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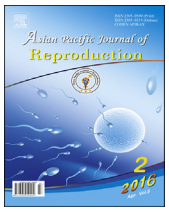

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Beneficial effect of Curcumin in Letrozole induced polycystic ovary syndrome

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ABSTRACT

Objective: To investigated beneficial effect of Curcumin (a phenolic curcuminoid derivative from *Curcuma longa*) in Letrozole induced PCOS in female Wistar rats.
Methods: Letrozole (1 mg/kg) was administered per orally (p.o) for a period of 21 days for the induction of PCOS, followed by dose of Curcumin (100 mg/kg and 200 mg/kg, p.o) for 15 days using 0.5% w/v CMC as vehicle.
Results: The administration of Letrozole led to abnormalcy in serum sex steroid profile, lipid profile, glucose and glycosylated hemoglobin levels and depletion in antioxidant activity. Curcumin was able to successfully exert its protective effect by restoring all the parameters to normal and disappearance of cysts in ovaries.
Conclusion: Curcumin showed beneficial effects in Letrozole induced PCOS in female Wistar rats. Its effect was comparable to that of Clomiphene citrate, most widely used treatment for ovulation induction in PCOS condition.

1. Introduction

Polycystic Ovary Syndrome (PCOS) is a common heterogenous endocrinological and metabolic disorder in women of reproductive age leading to infertility/subfertility. Women (5%–10%) of reproductive age are affected by PCOS [1,2]. Clinical manifestations of PCOS include infrequent or absent menses, abdominal obesity, acanthosis nigricans and signs of androgen excess (hyperandrogenism) which include acne or seborrhea and insulin resistance [3–7]. Long term consequences include increased risk of endometrial cancer, type 2 diabetes mellitus, dyslipidemia, hypertension and cardiovascular disorders [8,9]. The etiology of PCOS is not clearly understood, but lipid imbalance, oxidative stress, insulin resistance and genetics are some of the contributing factors.

Various experimental models for PCOS have been developed in rats like administration of Estradiol Valerate, DHEA and prepubertal androgen excess [10]. Even though these models induce PCOS, none of them are fully convincing and identify with the conditions of human PCOS completely.

Letrozole, a non-steroidal aromatase inhibitor produces a PCOS model which in numerous ways depicts human PCOS [11]. It blocks conversion of testosterone and androstenedione to estradiol and estrone respectively and simulates PCOS like condition [12] by causing hormonal imbalance, circulating hyperandrogenism and intra ovarian androgen excess leading to appearance of polycystic ovary. Follicular atresia and abnormal follicular development is observed due to induced elevation of androgen levels inside the ovary [17]. Letrozole induction was reported to cause hyperglycemic condition which may contribute to insulin resistance, hyperlipidemia leading to metabolic syndrome [14,15].

Currently, many therapies are in use to manage PCOS condition and to induce ovulation. But these therapies have been reported to cause severe side effects ranging from arthritis, joint or muscle pain [16] and psychological disturbances [17]. Therefore, now-a-days focus is being laid on medicines from natural sources which show minimal or no side effects.

Curcumin is a water-insoluble, low molecular weight, polyphenolic curcuminoid derivative found in rhizomes of Indian spice, *Curcuma longa* (turmeric). Turmeric is extensively used as a food additive and coloring agent in Asian cuisine [18] and also in Indian herbal medicine. Curcumin constitutes to about 2–8% of turmeric preparations.

Curcumin has been reported to possess a wide variety of biological effects like anti-inflammatory, anti-oxidant [19], hypoglycaemic [20] and antihyperlipidemic activities. Curcumin exhibits anti-proliferative and apoptotic activities in several human cancer cell lines, like those derived from cancers

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of prostate, breast and ovary [21,22]. Recent study showed protective effect of Curcumin on Porcine Ovarian granulosa cells [23]. Apart from these, estrogenic effects of Curcumin were observed on breast cancer cell lines [24]. In this study we hypothesized that Curcumin may be beneficial in management of PCOS induced by Letrozole due to the reported activities.

2. Materials and methods

2.1. Experimental animals

Virgin, cyclic, adult female Wistar Albino rats (160–200 g) were employed for the study. Animals were acquired from Animal house of Teena Labs, Kukatpally, Hyderabad and housed in our institution's animal house and allowed to acclimatise for two weeks. During the study all animals were caged in standard polypropylene cages and maintained in controlled environment of (22 ± 3) °C temperature, (55 ± 5)% humidity and a 12 h light/dark cycle. They were fed with standard diet and water provided *ad libitum*. The study was duly approved by Institutions Animal Ethics Committee for the use of animals and care of the animals was carried out as per the guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA) with protocol number. I/IAEC/LCP/016/2013/WR-30.

2.2. Drugs and reagents

Curcumin was acquired from Sigma Chemicals Co., St. Louis, MO, USA. Letrozole was obtained from Natco Pharma Limited, Hyderabad. Clomiphene Citrate (Fertyl-Super) tablets were procured from Ar-Ex Laboratories Private Limited, Goregaon (E), Mumbai. All other chemicals used were of analytical grade. The Glucose, Cholesterol, Triglycerides, HDL and Glycosylate Hemoglobin kits were obtained from ERBA Diagnostic, USA.

2.3. PCOS induction

All the experimental animals except control group, were orally administered with Letrozole at a dose of 1 mg/kg dissolved in 0.5% Carboxy Methyl Cellulose (CMC) once daily for 21 days [12]. Control group received vehicle only (0.5% CMC). Vaginal Smears were collected daily and evaluated microscopically using Giemsa stain to confirm the induction of PCOS.

2.4. Study design

The study consisted of 30 female Albino Wistar rats equally divided into five groups designated as group 1 (served as control group), group 2 (served as PCOS induced group), group 3 (served as standard group), groups 4 and 5 served as treatment groups. Following Letrozole administration, standard group was administered with Clomiphene Citrate at a dose of 1 mg/kg in 0.5% CMC per oral and treatment groups 4 and 5 were administered Curcumin with the dose of 100 mg/kg (Low dose) and 200 mg/kg (High dose) body weight respectively in 0.5% CMC per oral for 15 days i.e., from day 22 to day 36.

After 21 days, PCOS control group and after 36 days, animals from other groups were fasted overnight and anaesthetized with diethyl ether. Blood was collected by retino orbital puncture then serum was separated by centrifugation and was used for estimation of hormones, glucose, glycosylated hemoglobin and lipid parameters.

The animals were then sacrificed, ovaries and uterus excised, cleaned of fat and weighed. After excision, ovaries were freed from blood and cleaned with ice cold saline and homogenized using 10% ice cold potassium chloride for antioxidant and TBARS evaluation.

2.5. Measurement of invasive blood pressure and heart rate

At the end of the study, four animals from each group were anesthetized with Ketamine. Arterial blood pressure (BP) was recorded from carotid artery. A polyethylene catheter (PE-50 [1 mm O.D.]) for rats was attached to a pressure transducer (both were filled with heparinised saline), and inserted into the carotid artery and tied in place. Pressure fluctuations in the artery were transmitted along the catheter tubing to the transducer's diaphragm, which moved in response. The diaphragm movements were converted into a varying electrical signal that was amplified through a bridge amplifier and recorded by a Power lab system (AD instruments, Australia).

2.6. Biochemical estimations

2.6.1. Hormonal assay

Hormones were assayed by Competitive Chemiluminescent Immunoassay using automated instrument ADVIA Centaur, Siemens Healthcare Diagnostics Inc., USA. The testosterone was estimated using ADVIA Centaur TSTO kit, estrogen using ADVIA Centaur E2-6 kit, and progesterone using ADVIA Centaur PRGE kit.

2.6.2. Measurement of fasting blood glucose (FBG)

FBG was measured by Trinder's method using a commercial diagnostic kit from ERBA Diagnostics, USA.

2.6.3. Measurement of glycosylated hemoglobin (HbA1c) levels

HbA1c was assayed by cation-exchange method using a diagnostic kit from ERBA Diagnostics, USA.

2.6.4. Assessment of lipid profile

Lipid profile [total-cholesterol (TC), triglycerides (TG), and HDL-cholesterol (HDL-C)] were estimated by using enzymatic kits procured from ERBA Diagnostics, USA. LDL-cholesterol (LDL-C) was calculated by using Friedewald's equation.

2.6.5. Antioxidant assay

2.6.5.1. Superoxide dismutase

Superoxide dismutase activity was determined by the pyrogallol oxidation method. This is an indirect method that is based on the ability of the enzyme to inhibit the auto-oxidation of pyrogallol. Superoxide dismutase activity was determined by monitoring the rate of oxidation of pyrogallol by superoxide radicals. The reaction is initiated by adding pyrogallol and the change in optical density was recorded at 420 nm [25].

2.6.5.2. Catalase

Catalase activity was determined in 50 ml of sample mixed with 50 ml of substrate for 60s, then 100 ml of 32.4 mM ammonium molybdate solution was added and absorbance change was measured at 405 nm. One unit of the enzyme was defined as μmoles of H_2O_2 degraded/min/mg of protein [25].