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REMEDIAL INTERVENTION OF FERTILITY AGENT AND GENE 35 ON INDUCED CYSTIC OVARY IN RATS

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Abstract.

Infertility is a condition with a negative health state and cystic ovary disorder is one of it is causes. PCOS is a prevalent derange till now. Therefore, current study is designed to estimate the role of Gene 35 and fertility supplements (composed from chasteberry, green tea extracts and L-arginine along with some elements),as alternative therapy for induced cystic ovary with letrozole in rats. Thirty female rats were divided erratically into four groups: G1 negative control, G2 positive group, G3 fertility group, G4 Gene 35 group and G5 combination of both Gene 35 with fertility. Vaginal smear examination was done to ensure the occurrence of PCOS. Blood samples were obtained, and sera were separated for hormonal evaluation of luteinizing hormone (LH), testosterone (T), anti mullerian hormone (AMH), follicle-stimulating hormone (FSH), prolactin (PRL) as well as insulin and ovary samples were obtained for histopathological study. Current study reveals a significant ($p<0.05$) increase in serum AMH, FT, LH, PRL as well as insulin and significant ($p<0.05$) decrease in serum FSH in PCOS groups when compared to treated groups. However, treated groups experienced significant ($p<0.05$) decrease in serum AMH, FT, LH, PRL with insulin and significant ($p<0.05$) increase in in serum FSH in comparison to positive control. Histopathological study showed marked reduction of cystic follicle along with corpora lutea predominance that reflect a good response as compared to PCOS group. From the current result, there is a noticeable improvement in number of ovarian cysts and hormones levels. Therefore, it is recommended that Gene 35 and fertility supplement can be used in the treatment of PCOS patients as supporting and treatment.

Key words. Infertility, AMH, PCOS, fertility supplement, letrozole.

Introduction.

Polycystic ovary syndrome (PCOS) is a derangement of endocrine system, and it is knowing the sort of disruptive, unpleasant signs, which cause irregular menstrual cycle and acne to hair loss or excessive hair growth [1]. PCOS usually caused by higher androgen level, and it is the leading cause of infertility. Previous studies mentioned that hormonal variation such as hyperandrogenemia, increase in LH, insulin resistance and hyperinsulinemia might be playing a priority role [2].

Insulin resistance, cardiovascular disease risk, diabetes and other metabolic disorder are in a relation with PCOS, in addition, autonomic and central nervous systems play a pivotal function in controlling ovarian physiology and may have an interference in pathogenesis [3]. Changes in lifestyle as well as anxiety magnitude are consider a contributing agent in PCOS development [4].

PCOS characterized by irregular ovulation, infrequent menstrual cycle and infertility as reproductive features, along with higher concentration of male testosterone hormones, which is the leading cause of excess body or facial hair growth as well as acne [5].

Several research investigated the therapeutic potential of fertility component alone [6,7]. L-arginine reported to enhance reproductive characteristic, ovulation and pregnancy in PCOS women [8] as a consequent to normal nitric oxide level maintenance with L-arginine, which is significantly lower in women with cystic ovary [9,10].

Catechins is a robust antioxidant present in green tea extract with larger amount owing to their fertility function [11]. Reduced body weight, insulin resistance and diabetic effect related to green tea thereby PCOS metabolic alleviation [12].

The fruit Vitex used for treatment of gynecological disorder especially PCOS through controlling of prolactin hormone [13]. Vitex exert therapeutic potential with their apigenin, luteolin and casticin by interaction with dopaminergic receptor owing to suppress prolactin level, prevention of cyst formation and treatment of PCOS [14,15].

Shahnazi et al., [16] reported that 70 PCOS women treated with Vitex encountered a significant decrease in DHEA-S and normalization of menstrual cycle 60% without hormonal picture changes.

Ethinylestradiol and cyproterone acetate are component of Gene 35 drug, frequently administer in PCOS treatment fertility related to anti-androgenic effect, which inhibit the effects of androgens on the corresponding target organs, and anti-gonadotropic enhancement. They have a definite effect on reducing androgen secretion, but the effect of individual treatment is poor and needs to administer with other therapy to augment their efficacy thus sex hormones enhancement [17,18].

This study aimed to assess the efficacy of Gene 35 drug along with fertility agent that consisting from L-arginine, green tea (Camellia sinensis) extract, chasteberry (Vitex agnus-castus) extract along with some minerals in female rat with induced PCOS by letrozole.

Materials and Methods.

Animals: Thirty mature female rats weighing 200-210gm with regular estrus cycle for 10 days. These animals are maintained in a controlled environmental conditions such as temperature of 24℃, light cycle every 12 hours and provided with water ad libtum. All rats were acclimatized before the beginning of treatment and processed depending on the standard ethics of the Ethical Committee of College of Pharmacy, University of Basrah, number :(EC24).

Reagents: Jene-35 EDTM was obtained from Eris Pharmaceuticals (Australia), Fertility drug (consisting from L-arginine, green tea (Camellia sinensis) extract, chasteberry (Vitex agnus-castus) extract and some minerals) was purchased from Weliness Company/USA and Letrozole provided from Accord Healthcare Limited, Pharmaceuticals (United Kingdom).

Estrus period determination of female rats: The estrus cycle was monitored for 10 days before the beginning of the study through making vaginal smear to maintain a normal cycle state. Vaginal fluids were obtained by using a cotton-tipped swabs immersed in saline. Three types of cells can be recognized including (proestrus phase) round and nucleated ones which were epithelial cells, (estrus phase) irregular ones without nucleus which were cornfield cells and (met and diestrus phase) the little round ones which were leukocytes [19,20].

PCOS Experimental design: The study involved 30 female rats divided into five groups as **G1** negative control (Cont N), **G2** positive group (Cont P), **G3** fertility group (Fert.), **G4** Gene 35 (Gene 35) and **G5** combination of both Gene 35 with fertility group (Gene 35+Fert). All groups except negative control treated with Letrozole 1mg/kg/day, through oral gavage for 21 days to induced PCOS [1]. After induction of PCOS the positive control group G2 were sacrificed and blood sample were collected from vena cava and ovary preserved in 10% formalin, however, the reminder groups treated as follows: G3 treated with fertility 750mg/kg daily by oral gavage for 21days. G4 treated with Gene 35 (1.33mg/kg of cyproterone and 23.3µg/kg of ethinylestradiol) daily by oral gavage for 21days. The last group G5 treated with combination of fertility 750mg/kg combine with Gene 35 (1.33mg/kg of cyproterone and 23.3µg/kg of ethinylestradiol) daily by oral gavage for 21days.

Study parameters: After the end of treatment periods, all rats were sacrificed and blood samples were obtained from vena cava for hormonal measurement with Mindray CL-900 as well as ovary sample were preserved in 10% formalin for histopathological processing and examined with Genex light microscope.

Statistical analysis:

ANOVA were used to analyzed data and presented as mean ± standard Deviation. The significant differences determined with LSD with SPSS version 11.

Results.

Hormonal analysis:

Table (1) illustrated that there is a significant ($p<0.05$) increase in serum Testosterone, AMH and insulin concentration in the rats treated with letrozole compared with other experiment groups. However, there is a significant ($p<0.05$) decreased in serum Testosterone, AMH and insulin level in groups treated with Gene 35and fertility in comparison to other groups, although, serum insulin level does not affected as like testosterone and AMH levels by fertility drug. The level of these parameters presented with positive response in fertility and combination groups.

Table (2) reveals a significant ($p<0.05$) increase in serum LH and PRL hormonal level in rats with PCOS. In contrast, there is a significant ($p<0.05$) decrease in serum FSH in PCOS group compared with other study groups. On the other hand, the treated groups especially combination and fertility experienced an opposite result to that of PCOS group; there is a significant ($p<0.05$) increase in serum level of FSH hormone and a significant ($p<0.05$) decrease in both LH and PRL hormonal levels.

Table 1. *Effects of letrozole, Gene 35 and fertility on testosterone, AMH and insulin concentrations.*

Parameters/ Groups	Testosterone (ng/ml)	AMH (ng/ml)	Insulin (µlÚ/ml)
Cont N	0.61 ± 0.02^{c}	2.07 ± 0.30^{d}	3.15 ± 0.98^{c}
Cont P	3.96 ± 0.20^{a}	7.96 ± 0.84^{a}	5.78 ± 1.07^{a}
Fert.	0.58 ± 0.04^{c}	3.69 ± 0.35^{c}	4.67 ± 1.15^{b}
Gen35	0.99 ± 0.05^{b}	4.67 ± 0.39^{b}	3.18 ± 1.04^{c}
Gen35+Fert.	0.45 ± 0.04^{d}	2.04 ± 0.38^{d}	3.02 ± 0.56^{c}
P-value	**0.05**	**0.05**	**0.05**

Table 2. *Effects of letrozole, Gene 35 and fertility on serum level of FSH, LH and PRL hormones.*

Parameters/ Groups	FSH (ng/ml)	LH (ng/ml)	PRL (ng/ml)
Cont N	4.48 ± 0.18^{a}	3.37 ± 0.17^{c}	2.89 ± 0.55^{b}
Cont P	3.52 ± 0.20^{c}	7.44 ± 0.13^{a}	3.34 ± 0.07^{a}
Fert.	4.10 ± 0.05^{b}	3.87 ± 0.23^{c}	2.74 ± 0.05^{c}
Gen35	4.17 ± 0.05^{b}	4.52 ± 0.21^{b}	3.52 ± 0.05^{a}
Gen35+Fert.	4.52 ± 0.05^{a}	3.62 ± 0.22^{c}	2.46 ± 0.05^{c}
P-value	**0.05**	**0.05**	**0.05**

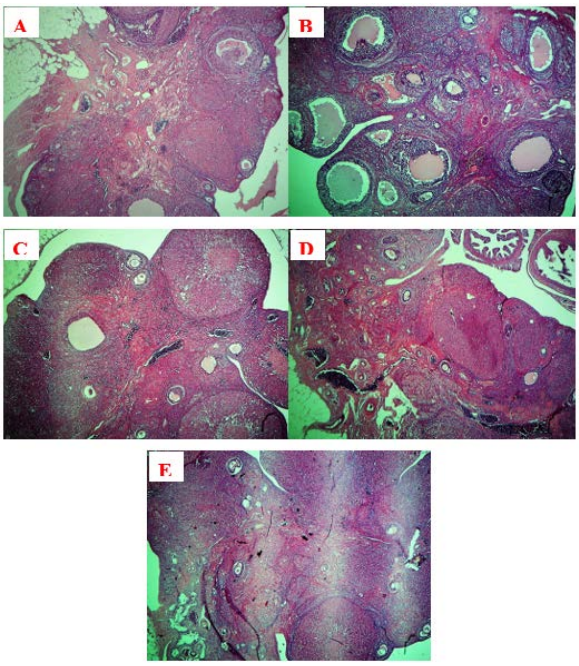

Figure 1. *Ovary section with H&E stain. X200. A) ovary section of control group shows normal follicle, graafian follicle and a healthy corpora lutea. B) Ovary of PCOS shows multi ovarian follicular cysts, thickened theca and granulosa layers, pyknotic oocyte within Graafian follicles and reduction in corpora lutea in stroma of ovary. C) Fert. group shows normal corpus luleum, newly follicles were noted. D) Gene 35 group shows healthy appearance of corpus luteum and graffian follicle. E) Gene 35+Fert. group shows normal dark dye granulosa and theca layers, corpus luteum. H&E. X200.*

Histopathological study:

Ovary section of control group presented with normal follicles, oocyte and graafian follicles as in (Figure A). PCOS ovary in (Figure B) notice a multiple follicular cyst with thick granulosa and theca layers. Histopathological examination of treated groups in (Figure C, D and E) reveals a marked reduction of ovarian cyst along with formation of newly follicles and normal corpora lutea.

Discussion.

Because the negative health impact of infertility and the wide occurrence of PCOS, this study is designed to evaluate the remedial potential of chasteberry, green tea extracts, L-arginine with some minerals as fertility drug and Gene 35 drug that consist of cyproterone and ethinylestradiol, on induced PCOS in rats by letrozole.

From the present result, the hormonal concentration of PCOS group showed increase in serum testosterone, AMH, LH, PRL and insulin level. However, there is a decrease in serum FSH concentration, this result is in line with Shehab et al. [2], this may be related to the effect of letrozole for induction of hormonal disturbance and thus PCOS evolution.

Paixao and his colleagues [21] reported that letrozole cause an excess of androgen by preventing it is transformation to estradiol and consequently higher LH level [22] through interaction with GnRH causing stimulation of theca interna cells to secrete more androgen and necrosis of granulosa cells thus cystic ovary development [21]. Several studies showed that hyperinsulinemia the primary source for evolution of ovarian hyperandrogenism, therefore, drug therapy for controlling glucose level used in PCOS to alleviate insulin concentration, controlling hyperinsulinemia and consequently hyperandrogenemia with ovulation restoration in PCOS women [23].

Cystic ovary progress due to sex steroid hormonal disturbances that controlling ovulation, follicular growth and maturation [24]. Several studies mentioned that the hormonal picture of PCOS consisting of higher level of LH and low concentration of FSH causing positive feedback on GnRH leading to greater secretion of LH hormone [25,26]. However, an opposite result was obtained in the treated groups as compared to PCOS. Increase AMH level and follicular arrest is a result of increase production of testosterone hormone due to over secretion of LH in PCOS [27]. Anti Mullerian hormone is used for identification and diagnosis of PCOS instead of number and follicular size on ultrasonography; research showed that women with PCOS have an increased concentration of AMH than those without PCOS [28,29].

The positive therapeutic potential obtained from treated groups reflected by reducing level of AMH, LH, PRL, testosterone and insulin level, on the other hand an increase FSH concentration this result is in similarity with [30,31]. The remedial effect acquired lead to attenuate the hyperandrogenism, excess LH, insulin resistance and enhance FSH effect on folliculogenesis, follicle growth, maturation and finally alleviating cystic ovary disorder [32].

Fertility drugs exhibit the beneficial effect on PCOS through their constituent; chasteberry extract is a notorious phyto-therapeutic agent used for management of PCOS [33] because of their possessing on casticin flavonoids that contribute to restoration of normal PRL hormonal level thus alleviating PCOS and enhancing fertility [34]. Green tea extracts another constituent of fertility drug that reduce insulin resistance through increase secretion of adiponectin from differentiated adipocyte by their catechins binding with peroxisome proliferator activated receptors. Green tea extracts also found to reduce theca cell thickness by promoting lipolysis and inhibiting their proliferation leading to reduce androgen production from this layer [30]. Furthermore, L-Arginine also present in fertility drug that provide antioxidant effect against oxidative stress induced cystic ovary and infertility disorder [35]. Finally, fertility drug also contains selenium that possess a pivotal role in reducing insulin level and insulin resistance associated with PCOS in addition to their antioxidant effect [36].

Gene 35 drug is a common medication used in treatment of ovulation disorder because of their ability to exhibit anti-gonadotropic effect, androgen receptor antagonist and limits production of androgen thus allowing single follicle development [37].

Histopathological analysis of PCOS revealed the presence of several sub capsular follicular cyst, thickened ovarian capsule, absence of corpora lutea and granulosa cells degeneration with hyperplastic theca cells [1,31,38], these results are in line with the current study. This result may be related to the oxidative stress provided by letrozole to induce PCOS [39,40]; it was found to cause a decrement in catalase, glutathione and superoxide dismutase level [41]. However, all treated group shows a marked reduction in cystic ovary, follicle development and appearance of corpus luteum. Group treated with fertility drugs along with Gene 35 drug provide an augmented result about fertility and improving PCOS condition.

Conclusion.

There is a remarkable enhancement in number of ovarian cysts and hormonal levels in relation to PCOS, therefore, this study is recommended that Gene 35 and fertility supplement can be used in treatment of PCOS patients as supportive and treatment due to their augmented effect in controlling several pathways of PCOS pathogenesis.

Conflict of interest.

All authors declare that they have no conflict of interest.

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