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Effects of Curcumin on GLUT4, Erα and Insulin Resistance Genes Expression in Polycystic Ovary Syndrome Rats

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ABSTRACT

Aims Polycystic ovary syndrome is one of the most common endocrine disorders in the women and animals. Some common hormonal therapies are used to treat the PCOS condition. This study was conducted to evaluate the effects of curcumin on rats with PCOS by evaluation of the gene expression of the GLUT4 and Erα and insulin resistance.

Materials & Methods Following induction of PCOS, sixty Sprague–Dawley female rats were divided into four groups including: (1) Control group (2) Control PCOS (3 & 4) those treated with 100 and 200 mg/kg of curcumin respectively. Body weight, fasting blood glucose (FBG), fasting insulin serum (FIS), homeostasis model assessment of insulin resistance (HOMA-IR) and gene expression of GLUT4 and Erα were evaluated.

Findings Induction of PCOS increased body weight, FBG, FIS, HOMA-IR and decreased gene expression of GLUT4 and Erα ($p<0.05$), but oral administration of curcumin could alleviate adverse effects of PCOS on the mentioned parameters ($p<0.05$).

Conclusion Curcumin alleviates adverse effects of polycystic ovary syndrome.

Keywords Curcumin; Polycystic Ovary Syndrome; Erα; Fasting Insulin; GLUT4; Rats

CITATION LINKS

[1] Chronic fructose consumption as a model of polycystic ovary syndrome in ... [2] Trace glucose andlipid metabolism in high androgen ... [3] Effect of Allium cepa seeds ethanolic extract on experimental ... [4] Activity of Corylusavellana seed oil in letrozole-induced polycystic ... [5] PI3K Akt mTOR and MAPK signaling pathways in polycystic ovarian ... [6] Up regulated expression of WNT5a increases inflammation and ... [7] Genetic polymorphisms of INS, INSR and IRS 1 genes are not associated with ... [8] Tyrosine kinase activity of insulin-like growth factor I and insulin receptors in human ... [9] In vitro binding of insulin and epidermal growth factor to human ... [10] Evidence for the presence of glucose transporter 4 in the endometriumand ... [11] Effects ofmetformin on the expression ofGLUT4in endometrium ... [12] Metformin augments the levels of molecules that regulate the expression of ... [13] Endometrial expression of estrogen receptors and the ... [14] Obesity,insulin resistance and diabetes: Sex differences ... [15] Delayed puberty and estrogen resistance in a woman with estrogen ... [16] Treatment options for polycystic ovary ... [17] Psychological side-effects of clomiphene citrate and human menopausal ... [18] Effect of tetrahydrocurcumin on blood glucose, plasma ... [19] Antihyperlipidemic effect of curcumin andtetrahydrocurcumin ... [20] Curcumin induces the degradation of cyclin E expression through ubiquitin-dependent ... [21] Curcumin-induced apoptosis in ovarian carcinoma cells is p53-independent ... [22] The effect ofCurcumin on secretory activity, proliferation and apoptosis ... [23] Evaluation of effects of Commiphora Wightii in dehydroepiandrosterone (DHEA) induced polyc-ystic ovary syndrome ... [24] Quercetin potentiates transdifferentiation of bone marrow mesenchymal stem cells into the ... [25] Amelioration by quercetin of insulin resistance and uterine GLUT4 and ERa gene expression in ... [26] Oral administration of menthol could alleviate adverse effects of polycystic ovarian ... [27] Defects in insulin signaling pathways in ovarian steroidogenesis and ... [28] Expression of tumor necrosis factor (TNF)-alpha protein in the ... [29] Cellular location of insulin-triggered signals and implications ... [30] Changes in the expression of insulin signaling pathway molecules in endometria ... [31] Estrogens protect against high-fat diet-induced insulin ... [32] The Effect of Curcumin on GLUT4 gene expression as a diabetic resistance marker ..

Introduction

Polycystic ovary syndrome (PCOS) is one of the most common endocrine disorders that induces anovulation in the women and animals [1].

Some metabolic abnormalities including dyslipidemia, insulin resistance, diabetes, obesity, and infertility occur in patients with PCOS [2, 3]. PCOS has been known with some signs including production of small arrested antral follicle, decreased estrogen and increased LH/FSH ratio [4].

Insulin signaling pathway could have major role in pathogenesis of PCOS which comprises pathways of phosphatidylinositol 3 kinase (PI3K) and protein kinase B/Akt signaling [5-7].

Selective insulin resistance can progress and in such cases, metabolic response to insulin action has been faulted including PI3K pathway, however, other actions are not only maintained but may be increased, including the mitogen activated protein kinase (MAPK) pathway, in insulin group target tissues and ovary [5, 6]. Insulin joins to its receptor and acts insulin receptor substrate (IRS) through the phosphorylation of tyrosine residues. The expression of IRS in the uterus has been reported [7] and glucose metabolism is essential for uterine cell differentiation [8].

It has been known that GLUT4 is the most important glucose transporter isoform in insulin-dependent tissues and modulates ininsulin-stimulated glucose transport in the uterus [9]. Different studies have reported decreased GLUT4 expression in the uterus of PCOS patients [10, 11].

Oestrogen receptor α (ERα) has been known to have an essential role [12], for example ER-knockout rats are infertile [13-15]. Some common hormonal therapies are used to treat the PCOS condition and to stimulate the ovulation. These treatments cause adverse effects on arthritis, joint or muscle pain [16] and psychological disturbances [17]. There is a great attention on medicines from natural sources that are safe and useful. Curcumin, is a natural active in turmeric, has biological effects including anti-inflammatory, antioxidant [18], hypoglycaemic [19] and anti-hyperlipidemic activities. It is known to have anti-proliferative and apoptotic activities in some human cancer cell lines, such as cells obtained from cancers of prostate, breast and ovary [20-22].

It seems that curcumin could improve the gene expression of GLUT4 and ERα and other related parameters in rats with PCOS. Therefore, this study was conducted to evaluate the effects of curcumin on insulin resistance (IR) and uterine gene expression of GLUT4 and ERα in rats with PCOS.

Materials and Methods

Animals

Sixty Sprague–Dawley female rats with weight of 180±5g were used for this study. All the animals were grouped in a 25°C a lighting diet 12:12 h light:dark cycle. The animals had ad libitum access to conventional feed pellets and water. To evaluate the body weight changes, we have weighed animals in start and end of the trial.

All animals were randomly grouped into 4 groups (n=15):

1) Control group without PCOS (Positive Control);
2) PCOS group without treatment (Negative Control);
3) PCOS rats treated with daily oral doses of 100mg/kg daily (Cur-100); and
4) PCOS rats treated with daily oral doses of 200mg/kg daily (Cur-200).

Curcumin (Sigma; USA) was dissolved in 0.5% carboxy methyl cellulose per oral for 30 days. To induce the PCOS, animals were subcutaneously administrated with 6 mg dehydroepiandrosterone (DHEA) per 100g day-1(DHEA dissolved in 0.2 mL sesame oil) for 21 consecutive days [23]. Following administration of curcumin (after 30 days), 5 animals per group were killed and some tissues including ovaries and uterus were collected and blood samples were collected to prepare the serum.

Evaluation of IR

The blood samples were used to evaluate the fasting blood glucose (FBG) and fasting insulin serum (FIS) levels. FBG was assessed by glucose oxidase procedure and FIS was assessed by a direct competitive enzyme-linked immunosorbent assay (ELISA) kit [24]. The optical values were read in the 450nm by a microplate reader and the induction of IR was investigatedby the homeostasis model assessment of insulin resistance (HOMA-IR) method. HOMA-IR was calculated using the following formula:

$$\text{HOMA-IR} = \text{FBG (mmol/L)} \times \text{FIS (mU/L)} / 22.50$$

Rat ovarian morphology

All the animals' ovaries were prepared by surgery fixed in 10% formalin and included in paraffin. Sections prepared were stained by haematoxylin and eosin then evaluated by two pathologists that did not information from the sample type for ovarian morphological properties.

Gene expression of GLUT4 and Erα

Endometrium RNA isolation and real-time polymerase chain reaction (PCR) were conducted as reported by others [25]. The primers sequences were GLUT4, forward (5'-GGGCTGTGAGTGAGTGCTTTC-3') and reverse (5'-CAGCGAGGCAAGGCTAGA-3'); Erα, forward (5'-CCAAAGCCTCGGGAATGG-3') and reverse (5'-AGCTGCGGGCGATTGAG-3'); and β-actin, forward (5'- AAGGCCAACCGTGAAAAGAT-3') and reverse (5'-ACCAGAGGCATACAGGGAC-3').

Statistical analysis

SPSS 21 software was used to analysis the data by tukey test (difference between groups). The level of statistical significance was set at $p<0.05$.

Findings

Body weight

Effects of experimental treatments on body weight of rats with PCOS are shown in Figure 1. As results show, body weight was significantly higher in rats with PCOS in comparison to those in control group ($p<0.05$). Oral administration of curcumin in the both levels (100 and 200mg) significantly decreased adverse effects of PCOS on body weight and better response was observed in higher dose ($p<0.05$).

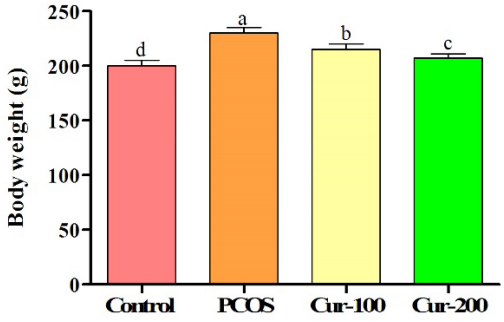

Figure 1. Effects of oral administration of curcumin on body weight (g) of rats with PCOS. Superscripts (a-d) show significant difference between groups.

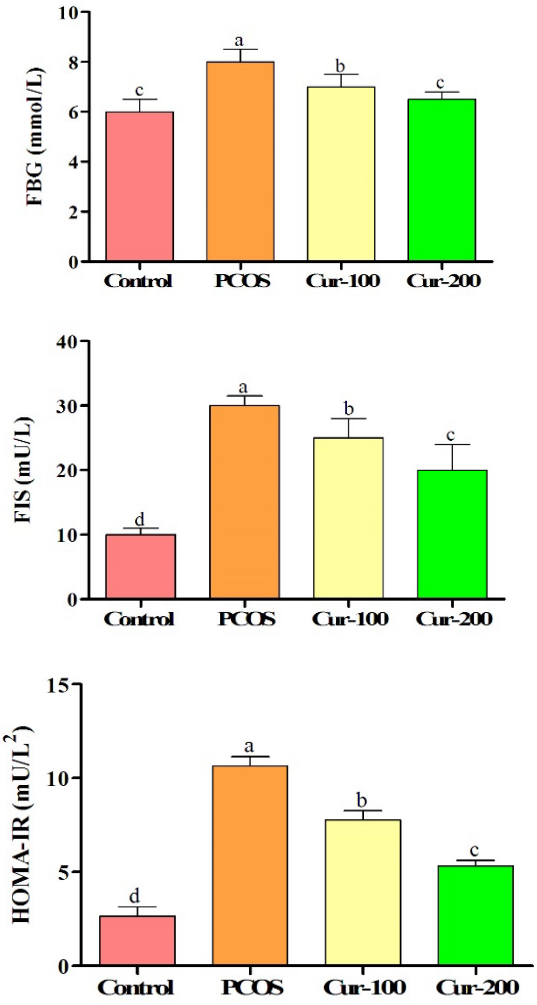

Figure 2. Effects of oral administration of curcumin on insulin resistance of rats with PCOS. Superscripts (a-d) show significant difference between groups.

Insulin resistance

PCOS increased levels of FIS, FBG and HOMA-IR in comparison to control group ($p<0.05$). Oral administration of curcumin could significantly decrease levels of FIS, FBG and HOMA-IR ($p<0.05$; Figure 2).

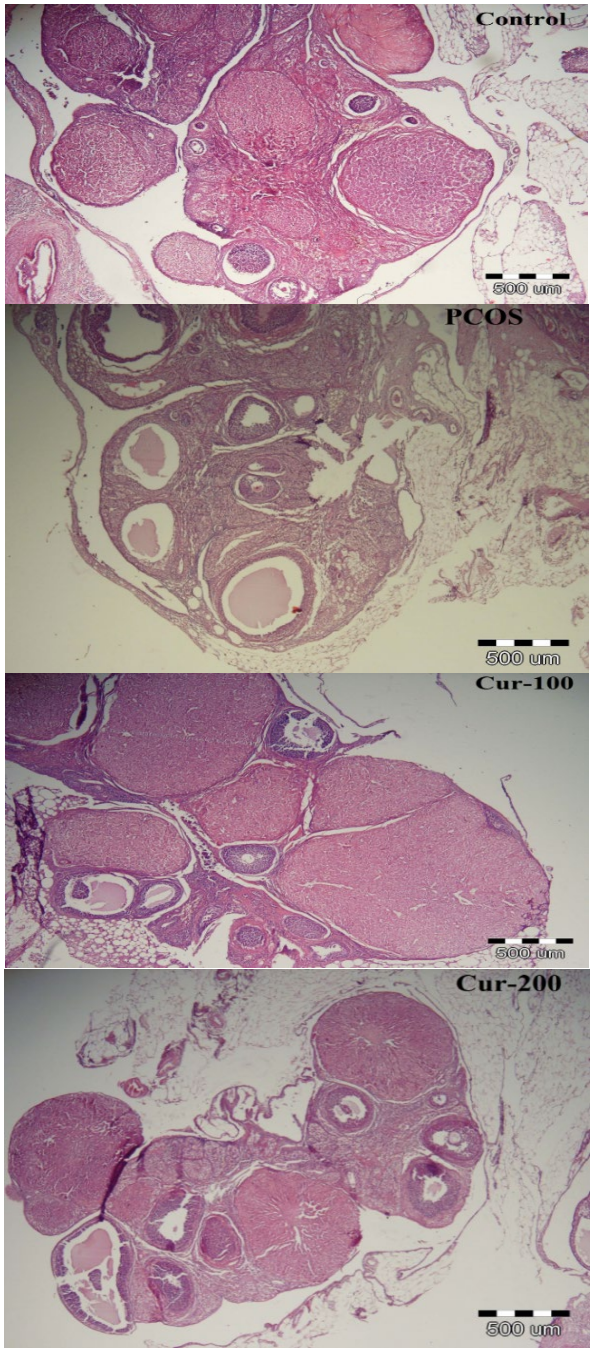

Figure 3. Effects of oral administration of curcumin on ovarian morphology of rats with PCOS

Ovarian morphology

In the control group, the follicles were normal, but ovaries of rats in the PCOS group had an increase in ovarian volume, decreased corpus luteum and theca layer hypertrophy and thickening in comparison to control group. Cystic follicles were also observed in PCOS group that means induction of PCOS. Oral

administration of curcumin alleviated cystic follicles and increased corpus luteum (Figure 3).

Gene expression

Effects of oral administration of curcumin on gene expression of GLUT4 and Erα of rats with PCOS are shown in Figure 4. Induction of PCOS decreased expression of GLUT4 and Erα ($p<0.05$). The use of higher levels of curcumin increased expression of GLUT4 and Erα ($p<0.05$). The use of lower level could also increase GLUT4 and Erα expression ($p<0.05$; Figure 4).

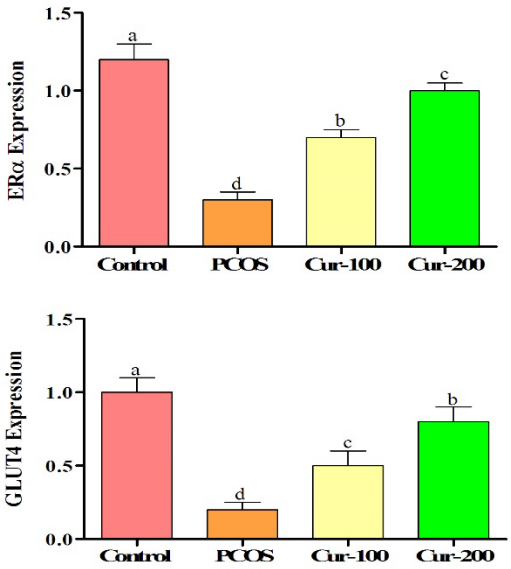

Figure 4. Effects of oral administration of curcumin on gene expression of GLUT4 and Erα of rats with PCOS. Superscripts (a-d) show significant difference between groups.

Discussion

PCOS is one of the disorder related with diabetes and causes with signs such as hyperglycemia in first stages that gradually induces IR [26]. In the current study, rats induced with PCOS indicated an increase in FIS, FBG and HOMA-IR. Hyperglycaemia is result from IR, but the exact mechanism of PCOS is still not known. Previous studies have shown faulted insulin intracellular signaling, particularly alterations in insulin receptor substance-1 (IRS-1) phosphorylation that may be a factor for PCOS-IR [27]. On the other hand, adipose tissue increases the release of pro-inflammatory cytokines [28] and changesIRS-1 tyrosine phosphorylation to serine phosphorylation, which initiates IR. Our findings also showed that the body weight was significantly higher in PCOS rats. It seems overweight promotes to produce pro-inflammatory cytokines and promotes IR. Oral administration of curcumin improved IR, especially in higher doses. Improved IR could be attributed to anti-inflammatory properties of curcumin that prevents production of pro-inflammatory cytokines [18]. On the other hand, curcumin decreased body weight that in turn decreases IR. As results showed, oral administration of curcumin decreased levels of FBG and FIS. Decreased levels of FBG could be attributed to increased expression of GLUT4 in rats treated with curcumin. Transformation on cell surface by GLUT4 relies on insulin signaling pathway [29] and IR decreases GLUT4 expression [30]. Not only GLUT4 but also ERα are involved in glucose metabolism. Increased ERα expression promotes the sensitivity of skeletal muscle cells to insulin and causes to consume the glucose [31]. It is clear that curcumin improves IR through involvement in the expression of GLUT4 andERα. Another study showed that curcumin increases the gene expression of GLUT4 but synergistic effects of curcumin and insulin is stronger in comparison to insulin that could be attributed to the competitive activity of insulin and curcumin in activation of gene expression [32]. It could be speculated that curcumin could improve insulin resistance by influencing on GLUT4 and ERα gene expression. Rats with PCOS showed a number of cystic follicles and decreased corpus luteum. Similar results were reported by previous studies [25]. Oral administration of curcumin could decrease cystic follicles and increase corpus luteum. Increased corpora lutea implicates ovulation and normal estrous cyclicity.

Follicles in the different steps of development with oocytes and clear, visible granulosa cell layer were seen in curcumin groups.

Conclusion

Curcumin has a protective effect in patients with PCOS and oral administration of curcumin prevents diabetes. Curcumin usage decrease the insulin resistance and increase expression of GLUT4 and Erα.

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