

Neem (*Azadirachta Indica* L.) and Oocyte Quality



Meenakshi Tiwari¹, Anumegha Gupta¹, Shilpa Prasad¹, Anima Tripathi¹, Pramod K Yadav¹, Ashutosh N Pandey¹, Karuppanan V Premkumar¹, Ajai K Pandey², Tulsidas G Shrivastav³ and Shail K Chaube^{1*}

¹Department of Zoology, Institute of Science, Banaras Hindu University, India

²Department of Kayachikitsa, Faculty of Ayurveda, Banaras Hindu University, India

³Department of Reproductive Biomedicine, National Institute of Health and Family Welfare, India

Submission: March 21, 2017; **Published:** April 19, 2017

***Corresponding author:** Shail K Chaube, Cell Physiology Laboratory, Department of Zoology, Institute of Science, Banaras Hindu University, Varanasi-221005, India, Fax: 091-542-2368174, Tel: 091-542-6702516; Email: shailchaubey@gmail.com

Abstract

Neem (*Azadirachta indica* L.) leaf has been used as a birth control in traditional as well as ayurvedic systems of medicine for a long time. The molecular mechanism by which neem bioactive ingredients regulate female fertility remains poorly understood. Studies carried out on experimental mammals suggest that neem leaf extract induces generation of reactive oxygen species (ROS) in ovarian follicles. The increased ROS level induces granulosa cell apoptosis through mitochondria-mediated pathway. Premature death of encircling granulosa cells directly affect the achievement of meiotic competence and thereby oocyte quality after ovulation. In addition, the increased levels of ROS enter in the follicular oocytes and deteriorate its quality by inducing mitochondria-mediated apoptosis. The granulosa cells as well as oocyte apoptosis within the follicular microenvironment deteriorate oocyte quality. Poor quality directly affects fertilization, implantation and pregnancy rates and increases abortion rate. Thus, based on existing animal as well as human studies, we are in opinion that bioactive ingredients of neem particularly neem leaf extract could be used as reversible herbal female contraceptive during fertility management in human.

Keywords: Aqueous neem leaf extract; Reactive oxygen species; Granulosa cell; Oocyte; Apoptosis; Fertility management

Abbreviations: GV: Germinal Vesicle; M-II: Metaphase-II; ROS: Reactive Oxygen Species

Introduction

Mammalian ovary is a dynamic organ that is responsible for the production of competent oocytes required for successful fertilization and early embryonic development [1,2]. The meiotic competency follicular is achieved within the follicular environment during final stages of folliculogenesis [3-7]. The achievement of meiotic competency starts in diplotene arrested oocytes, possessing germinal vesicle (GV) as well as nucleolus [1,5,8-12]. The diplotene arrest for such a long period is due to transfer of several molecules from encircling granulosa cells to the oocyte [1,6-8]. Pituitary gonadotropin(s) surge disrupt gap junction as well as cumulus-oocyte communication thereby transfer of meiosis inhibitory factors [13-15]. The interruption in the transfer of inhibitory molecules results in meiotic resumption of follicular oocytes *in vivo* in the presence of pituitary gonadotropin(s). The spontaneous resumption of meiosis can be achieved if cumulus-oocyte complexes are removed from ovarian follicles and cultured in appropriate medium for extended period of time [1,6-8]. Meiotic resumption from diplotene arrest occurs due to interruption in the transfer of inhibitory

factors from surrounding granulosa cells to the oocytes under *in vitro* conditions [13-15]. Although meiosis resumes due to physical removal of encircling granulosa cells, oocyte becomes more susceptible towards *in vitro* culture conditions mediated apoptosis [16-20]. This is supported by observations that GV stage (immature) oocytes are more susceptible towards oxidative stress-mediated apoptosis as compared to metaphase-II (M-II) stage (mature) oocytes [9,10,16-20]. The achievement of meiotic competency not only produces a right female gamete but also enhances its ability to resist any adverse changes under *in vitro* culture conditions during various assisted reproductive technology programs.

Achievement of meiotic competency in follicular oocytes starts with resumption from diplotene arrest [8] and ends with the achievement of M-II stage by extruding first polar body in most of the mammalian species [5,6,11,12,21-26]. The successful completion of meiotic competency is prerequisite for the determination of oocyte quality post ovulation. Several factors that are either released from encircling granulosa cells or

from oocyte itself regulate oocyte quality [3,6,7,20,23,26]. Thus, achievement of meiotic competency can easily be targeted to deteriorate oocyte quality post ovulation.

Oocyte is one of the most important factors that directly affect reproductive health of a female. Unfortunately, oocyte has not been targeted for the development of female contraceptives. Today, several drugs are available as female contraceptives but they are steroid based and they do not have 100% efficacy. Due to several side effects, these drugs are not easily accepted by reproductively active females. Hence, it is important to search herbal-based reversible contraceptives that are easily acceptable and show minimal side effects with 100% efficacy.

Neem plant (*Azadirachta indica* L.) is the most important medicinal plant globally. The utilities of this plant are listed in 'Charak-Samhita' and 'Susruta-Samhita' that are foundation of Indian system of natural treatment, ayurveda [27]. Various parts of neem plant are used for treatment of several diseases in traditional as well as ayurvedic systems of medicine globally. The aqueous extract of neem bark has been used to control gastric hypersecretion and gastroduodenal ulcer [28] and leaf extract is used to reduce oral infection and plaque index [29,30].

Neem leaf extract has anti-inflammatory, immunomodulatory, anti-ulcer, anti-hyperglycemic, antiviral, anti-bacterial, anti-fungal, nematocidal, anti-malarial, insecticidal, anti-oxidant and anti-mutagenic properties [31-36]. The apoptosis inducing ability of neem leaf extract has been investigated in cancer cells [37,38]. The neem leaf extract induces cytoplasmic granulation and deteriorates oocyte quality suggesting its potential use for female fertility regulation [39]. The anti-fertility properties of neem extracts have been reported in several mammalian species [34,40].

The neem leaves have been used to avoid pregnancy and as birth control in traditional system of Indian medicine [41-43]. However, the possible mechanism by which bioactive ingredients of neem control fertility remains to be elucidated. Few studies suggest that neem oil inhibits follicular development in rat and induces degeneration of oocyte [44,45]. Further, neem oil inhibits implantation in rats and bonnet monkeys and acts as a reversible contraceptive [42,46]. Neem oil has been used as vaginal herbal contraceptive in human [47]. However, due to unpleasant sharp odour, neem oil is not easily palatable. The aqueous neem leaf extract does not possess an unpleasant characteristic sharp odour of neem. Hence, it could be used as an alternative herbal medicine for fertility regulation in mammals including human [41].

The mechanism by which neem leaf extract acts at the level of ovarian follicles remains unclear. Studies using experimental mammals suggest that neem leaf extract inhibits folliculogenesis and antrum formation in follicles [40,44,45]. The neem leaves extract triggers apoptosis in granulosa cells, reduces number of granulosa cells encircling oocyte and induces oocyte apoptosis

in majority of ovulated cumulus oocytes complexes [17,18]. The aqueous neem leaf extract decrease catalase activity and increases ROS level in rat ovary [10,16-18]. The increased levels of ROS induce overexpression of p53 and Bax proteins. The increased Bax expression modulates mitochondrial membrane potential and induces cytochrome c release. Increased cytochrome c concentration induces DNA fragmentation and granulosa cell apoptosis [6,7,10,16-20].

The granulosa cell apoptosis leads to disruption of gap junctions between encircling somatic cells and oocyte within the follicle. Reduced intercellular communication deprives oocyte from nutrients, maturation-enabling factors, survival factors and induces susceptibility towards apoptosis [10,18-20]. The granulosa cell apoptosis reduces estradiol 17- β level required for development and maturation of oocytes in the ovary during final stages of folliculo genesis. The hypo-estrogenic condition may affect development and maturation of oocytes and trigger apoptosis. Studies from our laboratory suggest that quercetin induces cell shrinkage, membrane leakage, cytoplasmic granulation and cytoplasmic fragmentation in rat oocytes. These morphological apoptotic changes are associated with increased ROS level, overexpression of Bax protein, caspase-3 activation and DNA fragmentation [10,16-18].

Conclusion

Based on the existing information, we propose that neem leaf extract and its bioactive ingredients induce ROS-mediated granulosa cell apoptosis followed by oocyte apoptosis. The neem leaf extract-induced oocyte apoptosis deteriorates oocyte quality that reduces reproductive outcome. The apoptosis inducing property of neem leaf extract makes it a potential candidate for the development of reversible herbal contraceptive.

Acknowledgement

Authors are thankful to Department of Science and Technology, New Delhi, India, for financial assistance (EMR/2014/000702).

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DOI: [10.19080/GJORM.2017.01.555553](https://doi.org/10.19080/GJORM.2017.01.555553)

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