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Research Article

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Beluntas Leaves Extract (*Pluchea indica* L.) as an Antifertility in Male White Rats (*Rattus norvegicus*)

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ABSTRACT

Background: Beluntas (*Pluchea indica* L.) is a medicinal plant widely used in traditional herbal therapy due to its content of bioactive compounds such as alkaloids, flavonoids, tannins, chlorogenic acid, essential oils, saponins, and polyphenols. These compounds are known to have various biological effects, including potential influences on the male reproductive system.

Aims: This study aimed to determine the effect of beluntas leaf extract on the number of Sertoli cells and Leydig cells in male white rats (*Rattus norvegicus*).

Methods: This experimental study evaluated the reproductive effects of beluntas leaf extract through histological examination of testicular tissue. Male white rats were administered beluntas leaf extract at different dose levels. The observed parameters included histological features and the number of Sertoli cells and Leydig cells. Testicular samples were collected and examined microscopically to assess cellular changes.

Results: The results showed that the number of Sertoli cells and Leydig cells decreased with increasing doses of beluntas leaf extract. A dose-dependent relationship was observed, where higher doses resulted in a greater reduction in both Sertoli and Leydig cell counts. Histological observations supported these findings and indicated alterations in testicular tissue consistent with reduced cellular populations.

Conclusion: This study concludes that the administration of beluntas leaf extract can reduce the number of Sertoli cells and Leydig cells in male white rats. The higher the dose of beluntas leaf extract administered, the fewer Sertoli and Leydig cells were observed, suggesting a potential effect of beluntas on male reproductive function.

INTRODUCTION

Male antifertility has not been widely practiced. The limited number of contraceptives for men means that the family planning process among men is still very few people interested (Susetyarini et al., 2024). A good contraceptive must meet certain criteria and requirements to be safe. The requirements that must be met by a good contraceptive device are that it is harmless, reliable, cheap, widely accepted, secure, easy to obtain, reversible, and has few side effects. A truly ideal contraceptive method is still rare today. The use of contraceptives that have fewer side effects needs to be studied. The potential of traditional plant ingredients has not been scientifically proven to have bioactivity. Many plants contain phytochemicals that have the potential to be antifertility.

Phytochemicals are bioactive compounds from plants that can support animal performance (Jumadin et al., 2024). This compound is available in various forms. This compound is found in all parts of plants such as roots, tubers, leaves, and fruit (Jumadin et al., 2022). One of them is beluntas leaves.

Beluntas (*Pluchaea indica* L.) is a herbaceous plant from the Asteraceae family, that grows wild in dry and rocky areas and is planted as a hedge plant. Beluntas contains alkaloids, flavonoids, tannins, chlorogenic acid, sodium, essential oils, saponins, and polyphenols (Tuhumury, 2023). Fatah et al. (2024) stated that beluntas leaves also contain tannins, alkaloids, and flavonoids.

The substance content in plants, including beluntas, has properties and benefits for the physiological performance of body organs (Jumadin *et al.* 2023, 2022a). Beluntas leaves have been used for wound healing, and antioxidant and antimicrobial processes (Pertiwi et al., 2024; Purwani et al., 2024; Tuhumury, 2023).

Research on the potential of beluntas as an antifertility agent has been carried out previously, such as reducing the concentration and motility of spermatozoa after administering beluntas leaf stigmasterol (Fatah et al., 2024), reducing the concentration of spermatozoa after administering tannin extract (Susetyarini and Nurrohman, 2022), decreasing spermatozoa motility (Susetyarini et al., 2019) anti-spermatogenic activity of beluntas leaves (Susetyarini et al., 2023). However, its role has not been studied much in the testicular organs in white mice. This research will examine the effect of giving beluntas leaf extract (*Pluchea indica* L.) on the number of Sertoli cells and Leydig cells in male white rats (*Rattus norvegicus*).

MATERIALS AND METHODS

The procedures used in this study were in accordance with the rules of the Animal Ethics Commission of LPPM UHO (No. 1161a/UN29.20.1.2/PG/2023).

Preparation of Beluntas Leaf Extract

The collected beluntas leaves were removed from the stems, and dried in an oven at 55°C for 24 hours. Beluntas leaves are ground using a blender and sifted until they become a fine powder. Beluntas leaf flour was macerated with 96% ethanol for 1 x 24 hours, then evaporated using a rotary vacuum evaporator. The extraction of beluntas leaves was carried out according to work procedures (Junaidi et al., 2020).

Preparation and Care of Experimental Animals

The animals used were 24 male white rats (*Rattus norvegicus*) aged 8-10 weeks with an average body weight of 200 grams. Mice were obtained from a mouse breeder and acclimated for 2 weeks. Mice were kept individually, one in each cage. A square cage covered with wire. Bedding in the cage is wood shavings which are replaced twice a week. Feed is given as much as 20 grams/day in the form of pellets given in the afternoon. Drinking water is given as much as 100 ml/head/day.

Calculation of Beluntas Leaf Extract Dosage and Treatment Groups

The calculation of the dose of beluntas leaf extract in this study was a dose conversion from white rats according to Rahayu et al., 2012. The treatment given in this study consisted of 4 levels/doses of beluntas leaf extract, namely P0 (0 mg), P1 (1.5 mg), P2 (15 mg), and P3 (150 mg).

Treatment and Sampling Stage

A total of 24 white rats (*Rattus norvegicus*) were divided into four group cage units. Each replication, each treatment consisted of 6 animals. Feed is given once a day, namely 20 grams/head/day. Drinking water is given as much as 100 ml/head/day. Beluntas leaf extract was given to mice orally for 49 days.

Rat testicles were taken at the end of treatment. Mice were sacrificed by anesthesia. Mice were dissected from the abdomen to the chest. Rat testicles were taken and separated from attached fat, cleaned with NaCl, and testicular histology preparations were made. Making histology preparations of rat testicles was carried out according to working procedures (Jamili et al., 2021).

Research Variables and Data Analysis

Observations of Sertoli cells and Leydig cells were carried out using a microscope field of view and counted using a Hand Counter. The data obtained were analyzed for variance with Tukey's advanced test using IBM SPSS Statistics version 26.

RESULTS AND DISCUSSION

The histological reduction in Sertoli and Leydig cell populations observed in this study has important functional implications for male fertility. Sertoli cells play a central role in supporting spermatogenesis by regulating germ cell proliferation, differentiation, and survival; therefore, a decrease in their number is likely to result in disrupted or arrested spermatogenic processes. Concurrently, a reduction in Leydig cell populations may lead to diminished intratesticular testosterone production, a hormone that is essential for maintaining normal sperm development and maturation, ultimately impairing male reproductive capacity.

Sertoli Cells

Sertoli cells are one of the most important cells required for sperm production in male mice. Observation of Sertoli cells was carried out through histological images and the number of Sertoli cells. The results of these observations are presented in Figure 1 and Figure 2.

The number of Sertoli cells decreased and there were differences in the shape of the seminiferous tubules between P0, P1, P2, and P3. The seminiferous tubules in P0 are round as normal, while in P1, P2, and P3 the shape is no longer round and looks smaller (atrophic). According to Gabriel et al. (2018), the morphology of atrophied testes shows the absence of spermatogenic cell development, atrophy of the seminiferous tubules, thinning of the basement membrane epithelial layer, and a decrease in the number of interstitial cells. The number of Sertoli cells in white mice given beluntas leaf extract showed significantly different results. The higher the dose of beluntas leaf extract given, the fewer Sertoli cells were found. These results prove that giving beluntas leaf extract at a dose of 1.5; 15; and 150 mg/200 g BB orally can reduce the number of Sertoli cells in the seminiferous tubules of the testicles of white mice.

Leydig Cells

Leydig cells are crucial cells located in the testes and gonads of male mice. Observation of Leydig cells is carried out through histological images and the number of Leydig cells. The results of these observations are presented in Figure 3 and Figure 4.

The number of Leydig cells decreased between treatments P0, P1, P2, and P3. The area between the seminiferous tubules becomes smaller and the epithelium of the seminiferous tubules appears thin. The number of Leydig cells showed significantly different results at various levels of administration of beluntas leaf extract. Oral administration of beluntas leaf extract reduced the number of Leydig cells in the testicles of male white rats. The lowest value was obtained in treatment P3 at 2.83.

The results demonstrate a clear dose–response relationship between beluntas (*Pluchea indica* L.) leaf extract administration and the reduction in Sertoli and Leydig cell numbers. The control group (P0) exhibited normal testicular architecture, reflecting physiologically intact spermatogenesis and steroidogenesis. At the low dose (P1 = 1.5 mg/kg body weight), an initial decline in Sertoli and Leydig cell counts was observed, indicating an early biological response to exposure to beluntas bioactive compounds. This finding suggests that even minimal doses are sufficient to influence testicular cellular homeostasis.

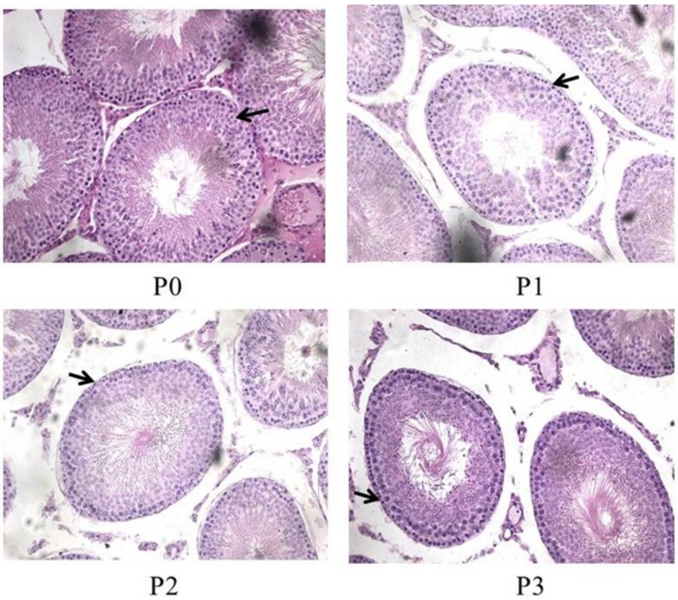


Figure 1. Histological appearance of the seminiferous tubules of the rat testis. Black arrows indicate Sertoli cells. P0 = beluntas leaf extract 0 mg/kg BW; P1 = beluntas leaf extract 1.5 mg/kg BW; P2 = beluntas leaf extract 15 mg/200 g BW; P3 = beluntas leaf extract 150 mg/200 g BW, using HE staining, magnification 100 time.

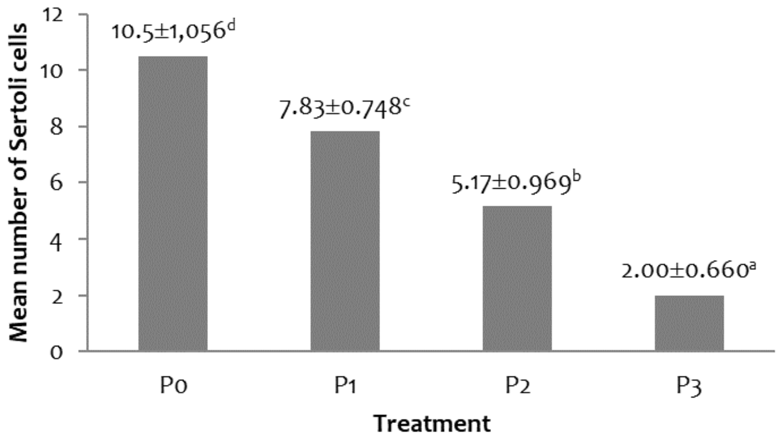


Figure 2. The mean number of Sertoli cells in mice given beluntas leaf extract for 49 days. Numbers accompanied by different letters for each treatment indicate significant differences ($p<0.05$). P0 = beluntas leaf extract 0 mg/kg BW; P1 = beluntas leaf extract 1.5 mg/kg BW; P2 = beluntas leaf extract 15 mg/200 g BW; P3 = beluntas leaf extract 150 mg/200 g BW.

The reduction in Sertoli and Leydig cells became more pronounced at the moderate dose (P2 = 15 mg/200 g body weight) and was most evident at the highest dose (P3 = 150 mg/200 g body weight), confirming a dose-dependent suppressive effect. Biologically, increasing doses likely intensify disruptions in FSH- and LH-mediated endocrine signaling, elevate oxidative stress, and potentially induce direct cytotoxic effects on testicular support cells. Consequently, the loss of Sertoli cells may impair spermatogenic progression, while reduced Leydig cell populations may compromise intratesticular testosterone production. This dose-dependent pattern underscores the antifertility potential of beluntas leaf extract and highlights the biological relevance of Sertoli and Leydig cell depletion as key indicators of impaired male reproductive function. Rahmawati, et al. (2025) reported that a dose of 0.4–2 mL/kg BW of beluntas leaf extract provided an initial safety threshold for the development of antifertility candidates.

Reduced Sertoli cell and Leydig cell counts are a critical marker of impaired supportive function for germ cells, as Sertoli cells and Leydig cell are essential for nurturing developing spermatogenic cells and maintaining the testicular microenvironment. This finding aligns with the broader pattern observed in antifertility research involving plant extracts, where phytochemicals such as tannins and flavonoids disrupt normal testicular cell populations.

In addition, these histological alterations suggest disturbances in endocrine regulation along the hypothalamic–pituitary–gonadal axis. Impaired Leydig cell function may reduce responsiveness to luteinizing hormone (LH), while compromised Sertoli cell activity can attenuate follicle-stimulating hormone (FSH) signaling and androgen-binding protein (ABP) production, both of which are critical for sustaining optimal testosterone concentrations within the seminiferous tubules. Such hormonal dysregulation may not only affect short-term spermatogenic efficiency but also compromise long-term reproductive potential, thereby enhancing the translational relevance of the observed histological changes.

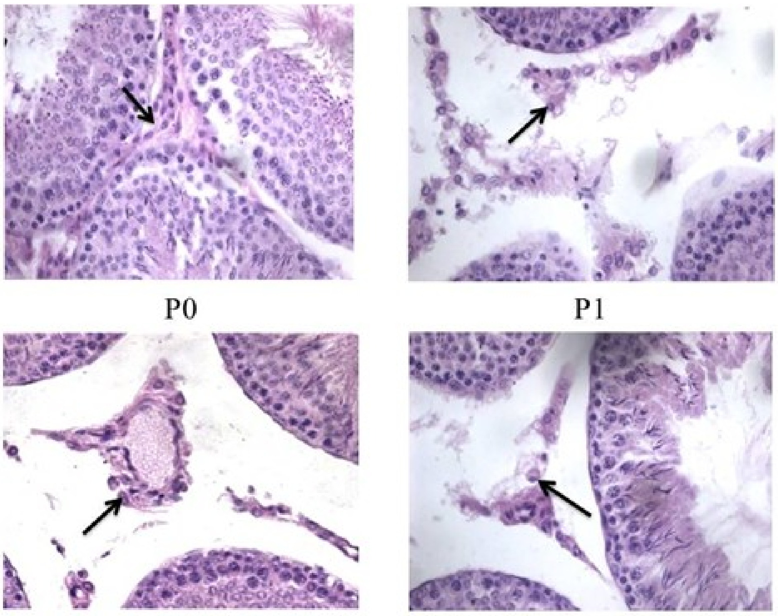
Studies specifically examining *Pluchea indica* corroborate antifertility activities, although many earlier investigations focused on other end-points of reproductive function. For instance, an earlier experiment demonstrated that beluntas leaf extract reduced the number of spermatid cells in mice, supporting a generalized suppressive effect on spermatogenesis (Amalina et al., 2010). Additionally, research on the stigmasterol component of P. indica leaves found significant effects on spermatozoa concentration and motility, further indicating antifertility potential in male rodents (Fatah et al., 2024). These results collectively suggest that multiple bioactive constituents in P. indica, including tannins and sterol fractions, may act synergistically to alter spermatogenic parameters.

Flavonoids, alkaloids, tannins, and steroids are bioactive phytochemicals that play a significant role in the regulation of male reproductive function by influencing spermatogenesis, Sertoli cell activity, Leydig cell steroidogenesis, and endocrine homeostasis. These compounds exert their biological effects through the integration of hormonal signaling pathways and modulation of oxidative stress within the testicular microenvironment. Excessive production of reactive oxygen species (ROS) is widely recognized as a major contributor to testicular dysfunction, leading to lipid peroxidation, DNA damage in germ cells, and impaired sperm maturation (Monageng et al., 2023; Kaltsas et al., 2023). In this context, phytochemicals with antioxidant properties are essential in maintaining normal spermatogenic processes.

Beluntas leaves have antifertility properties which can inhibit the development of Sertoli cells and Leydig cells because they contain phenolic compounds, flavonoids, tannins, steroids, and alkaloids. This is by the opinion of Jamili *et al.* (2021) giving beluntas leaf extract can reduce the number of normal spermatocyte cells because it contains antifertility compounds such as flavonoids, alkaloids, and tannins. Flavonoids and alkaloids have estrogenic abilities in inhibiting the hormonal process of spermatogenesis. This compound inhibits the release of Luteinizing hormone (LH) and Follicle follicle-stimulating hormone (FSH) in the anterior pituitary, thereby affecting the function of Leydig cells in producing testosterone (Setiawan et al., 2021). This is by the opinion (Nurlely et al., 2022) that alkaloids function as antifertility. Antifertility compounds have a mechanism of action through cytotoxic effects and hormonal effects, thereby inhibiting the metabolic rate of spermatogenic cells due to disturbances in the balance of the hormonal system. Alkaloids can inhibit antioxidant activity in Leydig cells, thereby reducing testosterone hormone production (Setiawan et al., 2021).

Flavonoids are thought to suppress the secretion of the hormone testosterone and Androgen Binding Protein (ABP) produced by Sertoli cells. This decrease in secretion causes levels of the testosterone hormone carried by Sertoli cells to the epididymis to decrease. Disturbed testosterone and ABP affect ultrastructural changes and cell metabolism (Setiawan et al., 2021). The flavonoid content inhibits the release of Luteinizing Hormone (LH) and Fillice Stimulating Hormone (FSH), thus affecting the function of Leydig cells in producing testosterone (Berlina et al., 2019).

Flavonoids are particularly important due to their strong antioxidant and anti-inflammatory activities, which protect germ cells and Sertoli cells from oxidative injury. Sertoli cells, which are directly regulated by follicle-stimulating hormone (FSH), are responsible for maintaining the blood–testis barrier



P0 P1 P2 P3

Figure 3. Histological appearance of interstitial cells. Black arrows indicate Leydig cells and white arrows indicate Sertoli cells. P0 = beluntas leaf extract 0 mg/kg BW; P1 = beluntas leaf extract 1.5 mg/kg BW; P2 = beluntas leaf extract 15 mg/200 g BW; P3 = beluntas leaf extract 150 mg/200 g BW, using HE staining, magnification 400 times.

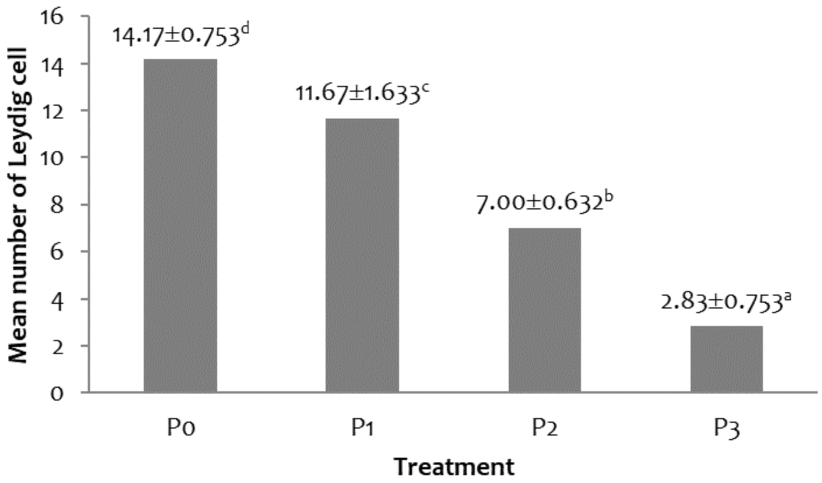


Figure 4. The average number of Leydig cells in mice given beluntas leaf extract for 49 days. Numbers accompanied by different letters for each treatment indicate significant differences ($p<0.05$). P0 = beluntas leaf extract 0 mg/kg BW; P1 = beluntas leaf extract 1.5 mg/kg BW; P2 = beluntas leaf extract 15 mg/200 g BW; P3 = beluntas leaf extract 150 mg/200 g BW.

and producing androgen-binding protein (ABP), a key factor that ensures high intratubular testosterone concentrations required for spermatogenesis (Chen et al., 2025). Several flavonoids have been reported to enhance Sertoli cell viability, reduce apoptosis, and preserve tight junction integrity by suppressing oxidative stress and inflammatory signaling pathways (Martin & Touaibia, 2020).

Leydig cell function is primarily controlled by luteinizing hormone (LH), which stimulates testosterone synthesis through the activation of steroidogenic enzymes. Flavonoids and plant-derived steroids have been shown to enhance Leydig cell steroidogenesis by upregulating the expression of steroidogenic acute regulatory protein (StAR), a rate-limiting factor in testosterone biosynthesis (Martin & Touaibia, 2020). Conversely, oxidative stress disrupts Leydig cell responsiveness to LH, resulting in reduced testosterone production and impaired spermatogenic progression (Monageng et al., 2023). Therefore, the antioxidant activity of these phytochemicals plays a crucial role in preserving Leydig cell functionality and hormonal balance.

Alkaloids and tannins exhibit dose-dependent effects on male reproductive physiology. At low to moderate concentrations, certain alkaloids and tannins may exert protective effects by reducing oxidative stress and supporting gonadotropin secretion, thereby contributing to the maintenance of FSH and LH levels (Kaltsas et al., 2023). However, excessive exposure may lead to cytotoxic effects, hormonal disruption, and decreased sperm quality due to protein precipitation or interference with cellular metabolism (Liman et al., 2022). Overall, the coordinated interaction between endocrine regulation—mediated by FSH, LH, testosterone, and ABP—and oxidative stress modulation underlies the influence of flavonoids, alkaloids, tannins, and steroids on spermatogenesis and male reproductive health.

CONCLUSION

The conclusion of this research is Administration of beluntas leaf extract can reduce the number of Sertoli cells and Leydig cells in male white mice. The higher the dose of beluntas leaf extract given, the fewer Sertoli cells and Leydig cells in male white mice.

This study has several limitations that should be explicitly acknowledged to enhance scientific transparency and editorial rigor. First, the relatively limited sample size may reduce statistical power and restrict the generalizability of the findings, and therefore the results should be interpreted with caution. Second, this study did not include comprehensive sperm quality parameters, such as sperm motility, morphology, and viability, which are critical functional indicators of male reproductive capacity. Third, the absence of hormonal measurements, particularly follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and androgen-binding protein (ABP), limits the ability to directly elucidate the endocrine mechanisms underlying the observed effects on testicular structure and spermatogenesis. In addition, the study did not assess the reversibility of the observed effects following the cessation of treatment, making it unclear whether the changes identified are transient or persistent. Future studies should therefore incorporate larger sample sizes, detailed sperm quality assessments, integrated hormonal profiling, and recovery-phase experimental designs to strengthen the biological interpretation and translational relevance of the findings.

AUTHORS CONTRIBUTION

D.N.Z., N.R., D., and M.S. contributed to data collection, laboratory experimentation, histological analysis, and manuscript drafting. L.J. served as the principal investigator and corresponding author, contributing to the study conceptualization, research supervision, data interpretation, and manuscript review and editing. All authors read and approved the final manuscript.

"The author declares that there is no conflict of interest with the parties involved in this research".

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