World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.com/
Research Article

Study the efficacy of anti-estrogenic drugs in the treatment of poly cystic ovary induced in female rats by estrogen valerate

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Received: 09-03-2014 / Revised: 24-03-2014 / Accepted: 29-03-2014

ABSTRACT

Polycystic Ovary Syndrome (PCO) is the most common endocrine disorder of women. It was characterized by irregular menstrual cycle chronic anovulation, polycystic ovaries and hyperandrogenism. The study designed to determine the efficacy of clomiphene citrate and tamoxifen in the treatment of PCO induced in rats by estrogen valerate. The level of serum LH and FSH was significantly decrease in PCO untreated group, while estradiol and testosterone levels were increased significantly in comparison with control group. Clomiphene citrate was significantly increased LH and FSH level and significantly decreased estradiol and testosterone level. Although the LH and FSH levels returned to their normal level in comparison with the control, but estradiol and testosterone levels after clomiphene citrate treatment, were still more than that recorded in control group. On the other hand, FSH, LH and estradiol levels were not significantly changed in tamoxifen treated group in comparison with PCO untreated group, while testosterone level was significantly decreased in comparison with PCO untreated group. The diameter of follicles was significantly increased, and the thickness of granulosa was significantly decreased in female rats with PCO induced by estrogen valerate. Follicles appeared to be severely atretic, with low numbers of new follicles and disappearance of corpora lutea. The estrus cycle, hormonal status and ovarian morphology were stored with antiestrogenic drugs especially clomiphene citrate.

Key Words: PCO, Antiestrogens, Clomiphene, Tamoxifen, Pharmacology

INTRODUCTION

Polycystic Ovary Syndrome (PCOS) is the most common endocrine disorder of women. It was characterized by irregular menstrual cycle chronic anovulation, polycystic ovaries and hyperandrogenism [1]. The incidence of the disease among women ranged from 2.5 to 7.5 % [2-5]. PCOS in teenage years characterized by hirsutism and menstrual abnormalities, while, in early adulthood and middle life, it characterized by infertility and glucose intolerance. However, in the late life, most patients showed diabetes mellitus and cardiovascular diseases [6]. PCOS was initially defined in 1990 as the combination of chronic anovulation or oligomenorrhoea and clinical or biochemical hyperandrogenism. The Rotterdam consensus in 2003 revised the diagnostic criteria, with two of the three following criteria declared as prerequisites for PCOS (chronic anovulation or oligomenorrhea, clinical or biochemical hyperandrogenism, and polycystic ovarian morphology) [7-8]. The pathophysiology of PCO remains uncertain, it was suggested that an excess of ovarian androgen production, either genetically or due to extra-ovarian factors such as hyperinsulinaemia or disturbances of the hypothalamic -pituitary-ovarian axis is the main cause in the pathogenesis of PCO [9-11]. The initial management of diagnosed PCO will depend upon the clinical problem – anovulation or hirsutism and avoidance of long-term sequelae of the syndrome. Antiandrogenic drugs are used for the treatment of hirsutism and many ovarian stimulants were used to induce ovulation and to restore fertility. Clomiphene citrate was widely used for this purpose. The advantages of clomiphene citrate were obvious; it was inexpensive, had low toxicity, and had few side effects. However, large percent of PCO were resistant to clomiphene citrate [12]. This study was designed to compare between two anti-estrogenic drugs in the treatment of PCO induced in female rats by estrogen valerate.

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MATERIALS AND METHODS

Forty adult Wistar albino female rats of 250±10 grams with regular estrus cycle were obtained from the animal house in Thi qar college of science and adapted for 2 weeks on the experiment condition (23±2^{o}C , 14 hrs light/10 hrs dark) before the study. They were fed a standard diet and water *ad libtum*. Thirty female rats were given a single intramuscular injection of 4 mg estrogen valerate (Riedeldehaen, Germany) in order to induce poly cystic ovary, while 10 animals were maintained on vehicle to serve as positive control [13-14]. The stage of cyclicity of estrus cycle was determined by microscopic analysis of the predominant cell type in vaginal smears obtained daily [15]. After 30 days, the animals with poly cystic ovary were divided into 3 groups (10 animals each) and treated for 20 days with clomiphene citrate 0.5mg/kg, tamoxifen 1mg/kg body weight, and vehicle to serve as negative control (PCO untreated group) respectively. After the treatment period, half of animals in each group were killed at the end of the treatment period, the blood samples from the positive control and experimental groups were immediately obtained for hormonal analysis. LH, FSH, Estradiol and testosterone levels were determined by ELISA (Kit CA-92627 Cod. No. EIA- 4K2G5, from American Co. Monobind, Inc. Costa Mesa). The ovarian tissues were fixed in 10% buffer formalin, embedded in paraffin wax, and five microns sections were stained with hematoxylin and eosin for descriptive and quantitative histology.

Single sided student -t- test was used to determine the significancy among groups.

RESULTS

The level of serum LH and FSH was significantly ($p< 0.0001$) decrease in PCO untreated group, while estradiol and testosterone levels were increased significantly ($p< 0.0001$ and $p< 0.001$ respectively) in comparison with control group. Clomiphene citrate was significantly increased LH and FSH levels and significantly decreased estradiol ($p< 0.05$) and testosterone level ($p< 0.001$) which restored to the normal limit. Although the LH and FSH levels returned to their normal level in comparison with the control, but estradiol and testosterone levels after clomiphene citrate treatment, were still more than that recorded in control group. On the other hand , FSH , LH and estradiol levels were not significantly changed in tamoxifen treated group in comparison with PCO untreated group, while testosterone level was significantly($p< 0.01$) decreased in comparison with PCO untreated group (table 1).

It appeared that the diameter of follicles was significantly increased, and the thickness of granulose was significantly decreased in female rats with PCO induced by estrogen valerate. However, there were no significant changes in the diameter of theca interna and theca externa. Both clomiphene citrate and tamoxifen were significantly decreased the diameter of follicles and increase the thickness of granulose in comparison with PCO untreated group. Clomiphene citrate restored both the diameter of follicles and the thickness of granulose to the normal limit. However both drugs didn't affect the thickness of theca interna and theca externa (table 2).

Histological sections of ovaries from control group showed follicles in various stages of development. Preovulatory tertiary follicles and growing secondary follicles were seen in proestrous, while secondary follicles and corpora lutea were seen in diestrous. In the section of ovaries of PCO untreated group, many of the follicles appeared to be severely atretic. Degeneration was observed in secondary follicles of all size. The degree of atresia ranged from nuclear pyknosis in periantral cells to degeneration and sloughing of the granulose which appeared as clusters in the antrum. No graafian follicles and corpora lutea were seen. In clomiphene and tamoxifen treated groups , the section appeared to be returned to the normal feature , the sections showed follicles of all stages with low number of cystic follicles, with the appearance of graafian follicles and corpora lutea which indicate recent ovulation.

After two days of induction, the vaginal smears of all female rats in PCO untreated group showed a mixture of all types (tout appearance), with a predominancy of nucleated cell (diestrus feature) and large unnucleated cornfield cell (estrus feature). After clomiphene and tamoxifen treatment all female return to normal cyclic rhythm.

DISCUSSION

The short estrus cycle of the female rats (4-5) days makes them ideal model to study the physiological and pathological changes in reproductive system. The follicular development, ovulation and development and regression of corpora lutea are affected by much ovarian and cyclic production of pituitary and hypothalamic hormones [16].

The PCO models in rats that have been used experimentally were included constant light exposure, hypothalamic lesions, sex steroid-induced models, and the mifepristone model. Estrogen valerate methods of producing experimental PCO models showed hormonal and

histological findings that support its resemblance to the human condition [17].

As in this study, the declined levels of LH, FSH in PCO were recorded by many studies and considered as a characteristic feature of PCO. Androgens and estrogens possessed a central position in PCOS, their elevation was closely related to the ovarian pathology, and they were used for induction of PCOS states in animal models [18-20].

Neuroendocrinological studies showed that the neuronal component responsible for the induction of the LH surge is located in the preoptic area which stimulated by estrogen [21-23].

Clomiphene citrate binds to both ERα and ERβ and acts as a pure estrogen antagonist in all human tissues, but the recemate displays weak agonistic action in rats. It induces GnRH secretion in females by blocking estrogenic feedback inhibition of pituitary. The amount of LH/FSH released at each secretory pulse is increased. In response, the ovarian follicles were developed in response to GnRH. By this mechanism clomiphene citrate increase LH and FSH secretion. Tamoxifen citrate is chemically related to clomiphene, it has complex actions; acts as potent estrogen antagonist in breast carcinoma cells, blood vessels and at some peripheral sites, but as partial agonist in uterus, bone, liver and pituitary. The specificity and its agonist effects on pituitary could be explain why tamoxifen didn't increase LH and FSH levels [24-25].

The restoration of normal histological features and cyclic rhythm of estrus cycle were a natural sequelae of the restoration of the hormonal status because the reproductive physiology including the follicular development , ovulation and development and regression of corpora lutea were guided by the cyclic production of pituitary and hypothalamic hormones [16,23].

According to these result we can conclude that antiestrogenic drugs especially clomiphene citrate are good therapeutic choice in the treatment of PCO.

Table 1: The effect of anti- estrogenic drugs on the serum levels of LH, FSH, estradiol and testosterone in PCO induced in female rats by estrogen valerate.

Groups	LH IU/ml	FSH IU/ml	Estradiol pg/ ml	Testosterone µg/ml
Control	1.361±0.48[a]	1.140± 0.24[a]	25.79±1.98[a]	0.043± 0.012[a]
PCO untreated group	0.260±0.09[b]	0.250±0.09[b]	119.12±4.26[b]	0.139±0.024[b]
PCO clomiphene treated group	1.234±0.52[a]	1.298±0.35[a]	34.76±1.14[c]	0.058±0.08[c]
PCO tamoxifen treated group	0.250±0.08[b]	0.278±0.13[b]	16.36±1.32[a]	0.056±0.018[c]

Vertically, similar letter means not significant

Table 2: The effect of anti- estrogenic drugs on the diameter of follicles , thickness of granulose , thickness of theca interna and thickness of theca externa (µm) in PCO induced in female rats by estrogen valerate .

Groups	Diameter of Follicles	Thickness of Granulosa	Thickness of Theca Interna	Thickness of Theca Externa
Control	582.8±82.6[a]	52.6±28.3[a]	14.8±4.2[a]	10.6±4.1[a]
PCO untreated group	746.8±94.6[b]	34.2±20.6[b]	14.6±3.9[a]	10.8±3.6[a]
PCO clomiphene treated group	612.2±78.6[ac]	46.2±12.6[ac]	14.2±4.1[a]	10.6±3.8[a]
PCO tamoxifen treated group	682.6±72.6[c]	46.8±18.3[ac]	14.8±6.2[a]	11.2±3.9[a]

Vertically, similar letter means not significant

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