

ABSTRACT

Some plant or plant extracts are used as the agent capable of lowering reproductive capacity among some people without causing adverse effects is one way to control population growth. One of the plants that has the potential as an agent capable of lowering reproductive capacity is the cashew (*Anacardium occidentale* L.), especially its nut shell. The nut shell contains laquer oil or Cashew Nut Shell Liquid (CNSL) which constitutes a phenolic compound, mainly composed of anacardic acid, cardol, methyl cardol and cardanol. Extract of cashew nut shell has an effect on the structure of female white rat (*Rattus norvegicus*) ovary, resulting in an inhibition of follicular development and the structure of granulosa cells thereby they are not compact. There are four factors involved in the follicular development, which are IGF-1, androgen hormone, aromatase and 17β -estradiol hormone. The purpose of this study was to investigate the effect of the administration of the cashew nut shell extract on levels of IGF-1, androgen hormone, CYP19aromatase and 17β -estradiol hormone. This research was conducted in three stages: first, toxicity was tested using Weil method. It used 20 two-month female rats. The extract was administered orally with five treatments, I. Control, II. The administration of cashew nut shell extract 2.5 mg kg^{-1} body weight (bw), III. 25 mg kg^{-1} bw, IV. 250 mg kg^{-1} bw, and V. $2,500 \text{ mg kg}^{-1}$ bw. The second stage was preliminary testing carried out using six two-month female white rats. Two treatments were the control and the administration of extract 500 mg/kg . The extract was administered orally for 25 days (five times estrous cycles). Ovaries were removed at the time of estrus in the 5th estrous cycle, and histological preparations were made by using paraffin method and HE staining with $4\mu\text{m}$ thickness. The third phase of research was confirmation of preliminary test results. There are three treatments which are control, the administration of extract 250 mg kg^{-1} bw and 500 mg kg^{-1} bw. Female white rats used were 10 six-week rats per treatment. The extract was administered orally for 45 days (nine times estrous cycles). Blood sampling for analysis of the levels of IGF-1, androgen hormone, and 17β -estradiol hormone was done three times at the time of 0, 5th, and 9th estrous cycle. On 9th estrous cycle, the rats were sacrificed to remove their ovaries, which were used for the analysis of the levels of IGF-1, androgen hormone, and 17β -estradiol hormone. Samples of blood and ovaries were analyzed by ELISA. The ovary was also used to make histological preparations by using paraffin method and Hematoxilin eosin (HE) staining in order to count the number of follicles, and immunohistochemical staining to observe CYP19 aromatase expression. Data were analyzed qualitatively and quantitatively. Qualitative analysis included the observation of ovarian histological structure and the observation of CYP19aromatase expression. Quantitative analysis included calculation of the value of LD_{50} by probit analysis. Calculation of the number of follicles, the levels of IGF-1, androgen hormone and 17β -estradiol hormone was

done using. Analysis of Variance (Anova), which was followed by *Duncan Multiple Range Test* (DMRT) at significance level of 5% ($p < 0.05$) using SPSS version 19. The results of toxicity test of the cashew nut shell showed LD_{50} values of $2,018 \text{ mg kg}^{-1} \text{ bw}$. Cashew nut shell extract can inhibit the growth of ovarian follicles, there were only a few follicles in the developmental stage, granulosa cells were not compact, and there was a gap between the granulosa cells. The extract did not affect the levels of IGF-1 and the levels of androgen hormones produced ($p > 0.05$). The influence of the extract on the expression of CYP19aromatase led to a decrease in the number of follicles ($p < 0.05$), and the aromatase protein was weak on the treatment of $500 \text{ mg kg}^{-1} \text{ bw}$, causing a decrease in levels of the 17β -estradiol hormone ($p < 0.05$). The level of ovarian estradiol hormone- 17β decreased by 16.17%. The research concluded that the cashew nut shell extract does not generate an affect on IGF-1 and androgen hormone. This extract influences folliculogenesis and steroidogenesis by decreasing the expression of CYP19aromatase, thereby reducing the levels of the 17β -estradiol hormone.

Keywords: cashew nut shell, IGF-1, androgen, CYP19aromatase, 17β -estradiol

INTISARI

Pemanfaatan tumbuhan ataupun ekstrak tumbuhan sebagai agen penurun kapasitas reproduksi di masyarakat merupakan salah satu cara pengendalian pertumbuhan penduduk. Salah satu tumbuhan yang berpotensi sebagai agen penurun kapasitas reproduksi adalah tanaman mete (*Anacardium occidentale* L.), terutama kulit biji. Kulit biji mete mengandung minyak laka atau *Cashew Nut Shell Liquid* (CNSL), merupakan senyawa fenolik, terutama terdiri dari asam anakardat, kardol, metil kardol, dan kardanol. Ekstrak kulit biji mete mempengaruhi struktur ovarium tikus putih (*Rattus norvegicus*) betina, menyebabkan terhambatnya perkembangan folikel dan kerusakan sel-sel granulosa. Sedikitnya ada empat faktor terkait dalam perkembangan folikel yaitu IGF-1, hormon androgen, aromatase, dan hormon estradiol-17 β . Penelitian ini bertujuan untuk mengkaji pengaruh pemberian ekstrak kulit biji mete terhadap kadar IGF-1, hormon androgen, CYP19 aromatase, dan hormon estradiol-17 β . Penelitian ini dilakukan dalam tiga tahap yaitu pertama uji toksisitas menggunakan metode Weil. Tikus betina yang digunakan berumur dua bulan sejumlah 20 ekor. Pemberian ekstrak secara oral, ada lima perlakuan yaitu I. kontrol, II. pemberian ekstrak kulit biji mete 2,5 mg/kg berat badan (bb), III. 25 mg/kg bb, IV. 250 mg/kg bb, dan V. 2.500 mg/kg bb. Tahap ke dua uji pendahuluan, tikus putih betina yang digunakan berumur dua bulan sejumlah enam ekor. Perlakuan ada dua yaitu kontrol dan pemberian ekstrak kulit biji mete 500 mg/kg bb. Pemberian ekstrak dilakukan secara oral selama 25 hari (lima kali siklus estrus). Ovarium diambil saat estrus pada siklus estrus ke-5, dibuat sediaan histologis dengan metode parafin dan sayatan setebal 4 μ m, diwarnai dengan Hematoksin Eosin (HE). Penelitian tahap ke tiga merupakan konfirmasi hasil uji pendahuluan, terdiri atas tiga perlakuan yaitu kontrol, pemberian ekstrak kulit biji mete 250 dan 500 mg/kg bb. Tikus putih betina yang digunakan berumur enam minggu sejumlah 10 ekor per perlakuan. Pemberian ekstrak dilakukan secara oral selama 45 hari (sembilan kali siklus estrus). Pengambilan darah untuk analisis kadar IGF-1, hormon androgen, dan hormon estradiol-17 β dilakukan tiga kali, yaitu pada siklus estrus ke-0, ke-5, dan ke-9. Pada siklus estrus ke-9 tikus dikorbkan kemudian ovarium diambil digunakan untuk analisis kadar IGF-1, hormon androgen, dan hormon estradiol-17 β dengan metode *Enzyme-linked Immunosorbent Assay* (ELISA). Ovarium juga digunakan untuk mengamati ekspresi CYP19aromatase dengan membuat sediaan histologis metode parafin dan pewarnaan HE guna menghitung jumlah folikel, serta pewarnaan imunohistokimia guna mengamati profil protein aromatase. Data dianalisis secara kualitatif dan kuantitatif. Analisis kualitatif mencakup pengamatan struktur histologis ovarium dan pengamatan profil protein aromatase. Analisis kuantitatif meliputi penghitungan nilai LD₅₀ dengan analisis probit; jumlah folikel, kadar IGF-1, hormon androgen dan hormon estradiol-17 β dengan analisis sidik ragam (Anava), dilanjutkan dengan *Duncan*

Multiple Range Test (DMRT) pada taraf signifikan 5% ($p < 0,05$) menggunakan Program SPSS versi 19. Berdasarkan hasil uji toksisitas kulit biji mete didapatkan nilai LD_{50} sebesar 2.018 mg/kg bb. Ekstrak kulit biji mete menghambat perkembangan folikel ovarium, hanya ada beberapa folikel dalam tahap perkembangan, sel-sel granulosa rusak, dan terdapat celah diantara sel-sel granulosa. Ekstrak ini tidak mempengaruhi kadar IGF-1 dan kadar hormon androgen. Pengaruh ekstrak terhadap ekspresi CYP19aromatase menyebabkan protein aromatase terpulas sedikit dan penurunan jumlah folikel preantral dan antral berturut-turut sebesar 46,58% dan 51,35%. Ekstrak ini juga menurunkan kadar hormon estradiol-17 β serum maupun ovarium sebesar 16,17%. Simpulan penelitian adalah ekstrak kulit biji mete tidak mempengaruhi kadar IGF-1 dan hormon androgen. Ekstrak ini menurunkan ekspresi CYP19aromatase dan menurunkan kadar hormon estradiol-17 β , akibatnya mempengaruhi perkembangan folikel, sehingga ekstrak kulit biji mete dapat digunakan sebagai agen penurun kapasitas reproduksi.

Kata kunci: kulit biji mete, IGF-1, androgen, CYP19aromatase, estradiol-17 β