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Clomiphene Citrate as Nanomedicine Assistance in Ovulatory Disorders and Its Hyphenated Techniques †

Tejasweeni Girase [1,*], Javesh Patil [2,*], Aayushi Tatiya [1], Devyani Patil [1] and Mamta Patil [1]

[1] Department of Quality Assurance, PSG Vidya Prasarak Mandal's College of Pharmacy, Shahada 425409, India; email1@email.com (A.T.); email2@email.com (D.P.); email3@email.com (M.P.)
[2] Department of Pharmacognosy & Phytochemistry, PSG Vidya Prasarak Mandal's College of Pharmacy, Shahada 425409, India
* Correspondence: tejasweeni20@gmail.com (T.G.); javesh4u@gmail.com (J.P.); Tel.: +91-9669424536 (T.G.); +91-9923441004 (J.P.)
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Abstract: Nanotechnology has prompted new aspirations for managing modern human challenges. Furthermore, it has been utilized for aid in the prevention, diagnosis, and treatment of ovulatory disorders. Women with ovulatory issues may benefit from formulation using nanotechnology as an alternative possible treatment. Clomiphene citrate is non-steroidal, ovulatory stimulant that acts as a selective estrogen receptor modulator (SERM). It is a triphenyl ethylene stilbene derivative that is primarily used to trigger ovulation in female infertility cases where there is anovulation. Anovulatory infertility is most frequently caused by polycystic ovarian syndrome (PCOS) which is a gynaecological endocrine disorder. Elevated serum concentrations of androgens, LH and insulin are the main features of its endocrine profile. The primary goal of treating PCOS-related infertility is to increase the amount of FSH that is exposed to the ovary, either by antagonizing the estrogenic effects of clomiphene citrate in the hypothalamus or by directly affecting the ovary using recombinant FSH. In about 80% of treated individuals, ovulation is recovered by clomiphene citrate. In this review, we discussed the chemistry and pharmacology of clomiphene citrate as well as the delivery of clomiphene citrate via nanosystems for improving solubility and limiting side effects. The hyphenated techniques for analysing and quantifying clomiphene citrate in solvents and biological samples are also overviewed.

Keywords: clomiphene citrate; nanomedicine; ovulatory disorder; PCOS; hyphenated techniques

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1. Introduction

Ovulatory disorders are the spectrum of conditions that have an impact on a woman's endocrine system and are a major contributor to female infertility. Polycystic ovary syndrome (PCOS) is a hormonal issue that affects women during their reproductive years [1]. Stein and Leventhal are generally acknowledged as the first researchers of polycystic ovarian syndrome; however in 1721 Vallisneri, an Italian scientist, reported a married, infertile woman with shiny, white-surfaced ovaries the size of pigeon eggs. Formal diagnosis criteria weren't offered and generally used until a PCOS meeting sponsored by the National Institutes of Health (NIH) in the early 1990s [2].

The compound currently known as clomiphene citrate was discovered in 1956 by Frank Palopoli and his colleagues in the Merrell chemistry department at the time. The initial outcomes of clomiphene's human clinical trials—at the time known as MRL-41—were published by Greenblatt et al. (1961) in the Journal of the American Medical Association (JAMA) in October 1961. It was the third drug to be submitted to the United States Food and Drug Administration (USFDA) for review in accordance with the regulations.

In 1965, a New Drug Application (NDA) was filed. Clomiphene was then approved in 1967, which marked the beginning of its prescription availability in the USA [3].

An emerging subject called nanomedicine combines medicine and nanotechnology to find novel treatments and enhance those that currently exist. Nanomedicine is the science and technology of applying molecular tools and insights into the human body at the nano scale to identify, treat, and prevent disease and traumatic injury, ease pain, maintain and improve human health. Over the past two decades, we have assisted in the translation of several nanomedicine applications into clinical practice, ranging from medical devices to nano-pharmaceuticals [4,5].

2. An Overview on Ovulatory Disorders

A major contributing factor to infertility is ovulatory disorders. Ovulatory disorder is a term used to describe a class of ailments in which ovulation either does not occur, occurs infrequently, or occurs irregularly. The hypothalamic-pituitary-ovarian (H-P-O) axis often indicates episodic or chronic dysfunction, which is related with ovulatory problems in girls and women of reproductive age [6]. A lack of effectors and dysfunctional ovaries are the results of abnormalities in the hypothalamic pulsatile release of GnRH. Similar to this, pituitary dysfunction prevents the ovaries from being stimulated, which restricts ovulation and follicular development [7].

For timely ovulation, a very precise hormonal balance is needed, including oestrogen, progesterone, luteinizing hormone, and follicle-stimulating hormone (release of egg from the ovary). Hormone imbalance is the primary cause of ovulation disorders. The adhesion of the embryo to the uterine lining can be interfered with by low levels of progesterone. It also raises the possibility of miscarriage. Infertility in women is also attributed to elevated amounts of oestrogen [8].

Anovulation (no ovulation) is a disease where eggs do not mature adequately or have not been released from the follicles of ovaries. Oligo-ovulation, a disorder in which ovulation does not take place on a regular basis and the menstrual cycle may last longer than the typical cycle of 21 to 35 days [9].

The following conditions frequently lead to the ovulatory disorders:

- Polycystic ovary syndrome (PCOS- most common);
- Hypothalamic dysfunction (either hypothyroidism or hyperthyroidism);
- Premature ovarian failure [10].

Polycystic Ovary Syndrome (PCOS)

One of the most frequent endocrine disorders in women appears to be the POLYCYSTIC OVARY SYNDROME (PCOS). Stein & Leventhal gave the first thorough description of the syndrome in 1935.It is defined by an endocrine condition that causes chronic anovulation and is characterised by elevated androgen production and abnormal gonadotropin secretion.It was previously thought to be a benign reproductive illness with the main clinical repercussions being hirsutism and infertility. It is now well known that PCOS has important metabolic effects connected to insulin resistance. In fact, PCOS is a significant contributor to type 2 Diabetes Mellitus (DM) in females. Thus, polycystic ovary syndrome is associated with significant reproductive, endocrine, metabolic, and cardiovascular, morbidity [11].

Menstrual irregularity, such as oligomenorrhoea, and indicators of hyperandrogenaemia, such as hirsutism, acne, and/or obesity, are the defining clinical signs of PCOS [12].

The major abnormalities in this condition are as follows:

1. Abnormal morphology of the ovary, identified bya characteristic hyperechogenic enlarged central stroma and >9 small follicles of 2–9 mm diameter on transvaginal ultrasound examination of the ovaries;

2. Abnormal steroidogenesis, which mainly involves increased androgen production from the ovary and also includes increased progesterone and estradiol production;
3. Hyperinsulinemia, which is strongly associated with anovulation than any other aspect of the syndrome;
4. Abnormal gonadotrophin secretion, which is most frequently seen in women with PCOS as confirmed by ultrasound and is characterised by elevated serum LH concentrations. Women with anovulatory PCOS appear to have a functional deficit in the endogenous activity of FSH [13].

Currently, the 1990 NIH/NICHHD conference's criteria are usually followed to decide if a patient has PCOS by examining and confirming Menstrual dysfunction, Hyperandrogenism (hirsutism, acne, androgenic alopecia), and/or Hyperandrogenemia, along with the exclusion of other associated conditions such hyperprolactinemia, nonclassical adrenal hyperplasia, or thyroid disease [14].

Clomiphene citrate (CC) treatment is a simple, economical, and efficient method of stimulating ovulation with no or little side effects, it has been widely utilised as a first-line treatment for anovulatory PCOS.The CC binds to oestrogen receptors on the hypothalamus, exhibiting an antiestrogenic action.This triggers a gonadotropin releasing hormone pulse that causes gonadotropin secretion from the anterior pituitary gland [15].

3. Drug Profile of Clomiphene Citrate

3.1. Physicochemical Properties

The IUPAC name of clomiphene citrate is 2-[2-chloro-1,2-diphenylethenyl)-phenoxy]-N,N-diethylethanamine2-hydroxy-1,2,3-propanetricarboxylate with empirical formula C26H28CINO.C6H8O7 and molecular weight 598.1. Clomiphene citrate is a white to pale yellow, odourless powder which is freely soluble in methanol and slightly soluble in water [16].

From a chemical aspect clomiphene is a tri-phenylene derivative that shows structural similarities with diethylboestrol. Two isomeric variants of clomiphene, cis and trans, are referred to as zuclomiphene and enclomiphene, respectively, in the current nomenclature. The racemic combination used in the commercial preparation contains 40% zuclomiphene and 60% enclomiphene [17]. Although enclomiphene is completely anti-oestrogenic, zuclomiphene exhibits moderate oestrogenic and anti-oestrogenic effects. The ability of zuclomiphene to trigger ovulation is almost five times greater than that of enclomiphene. Figure 1. describes the isomers of Clomiphene [18].

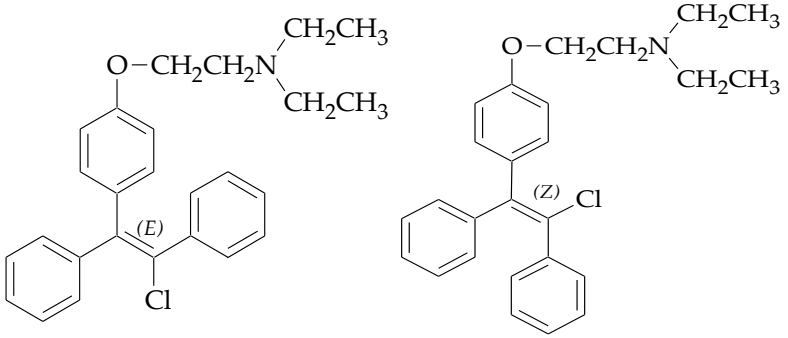

Figure 1. Enclomiphene and Zuclomiphene as trans and cis isomers.

3.2. Pharmacology and Mechanism

Clomiphene acts as an ovulatory stimulant and is a selective estrogen receptor modulator (SERM). It exerts estrogenic and anti-estrogenic effects by selectively binding to oestrogen receptors in the brain, ovary, endometrium, and cervix. Moreover, it has a

partial oestrogen agonistic effect in the hypothalamus, which inhibits estrogenic negative feedback and increases gonadotropin levels. It boosts luteinizing hormone and follicle-stimulating hormone secretion, raising serum levels of testosterone in the meantime. Besides that, it is employed as an adjuvant to minimize pituitary suppression [19].

After clomiphene administration in the follicular phase of the ovarian cycle, the pulse frequency of LH increases indicating that the primary action of the medication is to stimulate pulsatile secretion of gonadotrophin-releasing hormone (GnRH) by the hypothalamus [20].

4. Clomiphene Citrate Nanomedicine Assistance

Nanotechnology has prompted new aspirations for managing modern human challenges. Furthermore, it has been utilized for aid in the prevention, diagnosis, and treatment of ovulatory disorders. Intriguing characteristics and abilities of nanomaterials include their capacity to attain desirable sizes, to cross biological barriers more quickly, to be more soluble, and to be more reactive [21]. Clomiphene citrate as nanomedicine possess and exhibits following benefits:

4.1. Reduced Side Effect

Any change in the expression of the genes that influence how the embryo and endometrium interact during implantation may result in implantation failure and eventually infertility. Induction of ovulation with clomiphene citrate can have a number of negative effects, including anti-estrogenic activity on the endometrium and the potential suppression of endometrial receptivity. Hence, nano-sized formulation might be used as an alternate treatment. The influence of clomiphene citrate on the expression of genes involved in implantation and blood levels of estradiol were both improved by the drug's sustained release formulation, which also raised its targeting effectiveness. Thus, to lessen clomiphene citrate's negative effects on the endometrium, a novel CC-encapsulating PBF (Phosal-Based Formulation) in the nano-size has been developed. Prior research indicates that phospholipids, one of the major constituents of Phosal-Based Formulation (PBF), can be utilized to improve therapeutic efficacy and lower the overall dose needed, as well as to prevent adverse effects and protect drugs against hydrolysis. Moreover, phospholipids shield the drugs from being degraded down in the digestive system. This PBF could react better in implantation and avoid abortion by boosting genes involved in implantation. The formulation of PBF may offer potential substitutes to existing formulations for the oral administration of lipophilic drugs without any negative effects on non-target tissues [22].

4.2. Increased Solubility

One of the key factors in achieving the required drug concentration in the bloodstream for pharmacological response is solubility. Bioavailability and eventually solubility of drug molecules determine its effectiveness in treating a certain disease. A significant tool in this endeavor is nanotechnology. The drug clomiphene citrate has a moderate water solubility, but the use of nanotechnology has improved its solubility. Poorly soluble drugs can now be developed as nanosuspensions, either independently or in combination with other pharmaceutical excipients, because of recent developments in nanoparticle engineering. Pure drug particles colloidally dispersed at submicron (nanometer) sizes in an external liquid phase are known as nanosuspensions. When the saturation solubility as well as the surface area available for dissolution increased, nanosuspension emerged as a well-organized and efficient method of drug delivery for compounds that are not water soluble. The dissolution and solubility profile of clomiphene citrate had been tried to improved using the biodegradable polymers (chitosan and PLGA). However, no significant results were found. After that, Nano suspensions were created using PVP K-30 and Tween-80. The Nanosuspension (FF6) made by solvent diffusion method using

combinations of PVP k−30 (polymer) and Tween−80 (stabilizer) exhibited the highest improvement in solubility and also in vitro drug release [23].

5. Hyphenated Techniques

Pharmaceutical analysis represents one of the most crucial aspects of drug development because it is required at all stages of the process, which calls for precise and reliable analytical method. An analytical method analyses a sample qualitatively, quantitatively, or structurally for one or more analytes using a specific technique and detailed, step-by-step instructions. Analytical methods are primarily intended to ascertain the identity, purity, and effectiveness of drug substances or pharmaceutical formulations [24]. Technology, chemistry, and physics advancements have introduced numerous analytical and chemical procedures while also facilitating the expansion of currently employed ones. Hyphenated techniques are created through the technological fusion of various analytical methods, including separation methods (mainly chromatography) and detection methods (mainly spectroscopy) [25]. Analytical methods for the analysis of clomiphene citrate are listed below in Table 1. Where S.P. refers to Stationary Phase i.e., column and M.P. refers to Mobile Phase.

Table 1. Table of analytical methods for determination of Clomiphene citrate.

Method	Chromatographic Condition	Reference
RP-HPLC	S.P.- Shimadzu C18 column M.P.- Methanol: Acetonitrile (90:10) Flow Rate- 1.0 mL/min Detection Wavelength- 295 nm Retention Time- 3.44 min	[26]
LC-MS	S.P.-Luna C18 column M.P.- Methanol: Water containing 0.05% trifluoracetic acid (70:30) N-didesmethyltamoxifen as internal standard Flow Rate- 1 mL/min *m*/*z*-406.3 and 344.3 Retention Time-zuclomiphene: 3.35 min enclomiphene: 4.04 min N-didesmethyltamoxifen: 5.66 min	[27]
RP-HPLC	S.P.-Thermo cyano C18 column M.P.- Acetonitrile: 0.05 M Phosphate buffer (60:40) (pH-3 by OPA) Flow Rate- 1 mL/min Detection Wavelength- 245 nm Retention Time- 2.61 min	[28]

6. Conclusions

This study provides comprehensive information on clomiphene citrate's nanotechnological advancements as well as all analytical methods for CC assessment in different chemical and biological matrices. This thorough review of the literature led to the conclusion that the researchers could easily adapt the existing methods to suit their own needs.

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