ORIGINAL ARTICLE

Effect of Aqueous Extract of Withania Coagulans and Metformin on Gonadotropin Levels in Polycystic Ovarian Rats

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ABSTRACT

Various medicinal herb plants are being used in place of metformin for treatment of polycystic ovarian disease.
Purpose: To assess the influence of aqueous extract of Withania coagulans (aqWC) on serum follicle stimulating hormone and luteinizing hormone levels in polycystic ovary disease induced rats.
Study Design: Randomized controlled trial.
Methodology: Forty female Sprague Dawley were divided into 04 groups. Group A (Control) and Disease induced group. Standardized laboratory diet was fed to Group A while the disease induced group was given standardized diet and letrozole solution orally (1mg/kg) for 21 days for Polycystic ovary syndrome induction, which was established by observing estrous cycle of rats. Disease induced group was then distributed as Group B (PCOS control), C (Withania coagulans treated) and D (Metformin treated) for 14 days. Sampling was done from Group A and B (after 21 days) and Group C and D at the end of experiment in which serum Follicle stimulating hormone, Luteinizing hormone levels (µU/ml) and Ovarian weight (mg) were assessed.
Statistical analysis: Data analyzed by SPSS 21.0v.
Results: Follicle stimulating hormone showed significantly ($P<0.05$) increased levels and serum Luteinizing hormone showed significantly decreased levels in Group C and D rats in comparison to Group B rats. Ovarian weight also returned to normalcy in Group C and D.
Conclusion: This study concluded that Withania coagulans can be a substitute for ameliorating deranged gonadotropin levels inpolycystic ovary disease.
Key Words: Polycystic ovarian syndrome; Estrus cycle; Luteinizing Hormone (LH) and Follicle Stimulating Hormone (FSH).

INTRODUCTION

Increasing infertility rate among humans is now becoming a global health issue not only in Western countries but also in developing countries.[1] It has been estimated that almost one third of female infertility was due to ovulation problems because of hormonal imbalance of Luteinizing Hormone (LH) and Follicle-Stimulating Hormone (FSH).[2] Apart from pathological reasons, woman's ability to get pregnant is also affected by her lifestyle, occupational risk factors and chemicals exposure in environment and diet.[3] However, the concern regarding fertility from exposure to environmental contaminants is increasing. Fertility problems affect one in seven couples in the UK. No doubt social factors with better contraception awareness along-with role as working women contributed besides others.[4] Globally 26% couples have unsolved infertility. Polycystic ovarian syndrome (PCOS) is a commonly renowned reproductive ailment with prevalence of 20.7% in Pakistan. It is mostly observed in women of child-bearing age.[1] According to Androgen excess society, biochemical and clinical hyperandrogenism in combination with functional or sonographic dysfunction in ovarian characteristics are necessary for diagnosis of polycystic ovary syndrome. It is delineated by decreased serum follicle stimulating hormone, increased serum luteinizing hormone, serum glucose, serum insulin levels, body weight and variability in ovarian morphology and features.[2,3] Polycystic ovary syndrome is a confounding malady and its etiology is not clearly explained so far but is assumed to occur by intermixing of environmental elements for instance obesity, genetic predisposition, insulin resistance (IR), hyperandrogenism, disturbance of hypothalamic-pituitary-gonadal axis, ovarian dysfunction and follicular arrest.

Although polycystic ovary syndrome is an endocrine physiological disorder but it has reproductive, metabolic, and psychological consequences such as abdominal obesity which is directly linked to metabolic disease, hypertension, cerebrovascular disease, sub clinical atherosclerosis, obstructive sleep apnea, impaired glucose metabolism and type II diabetes mellitus. Studies have reported prevalence of dyslipidemia in 70% of females with polycystic ovary syndrome. There is also increased risk of development of endometrial carcinoma and emotional distress, anxiety disorders and depression.[4-6] The selection of treatment options for patients with polycystic ovary syndrome relies on the presenting symptoms. Disorders associated with menstruation are treated with oral contraceptive pills, androgen related disorders are treated by spironolactone and infertility is treated by weight loss by physical exercise (30 minutes/day) and moderate calorie restriction, clomiphene citrate for ovulation induction as it rises ovulation rate by 70-85%/cycle and metformin is widely used in polycystic ovary syndrome associated infertility as it augments ovulation and pregnancy rate.[6-9]

A novel animal model based on the use of non-steroidal aromatase inhibitor (Letrozole) is recently developed which is quite identical to human polycystic ovary syndrome. In the present study letrozole induced animal model has been used. Letrozole establishes the polycystic ovary syndrome rat model by inhibiting aromatase enzyme action and producing the state of hyperandrogenism and representing anovulation.[10]

Metformin being an insulin sensitizer is very efficient for improving the insulin resistance and hyperinsulinemia, the main culprit factors for hyperandrogenemia, folliculogenesis defects and metabolic syndrome.[11,12] But it is also linked with side effects such as gastrointestinal upset, lactic acidosis and vitamin B-12 deficiency.[13,14]

Due to these unwanted effects of metformin several medicinal herb plants are used for the treatment of polycystic ovary syndrome, offering minimum or no reported side effects along with cost effectiveness and easy availability.[15] Hence, we planned current project to assess the influence of aqueous extract of Withania coagulans (aqWC) on serum follicle stimulating hormone and luteinizing hormone levels in polycystic ovary disease induced rats.

METHODOLOGY

Forty female Sprague Dawley were divided into 04 groups. Group A (Control) and Disease induced group. The animals were kept at 22±2°C at a humidity level of 55±10%. Standardized laboratory diet was fed following the ethical guidelines at Islamic International Medical College in alliance with National Institute of health (NIH) to Group A while the disease induced group was given standardized diet and letrozole solution orally (1mg/kg) for 21 days for Polycystic ovary syndrome induction, which was established by observing estrous cycle of rats. Disease induced group was then distributed as Group B (PCOS control), C (Withania coagulans treated) and D (Metformin treated) for 14 days. Sampling was done from Group A and B (after 21 days) and Group C and D at the end of experiment in which serum Follicle stimulating hormone, Luteinizing hormone levels (µU/ml) and Ovarian weight (mg) were assessed. Blood sample (two ml) was collected in labeled gel tubes by intra cardiac sampling and stored at 2-8° C until further analyzed for gonadotropin levels by Sandwich-ELISA method. Rats were sacrificed by cervical dislocation and after dissection, ovaries were removed, cleaned off fat and weighed on weighing machine

Statistical Analysis: : Statistical analysis was performed by applying SPSS 21. Independent sample t-test was applied and P value <0.05 was measured significant.

RESULTS

Mean comparison of Serum FSH level (µU/ml) levels in groups A, B, C & D is represented in Table-1. Serum FSH level (µU/ml) of Group B rats showed significant reduction (P <0.05) (a*) than Group A rats. The rats in Group C and Group D presented statistically significant (P < 0.05) (b*), (c*) increase in contrast to Group B.

Group B rats showed significant increase (P < 0.05) in ovarian weight in comparison to Group A rats. However rats in Group C and Group D shown statistically significant (P < 0.05) decrease in weight in contrast to Group B as displayed in “Figure-1”.

Table-1: Hormonal levels among all Groups (µU/ml)

GROUPS (n=10)	S. FSH level Mean ± SD	S. LH level	P-value
Group A (Control)	1.41±0.66	1.66 ± 0.14	
Group B (PCOS control)	1.11 ± .023*	2.54 ± .10*	* = P<0.05 (a*)
Group C (Withania coagulans treated)	1.42 ±.018*	1.11 ± .03*	* = P<0.05 (b*)
Group D (Metformin treated)	1.42 ± 0.05*	1.11 ± .03*	* = P<0.05 (c*)

*Statistically significant ; *a= Group A against B ; *b= Group C against B ; *c =Group D against B

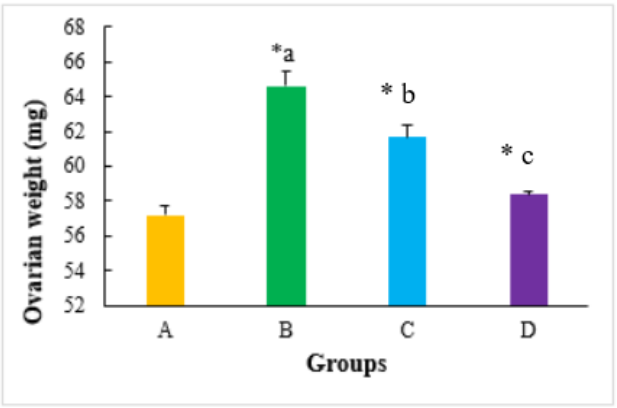

Figure-1: Mean comparison of ovarian weight (mg) of different groups

Note: *Statistically significant ; *a= Group A against B; *b=Group C against B ; *c =Group D against B

DISCUSSION:

In this study serum follicle stimulating hormone levels of the treated groups i.e. Group C and Group D rats were increased in comparison to Group B rats whereas serum luteinizing hormone levels and ovarian weight were lowered in Group C and D in comparison to Group B rats, which showed that aqueous extract of Withania coagulans does possess antiandrogenic potential. This effect might be due to the presence of active components present in it which are known as withanolides.

In the existing study results of the gonadotropin levels were similar to outcome of the study led by Saiyed et.al.,2016 who used hydroalcoholic extract of mixture of Withania somnifera dunal and Tribulus terrestris Lin. They concluded that this treatment increased the level of follicle stimulating hormone and decreased the level of luteinizing hormone and ovarian weight. According to them the antiandrogenic effects of the hydroalcoholic extract might be due to the presence of phytoestrogens.[16]

Another study conducted by Anbu et al also illustrated that administration of ethanolic extract of Sargassum Ilicifolium at doses of 100mg/kg, 200mg/kg and 400mg/kg for 21 days resulted in augmentation of serum follicle stimulating hormone and serum luteinizing hormone levels in testosterone propionate induced polycystic ovary

syndrome rats. Results observed in present study are similar to this study i.e. increase in serum FSH level and decrease in serum LH level,except that the animal model chosen is different as in the current study letrozole induced PCOD model was used. They have pointed out that antiandrogenic effect is due to presence of increased amount of iodine in the extract which helps in enabling normal fertilization process by meeting the daily requisite of iodine of women/pregnant women.[17]

Likewise a different study steered by Ghafurniyan et al established enhanced serum FSH level and reduction in serum LH level, in estradiol-valerate induced polycystic ovary syndrome rats after administration of hydroalcoholic Green tea extract (200mg/kg)for a period of 10 days. They suggested that alleviating outcome of Green tea hydroalcoholic extract on symptoms of polycystic ovary syndrome may be due to the antioxidant effect on oxidative stress caused by free radicals.[18]

Findings of the this study are also in concurrence to the study directed by Rajan et al inspecting the consequence of Soy isoflavones (100mg/kg) administration for 14 days on serum follicle stimulating hormone and luteinizing hormone levels and ovarian morphology in letrozole (1mg/kg) induced polycystic ovary syndrome rats. They revealed that Soy isoflavones (100mg/kg) administration considerably reformed the letrozole – induced polycystic ovary syndrome signs by decreasing serum luteinizing hormone level and increasing serum follicle stimulating hormone level. They also proposed that these antiandrogenic effects might be due to presence of phytoestorgens in isoflavones.[19] This study differed from current study as they administered two different doses of soy isoflavones i.e. 200mg/kg and 400 mg/kg and observed their effects on symptoms of polycystic ovary syndrome whereas in the current study aqueous extract of withania coagulans treated group was compared to metformin treated group which also showed similar significant results.

Limitations: Present study had number of limitations like small sample size, financial constrains and limited resources.

CONCLUSION:

This study concluded that Withania coagulans posseses significant antiandrogenic potential. Present study illustrated that it could be used as a possible substitute in ameliorating biochemical characteristics of polycystic ovary syndrome as its actions were similar to that of standard metformin.

Author's Contribution: HA & AFA: Overall supervision, write up and literature review. NAS & AS: Statistics application analysis literature review, help in write up. SA,AK & TL: Literature review help in write-up.

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