Rats testosterone level and reproductive organ weight treated by kapok (Ceiba pentandra Gaertn.) seed extract

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Abstract. Uncontrolled population of dogs can be avoided if pet owner can find easy and inexpensive methods to prevent pregnancy in their dogs. The use of natural ingredients for contraception derived from plants is called herbal contraception. Gossypol is a polyphenolic compound produced by pigment glands, especially in the seeds of cotton plants (Gossypium sp). Cotton seeds, a primary content of gossypol, is an antifertility substance that affects reproductive hormones control and has a cytotoxic effect. The purpose of this study was to determine the effect of kapok seed extract on male reproductive system by measuring blood serum testosterone level and weight of male reproductive organs. The administrations of kapok seed extract in male rats were given 0.5 ml/rat/day by gavage method for 33 days. Thirty two male rats were divided into 4 groups i.e. control group (only given aquadest) and treatment groups namely T1, T2 and T3 which were given kapok seed extract at the doses of 0.07; 0.12 and 0.24 mg/ kg bw/day, respectively. The administration of kapok seed extract (Ceiba pentandra Gaertn) decreased the testosterone level and the weight of the reproductive organs of male rats (Rattus norvegicus).

1. Introduction
The increase of rabies cases in Indonesia is inseparable from the uncontrolled population of dogs. The increase in the number of dog population is due to the large number of wild dogs on the road that are deliberately dumped by their owners, this results in an uncontrolled pattern of reproduction. Disposal of puppies on the road can be avoided if people can find easy and inexpensive methods to prevent pregnancy of their dogs. The use of natural material as contraceptive has been widely used and has also proven its efficacy through scientific studies. The use of natural ingredients for contraception derived from plants is called herbal contraception.

Gossypol is a polyphenolic compound produced by pigment glands, especially in the seeds of cotton plants (Gossypium sp)[1]. Cotton seeds have a primary content of gossypol, which is an antifertility substance that affects reproductive hormone control and has a cytotoxic effect [2]. Addition of cotton seed flour to animal feed caused an impaired fertility in bulls [3], and caused a decrease of goat sperm abnormal motility and morphology [4]. The administration of cotton seed extract in female mice also caused reproductive cycle disorders i.e. proestrus, metestrus and diestrus [5]. There was a decrease of sperm quality in local cats which were given kapok seed extract doses of 0.21, 0.36 and 0.71 mg/kg bw/day [6]. However, until now there was no study about the mechanism for reducing sperm quality due to the administration of kapok seed extract with this dose. Based on the background, it is necessary to study the effect of giving kapok seed extract on male production system by measuring the concentration of testosterone hormone blood serum and the weight of male reproductive organs.
2. Materials and Methods

2.1. Animal and management
This study used male rats (Rattus norvegicus), 3-4 months old and 175-180 g weight. During the research, the rats were kept in plastic cages (33x25x14cm), in a room maintained at 25-26°C and 50±10% humidity with a photoperiod of 12:12 hour light:dark. During acclimatization (7 days), rats were provided with standard feed (CP 551, produced by PT Charoen Pokphan Indonesia) and clean water (ad libitum). The study was approved by The Animal Ethic Committee, Faculty of Veterinary Medicine, University of Udayana, Bali, Indonesia.

2.2. Seed collection and extraction
Kapok seeds were obtained from Uluwatu Street at Badung District of Bali Province, Indonesia. The selected kapok seeds (rounded shape) were air-dried and blended into powder. Kapok seed powder was weighed (500 g), then soaked in 1000 ml of 96% ethanol for 72 hours. The macerate obtained by filtration was evaporated using a vacuum rotary evaporator. The crude extract obtained was then used as treatment doses [6].

2.3. Experimental design
This study used a Completely Randomized Design (CRD) consisting of 4 treatment groups (n=8). The control group, rats were given only aquadest. The treatment groups (T1, T2 and T3), rats were given doses of 0.07; 0.12 and 0.24 mg/kg bw/day of kapok seed extract, respectively. Kapok seed extract were given 0.5 ml/rat/day by gavage for 33 days. At the end of the treatments, all animals were euthanized and sacrificed to collect the blood samples and the reproductive organs (testis and epididymis).

2.4. Measurement parameters

2.4.1. Testosterone hormone level
The blood was collected were done through the retro-orbital sinus then put in tubes, then the tubes were centrifuged at 3000 rpm to separate the serum that will be used to measure the rat testosterone levels. The serum was stored in freezers at -20°C until the measurement day. Measurement of testosterone hormone levels was using by the Enzyme Linked Immunoassay (ELISA) technique. Hormone measurement procedures were carried out following the Kit Instruction Manual Procedure (Elabscience).

2.4.2. Weight of reproductive organs (testes and epididymis)
The testes and epididymis from the control and treatment groups were collected and the fat tissues attached to the organs were removed before weighing as absolute organ weight.

Data were represented in the form of mean ± standard deviations (SD) and were analysed by One Way Analysis of Variance (ANOVA) followed by a Duncan test for multiple comparisons test using SPSS statistics 22.0 software. We used the confidence level P<0.05.

3. Result and Discussion

3.1. Testosterone hormone level
Statistically showed that the level of testosterone hormone in the treatment group of 0.24 mg/kg bw/day decreased significantly compared to the control group. However, the testosterone hormone level in treated groups of doses 0.07 and 0.12 mg/kg bw/day decrease showed no significant difference compared to the control. Values were presented as means ± SD of 8 replicates per treatment. Means with different superscripts in the same row were significantly different (P<0.05). T0 = Control (only given aquadest),
T1 = 0.07 mg/kg bw/day of kapok seed extract, T2 = 0.12 mg/kg bw/day of kapok seed extract, P3 = 0.24 mg/kg bw/day of kapok seed extract.

3.2. Weight of reproductive organs (testes and epididymis)
The mean weight testicles treated 0.24/mg/kg bw/day of kapok seed extract showed significant different compared to the control group. However, the treatment groups of 0.07 and 0.12 mg/kg bw/day showed no significant difference to the control. The results of statistical tests on epididymal weights showed decrease in rats which were given kapok seed extract doses 0.12 and 0.24 mg/kg bw/day compared to the control. While The rats treated of 0.07 mg/kg bw/day decrease non-significant difference compared to the control.

Values were presented as means ± SD of 8 replicates per treatment. Means with different superscripts in the same row were significantly different (P<0.05). T0 = Control (only given aquadest), T1 = 0.07 mg/kg bw/day of kapok seed extract, T2 = 0.12 mg/kg bw/day of kapok seed extract, P3 = 0.24 mg/kg bw/day of kapok seed extract.

Kapok seed extract dose of 0.24 mg/kg bw/day caused a significant (P<0.05) decrease in testosterone levels compared to control and treatments T1 and T2 due to gossypol contained in the kapok seed extracts. The mechanism of decreasing testosterone levels occurred through the disruption of testosterone production in the Leydig cells. A study by [7] reported that rats fed a diet containing cotton seed meal, experienced a reduction in the expression of the androgen receptor gene found in the Leydig cells. Androgen receptors are transcription factors that play an important role in male sex development, spermatogenesis and maintained hormonal homeostasis [8,9]. Rats with spontaneous mutations in their androgen receptor gene failed to express genes that were very important in steroidogenesis such as those producing cytochrome P450 17α1-enzymes and hydroxyl steroid dehydrogenase-3. The absence of these enzymes in Leydig cells led to a failure of testosterone secretion in large quantities [10, 11]. Decreased androgen receptor activity could also occur due to disorders in the hypothalamic-gonadal axis. These events resulted in a decrease of testosterone secretion [12].

Gossypol has also been reported to reduce the steroidogenesis process, as well as decreasing progesterone and estradiol serum levels in female rats [2]. Testis is the gland where the spermatogenesis take place. Spermatogenesis is controlled by testosterone produced by the Leydig cells [13]. Decreased testosterone level causes disruption in the development of germinal cells in the testis, which lead to a decrease of spermatozoa count [14]. Decreased spermatozoa production could lead to decreased testicular weight [15]. Decreased in testosterone level also lead decreased in epididymis weight, because epididymis function as a place of spermatozoa maturation was also controlled by testosterone hormone. Gossypol was also reported to cause hormonal disorders causing decreased viability and increased the number of ovarian follicular cells that experienced atresia in sheep [16] and in chickens [17]. However, the negative effect of gossypol on the reproductive system was reversible if the administration was stopped [18].

4. Conclusion
The administration of kapok seed (Ceiba pentandra Gaertn) extract had decreased the testosterone hormone level and the weight of the reproductive organs of male rats (Rattus norvegicus).

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