AL-KAUNIYAH: Jurnal Biologi, 19(1), 2026, 11-20
Website: https://journal.uinjkt.ac.id/kauniyah/index
P-ISSN: 1978-3736, E-ISSN: 2502-6720

HISTOLOGICAL STRUCTURE OF RAT UTERINE AFTER TREATMENT WITH NANOCHITOSAN PREPARATION OF ETHANOLIC NEEM LEAVES EXTRACT

STRUKTUR HISTOLOGI UTERUS TIKUS SETELAH PEMBERIAN SEDIAAN NANOKITOSAN EKSTRAK ETANOL DAUN MIMBA

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Submitted: 28 Juni 2024; Revised: 4 September 2024; Accepted: 17 March 2025

Abstract

Neem leaves (*Azadirachta indica* A. Juss) contain compounds that have antifertility potential. The constraint in giving medicine orally is the low bioavailability and distribution of active compounds in herbal plants. This problem can be solved by packing herbal plant extracts in nanochitosan. The aim of this study was to analyze the effect of treating with a nanochitosan ethanol extract of neem leaves on the histological structure of the white rat uterine. This study was conducted for 8 months used a randomized design (CRD), which was divided into 3 treatment groups with 4 repetitions. Treatments is given for 21 days, including P0 (an aquades 2 mL/animal/day), P1 (an ethanol extract of neem leaves 2 mL/animal/day, and P2 (a nanochitosan preparation of neem leaves 2 mL/head/day. The variables measured were endometrial thickness, number of uterine glands, uterine diameter, and uterine weight. Numeric datas were analyzed statistically parametrically using the ANOVA test at a confidence level of 95%. The results showed that the endometrial thickness, uterine diameter, number of uterine glands, and uterine weight of P0 were normal, and did not significantly different (P >0.05) from P1 and P2. The conclusion of this study is that the treatment of nanochitosan ethanol extract of neem leaves has the same potential with ethanol extract of neem in affecting the histological structure of white rats uterine.

Keywords: Nanochitosan; Neem; Uterine

Abstrak

Tanaman mimba (*Azadirachta indica* A. Juss) merupakan tanaman herbal yang sering digunakan sebagai obat tradisional. Daun mimba memiliki kandungan senyawa yang berpotensi sebagai antifertilitas. Kendala dalam pemberian obat secara oral salah satunya disebabkan karena rendahnya bioavailabilitas dan distribusi senyawa aktif tanaman herbal. Hal ini dapat diatasi dengan mengemas esktrak daun mimba dalam bentuk nanokitosan. Tujuan penelitian ini adalah menganalisis pengaruh pemberian sediaan nanokitosan ekstrak etanol daun mimba (*A. indica* A.Juss) terhadap struktur histologi uterus tikus putih. Penelitian ini dilakukan selama 8 bulan menggunakan rancangan acak lengkap (RAL) yang terdiri dari 3 kelompok perlakuan dengan 4 kali pengulangan. Perlakuan diberikan selama 21 hari meliputi kontrol (P0) diberikan akuades 2 mL/ekor/hari, esktrak etanol daun mimba 2 mL/ekor/hari (P1), dan sediaan nanokitosan ekstrak etanol daun mimba 2 mL/ekor/hari (P2). Variabel yang diukur adalah tebal endometrium, jumlah kelenjar uterus, diameter uterus dan bobot uterus. Data tebal endometrium, jumlah kelenjar uterus, diameter uterus, dan bobot uterus dianalisis dengan uji ANOVA pada taraf kepercayaan 95%. Hasil penelitian menunjukkan bahwa tebal endometrium, diameter uterus, jumlah kelenjar uterus, dan bobot uterus P0 normal, berbeda tidak nyata (P >0,05) terhadap P1 dan P2. Kesimpulan dari penelitian ini bahwa pemberian sediaan nanokitosan ekstrak etanol daun mimba memiliki potensi yang sama dengan ekstrak etanol daun mimba dalam memengaruhi struktur histologi uterus tikus putih.

***Kata Kunci**: Mimba; Nanokitosan; Uterus*

Permalink/DOI: https://doi.org/10.15408/kauniyah.v19i1.39811

INTRODUCTION

Neem (*Azadirachta indica* A. Juss) is one of the plants that has been proven by the community to have many benefits (Cahyaningsih & Yuda, 2020). Supriyanto et al. (2017) state that neem leaves contain tannins, saponins, flavonoids, and terpenoids. These compounds have a variety of extensive biological activities and benefits, such as antimicrobial, anti-inflammatory, and antioxidant (Pokhrel et al., 2015; Agustin et al., 2016; Ravishankar et al., 2018). Widyaningsih et al. (2019) state that flavonoids, steroids, triterpenoids, alkaloids, tannins, and saponins have efficacy as antifertility compounds.

Antifertility compounds can prevent fertility through disorders in several normal reproductive mechanisms that can occur in males or females (Dabhadkar et al., 2015). Antifertility compounds have two ways of working in disrupting the reproductive system, among others, by damaging cells in organs that have a role in the reproductive system through the cytotoxic and cytostatic effects of these compounds. The compound antifertility also works by disrupting the function or the hormone system in the reproductive system so that the reproductive physiology process is disrupted (Handayani et al., 2018). Treatment of antifertility compounds for a long time can cause atrophy of the uterus and ovaries, leading to a decrease in the fertilization process, interference with cell division implantation disorders of zygote implantation (Rusmiati, 2010).

The uterine is a female reproductive organ that functions as a place to receive and develop the fertilized ovum. The uterine histological structure is composed of three layers: namely, endometrium, myometrium, and perimetrium (Harlita et al., 2015). The endometrium is a dynamic tissue layer or can change during the estrous cycle. Changes in the endometrium occur in response to the production of reproductive hormones by the ovary. These changes can be a marker of changes in the levels of reproductive hormones that occur in animals (Chumduri & Turco, 2021). The thickness of the endometrium thickness is influenced by fluctuations in the levels of the hormone estrogen and progresterone in each phase in the estrous cycle (Narulita et al., 2017).

The results of Alfiyanti et al. (2019)'s research prove that the treatment of ethanol extract of neem leaf at a dose of 14 mg/kg BW given orally for 21 days can affect the fertility of female mice by reducing the thickness of the endometrium. Rusmiati (2011) states that low endometrial thickness can cause disruption of zygote implantation. The thinner endometrial layer will reduce the occurrence of pregnancy.

The purity, stability, application, and target of sending bioactive compounds are influenced by the extraction and encapsulation process carried out. Encapsulation can protect bioactive components from environmental conditions and ensure sustainable release in effective locations. Encapsulation is a procedure for holding active compounds in other compounds known as wall materials, producing continuous film layers to protect active materials. This process results in making particles with sizes ranging from nanometers to millimeters (Bakshi et al., 2022).

Nanoparticles are colloidal particles or solids that have a diameter of 100–1,000 nm. Nanoparticles are used as a system of bioactive compound delivery to the target. The use of nanoparticles can increase the stability of compounds from environmental degradation (oxidation, enzymatic decomposition), improve the absorption of compounds such as macromolecules. Facilitate the handling of toxic materials, reducing the effect of irritation of active substances on the digestive tract, and modifying the release of active substances (Fajriyah et al., 2021). Chitosan is the result of the extraction of crustacean animal exosceleton waste. Chitosan has biodegradable, biocompatible, non-immunogenic, and non-carcinogenic properties that make chitosan suitable for pharmaceutical technology (Safitri et al., 2014). Chitosan as a nanoparticle packaging also has many advantages, such as being non-toxic, relatively stable during use, and can be a matrix of various types of drugs and plant extracts. Every material or extract of a compound can be packaged in the form of nanoparticles with specific formulas (Sonin et al., 2020).

Research by Estuningtyas et al. (2018) proves that the packaging of plant material as a drug in the form of nanoparticles using chitosan does not cause toxic effects on mice. The formula given to test animals is around 12.5–25 mg of plant extracts in 2 mL of chitosan nanoparticles. Zulfa et al. (2014) also prove that the treatment of traditional medicinal ingredients that are packaged in the form

of nanoparticles using chitosan with formula 2:1:0.1 (ethanol extract : chitosan : TPP) can increase the effectiveness of active components in the extract and not be toxic to a dose of 100 mg/kgBW/day on mice. Based on this description, it is necessary to conduct research on the effect of nanocitosan preparations, ethanol extracts of the neem leaf (*A. indica* A. juss) on the histological structure of the uterine of white rats (*Rattus norvegicus*).

MATERIALS AND METHODS

This study was conducted for 8 months in the Biology Laboratory of Animal Structure and Function of the Department of Biology, Faculty of Science and Mathematics, Diponegoro University, Semarang. Using a Complete Randomized Design (CRD) with 3 treatment groups, namely control with 2 mL/animal/day (P0) distilled water, ethanol extract of neem leaves with a dose of 14 mg/kgBW in 2 mL/animal/day (P1), and nanochitosan preparation of neem leaves 2 mL/animal/day (P2). Each treatment is carried out 4 repetitions. The test material is given orally for 21 days. Health Research Ethics Commission, Faculty of Medicine, Diponegoro University, Semarang, with No.50/EC/H/FK-Undip/VI/2022 has examined this research.

The tools used in this study are a set of maintenance cages and equipment, places of feed, drinking places, gloves, analytical balance, digital scales, magnetic stirrer, gavage needles, 3 mL syringes, measuring cups, petri dishes, a set of surgicals (dissecting set), a set of tools for making nanoparticle preparations, a set of tools for making uterine histological preparations by paraffin methods and hematoxylin eosin (HE) staining, and a set of observation tools of uterine histology structures.

The materials needed in this study are test animals in the form of white rats (*R. norvegicus*), 96% ethanol extract of neem leaves, a set of ingredients for making nanocitosan preparations, distilled water, rice husk, drinking water, mouse feed (in the form of standard feed (Charoen Pokphand Indonesia T-51)), physiological salt solution (NaCl), aluminum foil, a set of chemicals for making uterine histological preparations by paraffin method and hematoxylin eosin (HE) staining methods, a set of observation the uterine histology structure.

Preparation of Cage and Test Animals

The cage was washed using soap, then dried. The dry cage is given rice husk as a base and, given a place for feeding and drinking. Rats are acclimated for 1 week so that mice adapt to the new environment so that experimental animals are obtained with healthy conditions, then given fed and drink *ad libitum.*

Extraction of Neem Leaves

Neem powder is extracted by the maceration method using 96% ethanol solvent for 3 days. The extraction process is carried out until the extract becomes colorless. The extract is then concentrated by evaporation to obtain a half-solid mass and free of solvents. The extract is ready to be used as an experimental test material (Sitasiwi et al., 2017).

Preparation of Nanocitosan Ethanol Extract Neem Leaves

Chitosan is dissolved by thinning 2 mg of chitosan with 1% acetic acid using a magnetic stirrer. Sodium tripolifosphate solution (NATPP) is made by dissolving as much as 2 mg of sodium tripolifosphate in every 1 mL of distilled water using a magnetic stirrer. Nanocitosan preparations, ethanol extract of neem leaf is made by adding lactose to dry extract of ethanol extract with a ratio of 1 part of the thick extract and 2 parts of lactose. Making dry extracts is done by means of a mixture of thick extract and lactose, then as much as 10 g are dissolved in 200 mL N-Hexane. The mixture is evaporated at 80 °C for 1–2 hours. The manufacture of nanoparticles is done by dissolving ethanol extract powder into NATPP, homogenized with a magnetic stirrer at a speed of 1,200 rpm with a temperature of 60 °C for 5 minutes then ultrasonification at 30 °C for 30 minutes. Chitosan solution was added by dripping while homogenizing by using a magnetic stirrer (Sitasiwi et al., 2023a).

Giving Treatment

Rats are treated in a separate cage with a density of 4 animals per cage. The treatment is carried out after acclimation for 7 days. The treatment is done by gavage with a sonde needle, each with a volume of 2 mL/animal/day in the morning for 21 days. Measuring the rats weight is carried out at the beginning of the treatment, every 7 days once, and at the end of the treatment. Feed and drink consumption is measured once every day.

Weighing The Uterine Weight

The uterus were washed first with physiological salt (0.9% NaCl). The uterus were then dried using a tissue and placed on aluminum foil. The uterus were weighed with a digital scale.

Evaluating The Uterine Histology Structure

The uterine is made of histology preparations with the paraffin method using the staining of hematoxylin and eosin (HE). Observations were made by 3 tissue sections for each treatment unit with an optilab microscope at magnification 40×, 100×, and 400×. The data that has been obtained is averaged. Endometrial thickness is measured on the vertical uterine cross-section between the endometrium-miometrium and the endometrial surface (Zhao et al., 2015). Measurement of uterine diameter and endometrial thickness is calculated by adding the maximum size to the minimum size and then dividing it in two according to the Alfiyanti method (2019). The number of uterine glands calculated in the uterine cross-section at each observed slides.

Data Analysis

Data of uterine weight, uterine diameter, endometrial thickness, and the number of uterine glands obtained, normality and homogeneity tests are carried out. Data that follows the normal and homogeneous distribution pattern, followed by a Way ANOVA statistical test at a 95% confidence level. Data analysis was carried out using the SPSS (Statistical Product of Service Solutions) 23 program.

RESULTS

Observation of the histological structure of the rat uterine after treatment with the ethanol extract of neem leaves and the nanochitosan preparation of neem leaves is presented in Figure 1. The uterus consists of three layers namely the perimetrium, myometrium, and endometrium.

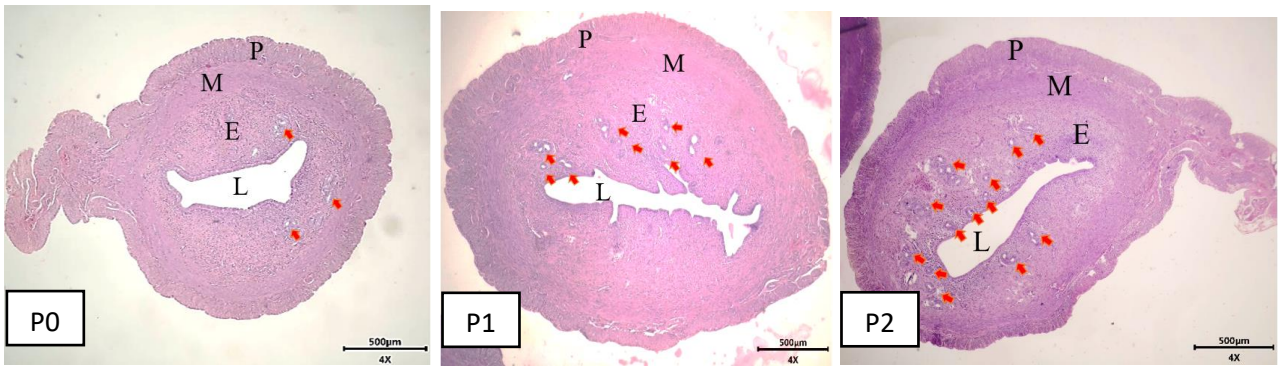

Figure 1. Histological structure of rat uterine (HE staining, 40× magnification). Note: P0 (control group aquades); P1 (treatment group ethanol extract of neem leaves); P2 (treatment group nanochitosan preparation ethanol extract of neem leaves). Perimetrium (P), miometrium (M), endometrium (E), lumen (L), and red arrows indicate the uterine glands

The results of the average statistical analysis of the uterine weight, uterine diameter, endometrial thickness, and the number of rat uterine glands after treated with nanochitosan preparation of neem leaves (*Azadirachta indica* A. juss). In each treatment group, with a treatment time of 21 days using the test One Way Anova at a 95% confidence level with SPSS version 23, can be seen in Table 1. The average of endometrial thickness, number of uterine glands, uterine diameter, and uterine weight of white rats based on the results of the ANOVA test were not significantly

different (P >0.05). These results indicate that the administration of ethanol extract of neem leaves and nanochitosan preparation ethanol extract of neem leaves, with a dose of 14 mg/kg/BW, gives the same effect on endometrial thickness, the number of uterine glands, uterine diameter, and uterine weight of rats.

Table 1. The average of uterine weight, uterine diameter, endometrial thickness, and the number of uterine glands after treating the nanochitosan preparation ethanol extract of neem leaves (Azadirachta indica A. juss) for 21 days

Variables	Treatment		
	P0 ($\bar{X}$ ± SD)	P1 ($\bar{X}$ ± SD)	P2 ($\bar{X}$ ± SD)
Uerine weight (g)	0.21 ± 0.10	0.29 ± 0.09	0.22 ± 0.09
Uterine diameter (µm)	2541.69 ± 720.05	3233.63 ± 307.71	2337.86 ± 716.93
Endometrial thickness (µm)	437.36 ± 116.81	531.97 ± 75.06	468.32 ± 123.59
Number of uterine glands uterus	21.66 ± 8.41	34.89 ± 10.72	41.65 ± 10.40

Note: P0 (control group with distilled water in 2 mL/animal/day); P1 (treatment ethanol extract of neem leaves with a dose of 14 mg/kgBW in 2 mL/animal/day); P2 (treatment group nanochitosan preparation ethanol extract of neem leaves 2 mL/animal/day)

DISCUSSION

The endometrium is a regenerative and dynamic tissue. Chumduri and Turco (2021) stated that the endometrium is a regenerative and dynamic tissue that can undergo changes throughout the estrous cycle. Endometrial changes occur in response to hormones produced in the ovaries. Hakameri et al. (2020) stated that the vascularization and thickness of the endometrium vary depending on the levels of ovarian hormones, namely estrogen and progesterone.

Neem leaves contain secondary metabolic compounds in the form of flavonoids, tannins, and saponins. Supriyanto et al. (2017) stated that neem leaves contain tannin, saponin, flavonoid, and terpenoid compounds. Setyawati et al. (2021) stated that flavonoids (especially isoflavones), saponins, and alkaloids have a structure that resembles the hormone estrogen, which is called phytoestrogen.

Phytoestrogens can stimulate the proliferation of cells in the endometrium by binding to estrogen receptors (ER). Paterni et al. (2014) stated that phytoestrogens can recognize and bind to estrogen receptors. Estrogen receptors are found in the nuclear membrane and plasma membrane of target organs and have almost the same activity as estrogen in the body. Farkas et al. (2022) stated that the estrogen receptor is mostly found in the cytoplasm, and after ligand binding it will be translocated to the nucleus to become a cytonuclear receptor. Phytoestrogens become competitors of estrogen in binding to the ER, which can make estrogen unable to bind to the ER which resulting in an increase in free estrogen in the uterus. Lephart (2015) states that phytoestrogens are competitors of endogenous estrogen in occupying estrogen receptors so that estrogen cannot bind to the receptor, resulting in an increase in free estrogen.

The estrogen receptor that plays a role in the proliferative activity of cells in the endometrium is ERα. This is in accordance with the opinion of Narulita et al. (2016) that ERα is the estrogen receptor most found in the endometrial epithelium and stroma. Estrogen will bind to ERα which makes the receptor active which leading the proliferation. Active receptors will stimulate mRNA expression to carry out protein synthesis needed for the cell proliferation process. Estrogen binds to the ER, then activates the receptor to bind to the binding site on the DNA chain. DNA that binds to ER will stimulate the synthesis and expression of mRNA in the form of protein synthesis so that target cell activity increases and proliferation occurs.

Estrogen binds to stromal ERα and binds to tyrosine kinase in the epithelium. The binding between the receptor and tyrosine kinase will activate the paracrine factor epidermal growth factor (EGF). The EGF complex and receptor tyrosine kinase will activate protein kinases in the cell cytoplasm. The activated protein kinase is thought to be mitogen-activated protein kinase (MAPK), which is the main signal for transcription and translation activation which resulting in protein synthesis. Synthesized protein is needed for the process of mitosis in epithelial cells. Mitosis that

occurs in epithelial cells will cause the epithelial cells to proliferate to an optimum level and increase the thickness of the epithelium (Narulita et al., 2016).

Elevated estrogen levels can cause negative feedback to the hypothalamus, resulting in decreased GnRH secretion. Decreased GnRH secretion will inhibit the secretion of the hormones LH and FSH by the anterior pituitary which causes disruption or reduction in estrogen secretion in the ovaries. This is in accordance with the opinion of Alfiyanti et al. (2019) that increasing estrogen levels will cause negative feedback on the hypothalamus-pituitary-ovary axis, which results in decreased FSH and LH secretion. The hormones LH and FSH play a role in the synthesis of estrogen and progesterone in the ovaries. Disturbed estrogen synthesis results in impaired proliferation of the cells that make up the endometrium.

Neem leaves contain various compounds that have cytotoxic properties. The cytotoxic effect of neem leaves can damage and disrupt the metabolism of the cells that make up the endometrium, which hinders the cell proliferation process. This is supported by the opinion of Alfiyanti et al. (2019) that changes in endometrial thickness are thought to also be caused by the presence of cytotoxic compounds in the ethanol extract of neem leaves.

Saponins can damage cells by increasing membrane permeability which results in cell leakage accompanied by the release of intracellular material. Saponins bind to membrane sterols. Sterols bound to the membrane will be released from the cell membrane, resulting in disruption of ion transport and cell membrane permeability (Mwangengwa et al., 2021). Alkaloid compounds are also thought to cause a decrease in endometrial thickness. Alkaloids have estrogenic properties and are toxic and antiproliferative. The antiproliferative properties of alkaloids are thought to inhibit proliferation in the endometrium (Hakameri et al., 2020).

The toxic compounds in neem leaves do not have a significant effect on the histological structure of the uterus. This insignificant effect is thought to be because the dose used in this study was still within safe limits. The dose given in this study was 14 mg/kg/BW. This is supported by research by Kupradinun et al. (2010), who explained that the aqueous extract of neem leaves was not toxic to mice up to the dose of 1 g/kg, and the LD50 value of the ethanol extract of neem leaf in male rats was 4.57 g/kg.

Nanoparticle testing on the nanochitosan preparation from ethanol extract of neem leaves using Particle Size Analyzer (PSA) showed that the extract material was successfully made into nanoparticles. NaTPP: chitosan has a size of 202.3 nm. 1 NaTPP-chitosan: 0.5 ethanol extract of neem leaves measures 324.9 nm, while the material 1 NaTPP-chitosan: 1 ethanol extract of neem leaves measures 297.3 nm (Sitasiwi et al. 2023b). The preparation of nanochitosan from ethanol extract of neem leaves is successful because it produces particles with a size ranging from 300 nm. This size can deliver the phytochemical compounds contained in the test material to the target organs. This is supported by the statement of Fajriyah et al. (2021) that *Moringa* leaf extract nanoparticles have a good particle size in a drug delivery system because they are <300 nm in size.

The use of nanoparticle preparations in neem leaf ethanol extract can increase the number of bioactive compounds in neem leaves which can reach targets such as flavonoid compounds which acts as antioxidants. Flavonoids can ward off free radicals so they can help reduce cell damage and cell regeneration. This is in accordance with the opinion of Simbolon et al. (2022) that the treatment material provided in nano size increases the number of bioactive compounds in the ethanol extract of neem leaves that reach the target organs. The flavonoids in neem leaves can ward off free radicals. Reducing free radicals in the tissue will help cells regenerate to their original state. Research by Handayani et al. (2022) showed that the improvement in the histological structure of the islets of Langerhans in the P3 treatment group (neem leaf ethanol extract nanochitosan preparation with a ratio of 1:1) was thought to be influenced by increasing neem bioavailability and the distribution of neem antioxidant content, which was able to be absorbed well by the pancreas. The treatment material given in nano size is thought to cause an increase in the number of bioactive compounds contained in the ethanol extract of neem leaves that reach the target organs.

Phytoestrogens enter the digestive tract through gavage treatment. These compounds are absorbed by the intestine and then enter the capillaries in the intestine. The capillaries will drain the

compound to the portal vein, which will carry the compound to the liver. Antifertility compounds will later be distributed throughout the body by the heart, including the uterus. Adani et al. (2017) in their research stated that the mechanism for the entry of antifertility compounds begins with the absorption of the compound by enterocyte cells containing capillaries. The capillaries will carry blood into the portal vein which then goes to the liver. Antifertility compounds from the liver are distributed throughout the body, including the endometrium. Phytoestrogen compounds that enter the liver will be detoxified. Singh and Cauhan (2014) stated that the active compounds in the ethanol extract of neem leaves will undergo detoxification in the liver. Detoxification in the liver can change the function of active compounds in the neem leaves extract, such tannins, saponins, and flavonoids, in regulating the reproductive hormones.

Flavonoids that enter the liver will be detoxified. This detoxification process means that flavonoids have a less significant effect and do not have a real influence on the regulatory function of reproductive hormones. The non-significantly different results in endometrial thickness, number of uterine glands, uterine diameter, and uterine weight of female white mice between the control and treatment groups were thought to be because the effects were less significant and did not have a real influence on the regulatory function of reproductive hormones. This mechanism is thought give an insignificantly different effect between the control and the treatment group. This is in accordance with the opinion of Avycena et al. (2020) that the function of the hypothalamus is not affected by the flavonoid content in the ethanol extract of neem leaves because the flavonoid compounds have undergone a detoxification process in the liver, which makes the secretion of gonadotrophins, such as LH and FSH, were not disturbed.

The number of uterine glands in the three treatment groups was normal. The normal number of uterine glands in rats is in the range of 21–46. Research conducted by Dair et al. (2008) and Salleh et al. (2013) showed that the number of normal rat uterine glands ranges from 10–52 per section. Uterine weights in the three treatment groups were normal. Normal uterine weight in rats are in the range of 0.21–0.29 g. Research conducted by Memudu and Oluwole (2021) and Muchtaromah et al. (2020) shows that normal uterine weight in rats are in the range of 0.20–0.34 g.

The size or thickness of the endometrium is in line with the uterine glands, the diameter and the weight of the uterus. The diameter and weight of the uterus are influenced by the thickness of the endometrium. The endometrium is a tissue that is very responsive to the estrogen hormone. Busman et al. (2021) in their research stated that endometrial thickness is the main factor that influences the weight and diameter of the uterus. The endometrium layer of uterine is the most sensitive tissue to estrogen. Estrogen can cause proliferation of the endometrium. Endometrial thickness is a factor influencing the diameter and weight of the uterus. Schweikart et al. (2014) stated that phytoestrogens as exogenous estrogens originating from outside the body, at certain doses can cause significant changes in uterine weight in uterotrophic bioassay studies. Factors that can cause differences in uterine weight such as estrogen agonist properties, cell proliferation, drug exposure pathways, vascular permeability, water retention, and animal strain.

CONCLUSION

Treatment of nanochitosan preparations from ethanol extract of neem leaves (*Azadirachta indica* A. Juss) had the same potential as ethanol extract of neem leaves on uterine weight, uterine diameter, endometrial thickness, and number of uterine glands between the treatment groups. Future research needs to analyze the levels of ovarian hormones, namely estrogen and progesterone, and observe the estrous cycle that occurs in female mice after administering nanochitosan preparations from the ethanol extract of neem leaves (*A. indica* A. Juss), which plays a role in regulating the estrous cycle and changes in the structure of the reproductive organs in rats.

ACKNOWLEDMENTS

Thanks to the Faculty of Science and Mathematics, Diponegoro University, for supporting facilities and costs through funding sources other than the APBN, Faculty of Science and Mathematics, Diponegoro University for the 2022 fiscal year in accordance with the Assignment Letter for Implementing Research Activities Number: 30191D/UN7.5.8.2/PG/2022.

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