the Faculty of Veterinary, Udayana University no: 194/KE-PH-LH-1/VI

AND extraction and separation from *A. paniculata*

A. paniculata dried powder was macerated in 96% ethanol for 24 hours, filtered, and its filtrate was then re-macerated three times. The macerate was then evaporated in a rotary evaporator to produce concentrated product. This extract was mixed with silica powder before extraction using chloroform organic solvent soxhletation. The process will end if the chloroform organic solvent became clear[8].

Recrystallization AND

The extract obtained was reconstituted with hot methanol p.a, then immediately cooled, so that it would immediately form AND crystals. To obtain a cleaner AND crystal, it could be washed with cold n-hexane[8].

Test animal treatment

Twenty 4-5 weeks old Wistar male albino rats (250 g) were divided into four groups. Animals were then grouped as following:

- Group A was the normal group; male rats were given standard food and water
- Group B was 2 mg/kg BW AND dose group, male rats only fed with standard food, water and 2 mg/kg BW AND dose for 5 weeks.
- Group C was 6 mg/kg BW AND dose group, male rats only fed with standard food, water and 6 mg/kg BW AND dose for 5 weeks.
- Group D was 18 mg/kg BW AND dose group, male rats only fed with standard food, water and 18 mg/kg BW AND dose for 5 weeks.

After 5 weeks, each male Wistar rat sperm was taken; the sperm morphology and amount were then observed. The testicular organ was obtained to observe the seminiferous tissue form of male Wistar rats.

Histopathology of testicular organs

The testicular organ was fixed in formalin solution and then sent to the Histopathology section of Denpasar Hall Veterinary. The next process was according to standard histopathology procedure which was testicular organ was sliced transversely in the middle with 5 micron-thick microtome. Preparations were placed on top of the object glass and were stained with HE dye. It was then covered with a glass desk and covered with canard balm at the edges. These testicular testicles were ready to be observed with a microscope. Testicular histopathologic preparation was observed under a microscope with 400 times magnification (40x10).

Results Analysis

The number of spermatozoa was calculated by the suction of spermatozoa from cauda epididymis using a hematocrit pipette until it reached 0.5 then diluted with a physiological NaCl solution until it reached 101 (200 times dilution), and the pipette was shaken. A few drops of spermatozoa from the hematocrit pipette were first dropped on a tissue, then on a hemocytometer that were closed with a cover glass. Spermatozoa were observed and calculated by using a microscope. The number of spermatozoa was calculated using the formula:

$$number\ of\ spermatozoa\ x\ 200\ x\ 10^4 = \cdots\ million/ml$$

The obtained data of tubular seminiferous tissue were analyzed descriptively by comparing them with normal mouse conditions and with the literature.

RESULTS

The obtained AND compounds were amorphous white crystals. AND recrystallization was repeated to produce crystals with less chlorophyll content. Chlorophyll elimination was performed by using a cold n-hexane solvent and dissolved with methanol. The obtained AND crystal was then administered to rats for 5 weeks.

The rats' testicular weight for the 4 groups (A, B, C, and D) is shown in the following table (Table 1). It appeared that there was no significant difference for the testicular weight of each group ($p>0.05$). There was no significant effect of giving 2, 6 and 18 mg/kg BW AND dose for 5 weeks to the number of testicular weight. AND also did not cause changes in spermatozoa number. Statistic result showed that there was no difference between each group ($p>0.05$). The mouse sperm appearance that was shown in figure 2, was in one field of view by using microscope. This indicates that AND did not cause changes in testicular weight and number of spermatozoa.

Table 1: Parameters of testicular weight and number of spermatozoa

Parameter	A	B	C	D
Testicular weights (Average±SD) g	1.316 ± 0.146	1.427 ± 0.135	1.344 ± 0.150	1.510 ± 0.065
Number of spermatozoa (average±SD million/ml)	32.0 ± 1.414	30.4 ± 1.673	29.6 ± 1.673	30.2 ± 1.483

A = normal group of rats; B = rats with 2 mg/kg BW AND dose; C = rats with 6 mg/kg BW AND dose; D = rats with 18 mg/kg BW AND dose

The morphology of spermatozoa of male Wistar rats in all groups was normal and no difference in each group. Sperm head abnormalities include double head, flat head, head without chromosome or thickened central head. The tail abnormality was characterized by the presence of curved tail, circular tail, loop-like tail, or a broken tail[12]. The normal male rats' sperm morphology also looked the same; there was a curved part on the sperm head and the tail also looked the same (Fig. 2). The administration of 2, 6 and 18 mg/kg BW AND did not affect the morphology of male Wistar rats spermatozoa.

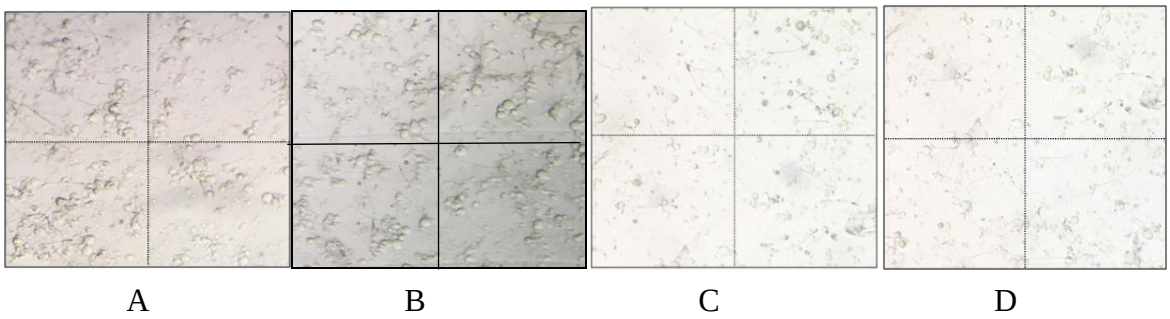

Figure 1: Spermatozoa appearance under a microscope (10x). A = normal group of rats; B = rats with 2 mg/kg BW AND dose; C = rats with 6 mg/kg BW AND dose; D = rats with 18 mg/kg BW AND dose

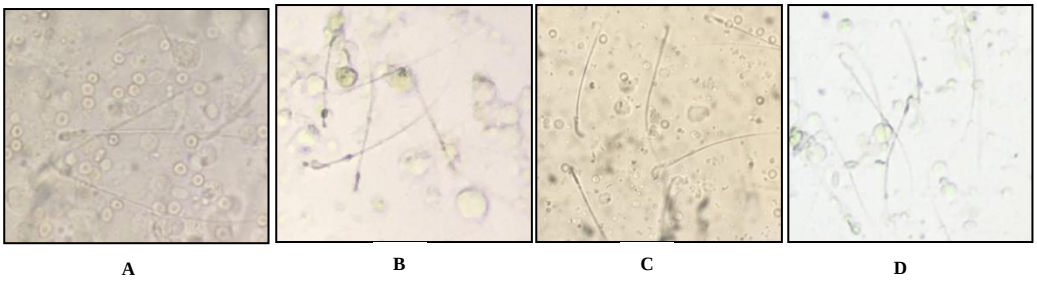

Figure 2: Morphology of sperm rats, A = normal group of rats; B = rats with 2 mg/kg BW AND dose; C = rats with 6 mg/kg BW AND dose; D = rats with 18 mg/kg BW AND dose (400x)

The seminiferous tubules morphology of male Wistar rats that were not given AND and given 2, 6 and 18 mg/kg BW AND for 5 weeks were shown in Fig. 3. There was no difference in the somniferous tubules morphology between normal groups and rats that were given 2, 6 and 18 mg/kg BW AND.

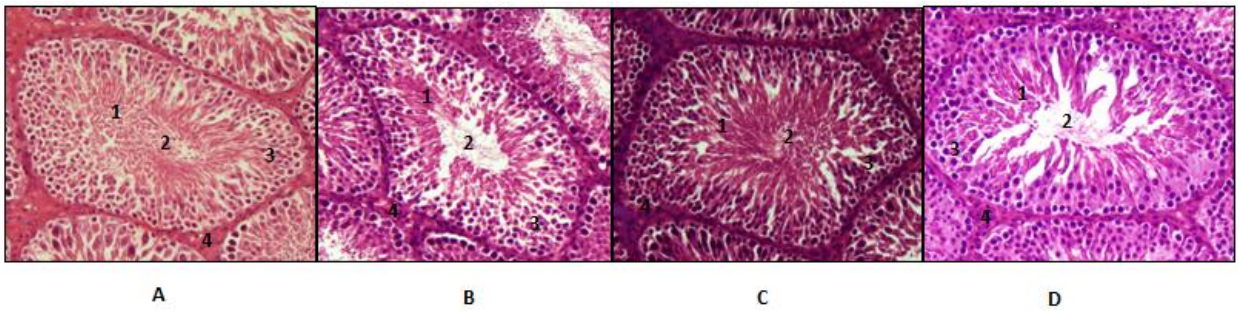

Figure 3: Seminiferous tubules morphology of male Wistar rats , A = normal group of rats; B = rats with 2 mg / kg BW AND dose; C = rats with 6 mg/kg BW AND dose; D = rats with 18 mg / kg BW AND dose (100x); 1. Spermatozoa, 2. Lumen tubules, 3. Primary Spermatocytes, 4. Leydig Cells

DISCUSSION

Based on the results above, AND did not give antifertility side effects in male Wistar rats that were given 2, 6 and 18 mg/kg BW AND for 5 weeks. In another study, *A. paniculata* ethanol extracts with 11.25, 22.5 and 45 mg /mice were able to destroy seminiferous tubular tissues. The morphology of seminiferous tubular of the mice showing degradation in spermatogonia cells[10]. The most major content in *A. paniculata* ethanol extracts was AND, so it was suspected that AND caused the destructive effects towards seminiferous tubular tubes. But based on these results, it was shown that AND 2, 6 and 18 mg/kg BW AND for 5 weeks did not damage seminiferous tubules. Seminiferous tubules morphology of male Wistar rats (Fig. 3 A, B, C, D) showed normal round with complete spermatogenic cycle. Adminsitration of AND 2, 6 and 18 mg/kg BW AND for 5 weeks is save for male rats.

CONCLUSION

2, 6 and 18 mg / kg BW AND dose for 5 weeks did not affect number of spermatozoa, testicular weight, degradation and spermatogenic cells which spread in seminiferous tubules.

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