Neem leaves extract (*Azadirachta indica A. Juss*) on male reproductive system: a mini-review

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Abstract. Neem (*Azadirachta indica A. Juss*) is the tropical plant of the family *Meliaceae* that has been used as medicine. Almost all parts, i.e., leaves, stems, bark, roots, seeds, and flowers can be used to medicate multiple diseases. Apart from medicine and other health benefits, neem is a very promising plant as it could be used to reduce the rate of population growth. The aim of this review is to figure out the effect of neem leaves on the male reproductive system which can be developed into safe and reversible male contraception. The article review used online databases to conduct keyword searches and investigate the active compounds in neem leaves that could serve as antifertility, the effect of neem leaves on reproductive hormones and spermatogenesis. Several studies show that neem leaves can affect the male reproductive system by interfering spermatogenesis, e.g., reducing the amount, motility, and morphology of the spermatozoa, affecting the structure and function of the testes which cause a decrease in the quality of spermatozoa. Abnormal spermatozoa could reduce spermatozoa ability to fertilize. Thus neem leaves can be proposed as male contraceptives.

Keywords: neem leaves, testosterone, follicle-stimulating hormone, spermatogenesis, contraception.

1. Introduction

Controlling population growth is vital to improving human well-being [1]. Contraception is needed to control the rate of increase in the world population mainly in developing countries. Neem is one of the plants that can be developed into male contraception. The use of traditional neem to treat diseases has been done for a long time [2]. Neem (*Azadirachta indica A. Juss*) is a highly beneficial plant found in a country with a tropical climate like Indonesia [3]. Neem is part of tropical plants from the Meliaceae family and has been used for thousands of years as medicine. Some parts of neem plants can be
developed to be medicine to treat multiple diseases [4] including acute and chronic diseases, especially in Asia and Africa [5].

Neem plants grow in various countries in the world, including Asia, Africa, America, and Australia [6]. The botanical name of the neem plant is Azadirachta indica A. Juss. The Latin name for neem is Azadirachta indica. Neem has been widely used by humans since prehistoric times to treat various diseases. The plant is extraordinary, and it is referred to as "21st-century trees" by the United Nations. Neem is also referred to as "a tree for solving global problems" because it has many benefits, and it can treat various diseases [7].

Neem Leaves serve as antibacterial, antifungal, anti-inflammatory, immunomodulatory, antihyperglycemic, antiulcer, antifungal, antibacterial, antimutagenic, anticancer, antimalarial, antiviral, antioxidant [5,8,9], antifertility [3,10,11], contraception [9], herbal contraception through spermicidal and non-hormonal activities without any side effect [12]. Various active compounds contained in the neem leaf have an antifertility effect [13]. Women in the northwest Madagascar village consume neem leaves to prevent pregnancy. In Gambia and Ghana, neem leaves are made as tea drink to stop pregnancy at the age of the first 2-3 [14].

Neem plants can grow on rocky and dry soil, in different climatic conditions up to 700m in height and could grow for more than 200 years. Neem plants grow a lot in areas with rainfall from 450 to 1200 mm, temperatures from 0 to 49 °C and pH varies from and 4-10. Neem can also grow in areas with very low rainfall of 150-200 mm [3].

Neem plants can adapt to climate, geographical, and topographic conditions. It does not require much sunlight and water for growth. Neem plants grow in almost all types of soil, including alkaline, clay, and saline soils [6]. It is one of the most studied medicinal plants and is used for health. This plant is widely cultivated throughout the world due to its adaptive to various climate conditions [15].

The aim of this study is to determine the effect of neem leaves on testosterone, follicle-stimulating hormone (FSH) and the process of spermatogenesis, thus, neem leaves can be developed into one of the male contraception.

2. Neem leaf active compound
Neem plants contain more than 300 primary and secondary metabolites. The main compounds consist of carbohydrates, proteins and fat derivatives, while secondary metabolites include flavonoids, steroids, saponins and alkaloids [9]. The neem active compound is classified into two main groups, namely isoprenoid and non-isoprenoid groups. The isoprenoid group includes diterpenoid and triterpenoid, namely protomeliasin, limonoid, neem, gedunin, azadiron, and azadiractin derivatives. Some examples of non-isoprenoid are proteins, sulfur, carbohydrates, dihydrochalcone, polyphenols, and glycoside [3].

The other examples of non-isoprenoid are coumarin, tannins, aliphatic compound and phenolic acids [16]. Neem also contains flavonoids, alkaloids, and saponins [17]. Phytochemical test results show that Neem leaf extract contains phenol compounds [18], flavonoids, saponins, steroids, alkaloids, amino acids, and tannins [19]. Furthermore, in the gas chromatography-mass spectroscopy (GC-MS) analysis shows that neem leaf extract contains hydrocarbon, phenolic, terpenoid, alkaloids, and glycoside [20].

The results of phytochemical evaluation using high-performance thin-layer chromatography (HPTLC) shows that the neem leaf extract contains stigmasterol, terpinene-4-ol, sugiol, 4-cymene, nimbol, α-terpinene, and vitamin E [21]. Neem leaves also contain isoprenoid, flavanones e (8,3-di-isoprenoid-5,7-dihydroxy-4'-methoxyflavanone), non-isoprenoid, and meliacin (2,3-dehydrosalanol) [22,23]. The results of the methanol extract of neem leaves contain triterpenoids, 22, 23-dihydrodronimocinol, and desfurano-6 α hydroxyazadiradione [24]. In addition, neem leaves also contain azadiradione [25], tetraterpenoids (14.15-epoxynimonol) [26], and flavonoids [27].
3. Method
This research is based on the ScienceDirect database, Springer Nature and other databases using a combination of different keywords such as neem leaf active compounds and others. Also, relevant articles are selected to be reviewed.

4. Result and discussion

4.1. The effect of neem leaves extract on testosterone
Testosterone is the main hormone that regulates spermatogenesis in the testis. Testosterone is produced by Leydig cells as a result of stimulation of luteinizing hormone (LH) and acts as a paracrine which diffuses into the testicular seminiferous tubules [28]. In spermatogenesis, testosterone regulates the proliferation of spermatogonia. Testosterone allows spermatocytes to complete the division of meiosis to form spermatids. Lack of testosterone receptors in animals causes disruption of germ cell formation and becomes infertile and become infertile. Besides, androgenic stimulation in peritubular myoid cells is also crucial for the normal development of spermatozoa germ cells [29].

The process of maturation of germ cells into spermatozoa in spermatogenesis requires testosterone. Low testosterone levels in the testes cause cessation of spermatogenesis before the completion of the meiosis process, which disrupts spermatozoa production. Testosterone acts by spreading to target cells and binding to specific intracellular receptor proteins in the cytoplasm and nucleus [30]. Testosterone is maintained at a high level in the testes which is around 25-125 (340-2,000 nM) times that of plasma (8.7-35 nM) and acts through its receptor (AR) in Sertoli cells to support germinal cell development. The absence of testosterone causes infertility due to disorders of meiosis in the diploctene or pachyctene stages in spermatogenesis [31,32]. Testosterone is needed for a critical process in spermatogenesis, supports the completion of meiosis, elongated spermatid adhesion, and maturation of spermatozoa [33].

The results show that neem leaf extract affects testosterone concentration. The male rats that were given daily intraperitoneal doses of neem leaf with doses of 50, 100 and 150 mg/kg of body weight for 15 days causing a decrease in testosterone concentrations [34]. In addition, neem leaf extract given to male rats with a dose of 200 mg/kg of body weight for 28 days also causes a decrease in testosterone concentration [35].

Other studies also indicate that the use of neem leaf extract causes a decrease in the concentration of luteinizing hormone (LH) and histological changes of the anterior pituitary. This finding shows that neem leaf extract reduces the concentration of the LH hormone, which will affect testosterone in male rats and will influence spermatogenesis [36]. Joshi et al. [37] found that there were changes in tissue and biochemistry in the testes of rats after taken neem leaf extract; however, when the extract was stopped after 8, 16 and 24 days the changes returned to normal. This finding shows that the antiandrogenic properties of neem leaf extract are reversible.

4.2. The effect of neem leaves extract on follicle-stimulating hormone (FSH)
Follicle-stimulating hormone (FSH) is a group of glycoprotein hormones, and FSH receptors (FSH-R) are the connected receptors of G proteins; it is also called seven-transmembrane receptor or heptahelical receptors [38,39]. Sertoli cells have receptors for the hormone FSH in regulating spermatogenesis. FSH receptor mutations are associated with a decrease in the number of spermatozoa. FSH hormones, both singly and synergistically with testosterone, can prevent apoptosis of testicular germ cells [40].

FSH hormone plays an important role in all spermatogonia cycles and acts to optimize the production of spermatogonia germ cells [29]. The FSH hormone binding to receptors in Sertoli cells activates all the factors needed for survival and differentiation of germ cells [41]. The FSH function is the most important part of the complex hypothalamus-pituitary-gonadal axis, and its feedback control mechanism regulates testicular function. The FSH hormone plays an important role in determining the number of Sertoli cells and maintaining spermatogenesis. In addition to the proliferation and differentiation of Sertoli cells, FSH also regulates interactions between cells and genes needed for metabolism and transport of regulatory substances and nutrients from Sertoli cells to germ cells. In rat with FSH
deficiency, the number of spermatozoa was significantly reduced. In humans, FSH-R receptor inactivation causes azoospermia and inhibits spermatogenesis [42].

The results showed that the neem leaves extract affected FSH concentrations. The administration of neem leaf extract in male rats with a dose of 50, 100 and 150 mg/kg body weight every day for 15 days showed a decrease in FSH concentration [34]. Other studies found that the neem leaves at a dose of 200 mg/kg body-weight that was given to male rats for 28 days also decrease the FSH concentration [35]. The administration of 200 mg and 400 mg/kg bodyweight of neem leaf extract in male rats also decrease the FSH concentration. Decreasing FSH concentration will disrupt the process of spermatogenesis, which will affect spermatozoa production [36].

4.3. The effect of neem leaves extract on spermatogenesis
Neem leaves have an antifertility effect [11]. Neem leaf extract can affect spermatogenesis through the antifertility activity. This occurs because of several histological changes, disorders of spermatogenesis, and changes in reproductive hormone levels after the administration of neem leaf extract [9].

The results of the study by Mishra and Singh [43] showed that administration of Neem leaf extract in male rats orally at a dose of 50 and 100 mg/kg body weight did not cause changes in the histological epididymis, but changes occurred at doses of 200 mg/kg body weight. Neem leaf extract caused damage to the seminiferous tubules, chromatin condensation disorders, germinal cell degeneration, which caused disruption to spermatogenesis and thus reduced motility, morphology, and the number of spermatozoa of male rats. But after 42 days of cessation of treatment, the male reproductive organs returned to normal. Giving extract of neem leaves causes a reversible change in the reproductive organs of male mice.

Other studies have shown that administration of neem leaf extract in male rats at a dose of 100 mg orally caused intracellular abnormalities and vacuolization within Sertoli cells, reduced cytoplasmic inclusion in Leydig cells and disruption in the final stages of spermatids. Ultrastructural changes due to the administration of neem leaf extract can affect spermatogenesis [44].

Giving extract of neem leaves at a dose of 500 mg/kg body weight causes atrophy of the seminiferous tubules with widened space between cells, Leydig cells degenerate, the number of Leydig cells and their core diameter decreases significantly. The antiandrogenic and anti-spermatogenic properties of neem leaves cause a reduced fertilization ability of spermatozoa [45]. The effect antifertility of neem leaf extract on male rats at a dose of 100 mg/kg body weight for ten weeks caused impaired spermiogenesis, deformity, and sperm motility [46].

Giving ethanol extract of neem leaves orally in male rats at doses of 0.5 mg, 1.0 mg, and 2.0 mg/kg of body weight for six weeks causes chromosome damage in the meiosis stage, disruption of gene regulation responsible for spermatozoa formation. In addition, there was a decrease in the number of spermatozoa and an increase in spermatozoa abnormalities [47]. Another study found that provision azadiractin in neem leaves with a dose of 5 mg, 10 mg, and 50 mg/kg body weight showed no toxicity in male mice reproduction [48].

Neem leaf extract can inhibit spermatogenesis which in turn reduces the motility and concentration of spermatozoa. After 4-6 weeks of cessation of the administration, the changes become normal again [49]. In rabbits fed neem leaf-based food caused a decrease in semen volume and the number of spermatozoa and an increase in morphological abnormalities of spermatozoa. In addition, it was reported that there was no decrease in libido in rabbits fed neem-based foods [50]. Neem leaf extract also had a strong spermato-toxic effect. The effects of spermato-toxic extracts of neem leaves caused 100% immobilization and mortality of human spermatozoa in a dose of 3 mg within 20 seconds [51,52]. Khan et al. [53] study found that giving neem leaf extract caused changes in Leydig cell structure and seminiferous tubules of the testes, reducing sperm motility and sperm density.

Several other studies suggest that the antifertility of neem leaf extract is not associated with changes in testosterone concentration. The mechanism of neem leaf extract in inhibiting spermatogenesis is not clearly understood [54]. Possible mechanism of action of neem leaf extract in inhibiting spermatogenesis by acting through Sertoli cells [43].
5. Conclusion
Neem leaf extract has an antifertility effect on male rat reproduction. Neem leaf extract affects the concentration of testosterone, follicle-stimulating hormone (FSH), and spermatogenesis. The antifertility effect of neem leaf extract is reversible to the male rat reproduction so it can be developed into a male contraceptive candidate. It needs to be studied further through clinical trials of the effects, and the right dose of neem extract affects the male reproductive system.

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