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Biological Investigation of Aqueous Extract of Funnel Seeds to Check its Therapeutic Effects in the Management of Polycystic Ovarian Syndrome in Induced PCOS Female Albino Rats

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ABSTRACT

About every one out of nine women around the globe is living with polycystic ovarian syndrome. Polycystic ovarian syndrome is characterized by increased male hormone (androgen) in female body that results in delayed ovulation by unbalancing follicle stimulating hormone, luteinizing hormone, testosterone, and insulin levels. Other associated signs are increased insulin levels due to elevated androgens, weight gain, male pattern hair growth, and depression. Funnel seed is a wonder in regulating the imbalanced reproductive hormones. The purpose of Current study was to evaluate the hormone regulating properties by using funnel seed as source element. For this purpose, 14 female albino rats with induced polycystic ovarian syndrome were divided into two group. Treatment group was given funnel seed extract in dose of 50mg/kg of the body weight for sixty days. Levels of follicle stimulating hormone, luteinizing hormone, testosterone and insulin levels were measured and compared in blood samples on 0th day and 60th day in study groups. Insulin and testosterone levels were significantly ($p < 0.05$) lower in the treatment group, whereas ovulating hormones, such as follicle stimulating hormone and luteinizing hormone, were significantly ($p < 0.05$) higher.

INTRODUCTION

Polycystic ovarian syndrome (PCOS) was first hypothesized as over-production of androgen hormone i.e., hyperandrogenism from functional ovaries in female of reproductive age (Norman, Robert J., et al. 2007) Hyperandrogenism leads to absence of ovulation. Thus, leads to infertility and irregular reproductive cycle. Sleep apnea, male pattern hair growth, hirsutism, abrupt increase in weight, and insulin resistance are observed in females with PCOS. Females with PCOS develop

insulin resistance which leads to the development of hyperglycemia and metabolic irregularities (Franks, Stephen., 1995).

These metabolic irregularities lead to the development of type 2 diabetes mellitus and dyslipidemia. Hormone dependent cancers and cardiovascular disorders are also observed in females with PCOS. PCOS can be due to genetic, environmental and lifestyle (Guzick, David S., 2004) According to the studies around 4-20%



women of reproductive age are suffering from PCOS around the globe (Ommen, Steve R., et al.,2020) . However, some prevalent studies have reported 3-10% prevalence of PCOS in various regions of the world and it greatly depends on regions and ethnicity (Wolf et al., 2018). Many drug therapies and herbal medicines are used to manage PCOS and to restore fertility. These include the use of inositol which are second messenger like insulin and helps in regulating reproductive cycle and normal ovulation(Azziz, Ricardo. 2020)However, people now days females are inclined to the natural existing solution to manage symptoms of PCOS. Fennel seeds (*Foeniculum vulgare*) is one of the native plants of family *Apiaceae* which is grown worldwide which contain vital oil including fenchone, estragole, and trans anethole (Khani, Samira, et al..2020).

Fennel (*Foeniculum vulgare*) is a plant in the *Apiaceae* (*Umbelliferae*) family that provides flavor to numerous meals, including soups, sauces, pickles, breads, and cakes. The species is native to the Mediterranean and grown in other countries including England, Germany, China, Iran, Vietnam, and South America. Fennel seed has been shown to have diuretic, analgesic, antipyretic, and antioxidant properties. The essential oil has been extensively studied for its antioxidant, antibacterial, and hepatoprotective properties (Lucinewton et al., 2005)

Fennel seeds also contain flavonoid glycosides, non-flavonoid phenols, hydroxycinnamic acid, phenolic acids, coumarins, and tannins. All these compounds of therapeutic importance impart medicinal properties to fennel seeds. Fennel seeds have tremendous anti-oxidant, anti-inflammatory, antibacterial, hypotensive, and estrogenic potential. (Kalleli, Fatma, et al., 2019).

Fennels also have role in regulating insulin action, thus, it can be helpful in lowering the insulin resistance and elevated levels of insulin in female body which leads hyperandrogenism i.e., the main pathophysiology behind PCOS (Ahmad, Bouchra Sayed, et al.,2018). The purpose if this research was to check the effects of fennels seeds against poly cystic ovary syndrome that is very major problems of females that leading to different types of complications so the novelty of this work was to check the bio active ingredients that are present in fennel seeds and then applied it on albino

rats to check the positive association of phenolic of fennels for the management of PCOS in females

MATERIAL AND METHODS

Procurement of Materials

Fennel seeds were purchased from the local market and packed into polythene bags to avoid any air contamination

Handling of Animals

Ten-week-old female wistar albino rats weighing between 200 and 220 grams were acquired from the National Institute of Health (NIH), located in Islamabad. Rats were housed in glass cages in an animal house and supplied water and food at will. At the animal home, a 12-hour light and dark cycle was maintained.

Induction of PCOS

Via vaginal discharge sampling and cellular observations, estrous phase of rats was assessed. PCOS was induced on the 14th day after injecting estradiol valerate. 2mg dose of estradiol valerate injection was administered muscularly to rats in estrous phase for the induction of PCOS.

Preparation of Fennel Seed Extract

Tap water was used to wash the fennel seeds first, followed by distilled water. Fennel seeds were crushed into a powder after being dried (Chen et al., 2015). The fennel seed extract was made using 70% ethanol. 500 ml of ethanol was used to dissolve 100g of fennel seed sample, which was then stored in a water bath at 40 °C for 48 hours. After filtering and evaporating the solution, it was left to condense at room temperature. The extract was stored in amber-colored vials at 4 °C (Hossain, Md Munir, et al.2013).

Study Duration and Study Design

The trial had a totally randomized design and lasted for 60 days (Bhide et al., 2018).

Treatment Groups and Treatment Plan

Rats were split into two groups: group 1 consisted of PCOS-afflicted rats that were not given any medication, and group 2 consisted of rats with PCOS that were given 50 mg/kg of fennel seed extract by body weight. A catheter was used to provide the dose orally (Damjanović, Biljana, et al. 2009).

Table I*Treatment groups and treatment plan*

Treatment Groups	Title	Treatment
T ₀	Control group with PCOS	No treatment
T ₁	Treatment group-1 with PCOS	50 mg/kg of body weight of funnel seeds extract

Sample Collection and Biochemical Analyses

Rats were anesthetized with chloroform, and blood samples were taken both at the end of the trial after the rats were given funnel seed extract and after PCOS was induced. Ha, Kee-Yong, and others (2011). The levels of LH, FSH, TST, and insulin were measured using commercially available ELISA kit. Chloroform was readily available in the university lab that was used during anesthesia to animals.

STATISTICAL ANALYSIS

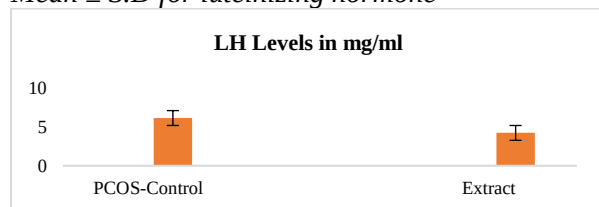
Under a completely randomized design (CRD), descriptive statistical analysis was performed to examine the degree of significance ($p < 0.05$) by comparing the means before and after the dosage using a one-way ANOVA followed by a two-sample t-test. The results are displayed as mean $\pm$ S.D. According to D'Agostino (2017), IBM SPSS Statistics 20 is used for all statistical studies.

RESULTS AND DISCUSSION

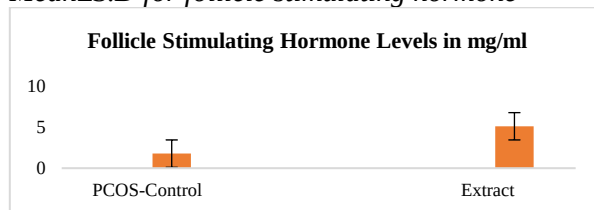
This research study was designed to investigate the medicinal effect of funnel seeds extract on female reproductive hormones LH, FSH, TST and insulin. Parameters including LH, FSH, TST, and insulin were measured and compared between the 0-day and 60th day in control group and treatment group.

Biological Effect of Funnel Seeds Extract on Luteinizing Hormone Level

Results showed significant ($p < 0.05$) reduction in luteinizing hormone (LH) levels in treatment group after administration of funnel seeds extract as shown in figure 1.

Figure 1*Mean $\pm$ S.D for luteinizing hormone***Effect of Funnel Seeds Extract on Follicle Stimulating Hormone Level**

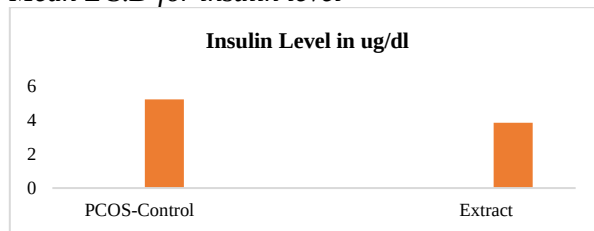
Results showed significant ($p < 0.05$) increase in follicle stimulating hormone (FSH) levels in treatment group after administration of funnel seeds extract. However, FSH level is very low in PCOS control which showed delayed ovulation as shown in figure 2.

Figure 2*Mean $\pm$ S.D for follicle stimulating hormone***Effect of Funnel Seeds Extract on Testosterone Hormone Level**

Results showed significant ($p < 0.05$) reduction in male testosterone levels in treatment group after administration of funnel seeds extract. However, TST level is high in PCOS control which resulted in delayed ovulation as shown in figure 3.

Figure 3*Mean $\pm$ S.D for testosterone hormone***Effect of Funnel Seeds Extract on insulin ug/dl Level**

Results showed significant ($p < 0.05$) reduction in hyperinsulinemia in treatment group after administration of funnel seeds extract. However, insulin level very high in PCOS control which increase production of male hormones in female body as shown in figure 4.

Figure 4*Mean $\pm$ S.D for insulin level*

DISCUSSION

The study was designed to investigate the effect of funnel seed extract on the hormones including LH, FSH, TST, and insulin in response to the 50mg funnel seeds extract per kg of body weight of rats. Females with PCOS have decreased levels of follicle stimulating hormone. Study reported a significant increase in follicle stimulating hormone which is responsible for normal ovulation. Study also reported a significant reduction in testosterone and luteinizing hormone levels which were high in PCOS control. Similar findings were reported in a study that investigated the effect funnel seeds phytochemicals incorporation in chitosan capsules in order to study its effect on the various parameters of PCOS. The study reported reduction in testosterone, luteinizing, and insulin hormone levels while an increase in follicle stimulating hormone level which is similar to the finding of this study. Study explained that chitosan alone could not show such results and improvement in PCOS symptoms was due to phytochemicals present in funnel seeds (Bayrami et al., 2020). Another study that investigated a herbal composition containing funnels seeds, anise and bamboo cane reported that this composition was as effective as metformin in the patients of PCOS. Study reported that this composition that showed effective medicinal potential against PCOS and it could be as beneficial as the drugs used to manage PCOS symptoms. However, as the study was investigated the therapeutic effect of composition of funnel seeds, anise and bamboo cane so it could not be claimed

that which played more vital role in these results (Khanage, Shantaram Gajanan, 2019). Funnel seeds decreased high insulin level in rats which showed that insulin sensitivity way increased, and insulin resistance was decreased. A study that investigated the medicinal effect of funnel seed essential oil reported that 100mg per kg of body weight per day improved insulin resistance in rats which is like findings of the study. However, it also did not investigate the funnel seeds extract (Poojar, Basvaraj, et al, 2017). This study did not study the effect of funnel seeds extract in combination with any other herb and plant which was not studied much. However, which phytochemical influenced and controlled the symptoms of PCOS could be further investigated.

CONCLUSION

A large number of women around the globe are suffering from reproductive health issue. Many therapeutic and medicinal approaches are adopted for the treatment of reproductive issues. Funnel seed is a source of medicinal properties which are beneficial against polycystic ovarian syndrome. Funnel seeds contain a large number of polyphenols which are useful in lowering the oxidative stress and hormonal imbalance. In this study, funnel seeds reported benefits in lowering testosterone levels and elevating follicle stimulation hormone and progesterone levels. Funnel seeds can be a potent natural solution against hormonal imbalance.

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