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SİLYMARİN İMPROVED IVF OUTCOME İN WOMEN WİTH POLYCYSTİC OVARY SYNDROME

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ABSTRACT

Background: The most feature of polycystic ovary syndrome (PCOS) is oligomenorrhea, enlarged cystic ovaries and it is the most common cause of chronic anovulation. Because of high level of oxidative stress and inflammation in this patient, we usually have poor IVF outcome. In this study we investigate the effect of Silymarin, the known anti-oxidant agent, on IVF outcome in patient with PCOS.
Methods: In this clinical trial, 40 PCOS patients whom underwent IVF were enrolled. All patient received metformin 1500mg/day and case group received additionally Livergol (Silymarin) 210mg/day. Oxidative stress and inflammation parameters were measured in the base and after intervention. Also, the Oocytes retrieved, transferred embryos and pregnancy rate were observed.
Results: Our results have shown that Silymarin can reduce oxidative stress and inflammation in PCOS patients. Also, it can improve IVF outcome in this patients.in addition we have shown that this improving effect was independent to body weight.
Conclusion: Since, Silymarin is pharmacologically safe and well tolerable, we suggest to improve IVF outcome in PCOS patients, we can use Silymarin as a good adjuvant therapy.

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Introduction

Polycystic ovary syndrome (PCOS) is a heterogeneous syndrome that affects a large number of female populations in childbearing age. The prevalence of PCOS in this groups of women is 5-10% (1).It is characterized by hyperandrogenism, oligomenorrhea, enlarged cystic ovaries and chronic anovulation .Also, this disease accompanied with diabetes, insulin resistance. Laboratory finding shows lipid profile abnormality, chronic inflammation and increases in oxidative stress (2; 3). Oxidative stress is caused by an imbalance between oxidants and antioxidants and associated with cellular and molecular damage(4). It was well established that increasing of it is one of the main pathogenesis in some disorder such as cardiovascular abnormality, diabetes, hyperlipidemia, obesity and some reproductive disorders such as endometriosis, PCOS, and unexplained

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infertility(5,6,7).There have been evidences that infertile women with PCOS have imbalance between oxidant production and antioxidant defense(8).

According to previous studies, the level of oxidative component in follicular fluid is even more than plasma, suggesting it has important role in follicular development (9).Also, there have some evidences indicated that if oxidative stress decreased, outcome of IVF would be better (10;11; 12). So, oxidative stress not only in events of natural reproduction, but also has a negative impact on the success of assisted reproductive techniques.

Silymarin is a standard product extracted from the herb Milk Thistle which is known as an antioxidant against oxygen free radicals. Also, it acts as a membrane stabilizer and preventing lipid peroxidation and cell damage. The anti-inflammatory effects of Silymarin have been also shown in previous studies (13; 14; 15; 16).

The confirmed effect of Silymarin in lowering glucose, insulin, testosterone, progesterone, and lipid profile according to the known pathophysiology of PCOS can have favorable effects in reversing the vicious cycle of hyperandrogenism, hyperinsulinemia, and anovulation in women with PCOS (17; 15). Therefore in this study the effect of Silymarin on oxidative stress and IVF outcomes in patient with PCOS will be evaluated.

Materials and Methods

Medication

Livergol was purchased from GOLDARU Co, Esfahan, Iran. These tablets were standardized with 70 mg of Silymarin extract.

Study protocol

In this clinical trial, 40 women with PCOS undergoing IVF-ICSI in IVF center of the Mirza- Kouchak Khan hospital were enrolled. The age of patients was between 20-35 years old and PCOS were diagnosed based on Rotterdam 2003 consensus criteria(18). Patients with diabetes mellitus, hypertension, Cigarette smoking, using antioxidant drugs currently or in the past 3 months and with multifactorial infertility was excluded from the study. The study was approved by the ethics committee of Tehran University of medical sciences and registered with the number of IRCT2015041321743N1 in Iranian Registration of Clinical Trials.

After documentation of demographic data, the patients were received Metformin 500 mg, three times a day and then they randomly divided into two equal groups. At the same time with long agonist cycle, First group received one tablet of Livergol 70 three times a day and the control group received the similar placebo tablets. The subjects and the researcher were not aware of the grouping. At baseline and on the day of HCG injection, 10 cc peripheral venous blood samples were taken and immediately blood sample centrifuged and separated serum from it, kept at -80 until the day of assessment.

Ovulation induction and IVF procedure

GnRH agonist was started at day 21 of the cycle and on the third day of the next menstrual cycle, patients again underwent ultrasonography and the gonadotropin was begun. Patients also underwent ultrasonography at days 5-6 of gonadotropin to evaluate the response rate. When at least three follicles bigger than 18 mm in diameter were observed, 10000 IU of HCG administered and serum estradiol level was measured on the same day. Approximately 34-36 hours after the HCG injection, oocyte retrieval was performed and the number of retrieved oocytes was recorded. Patients were monitored up to 14 days later after recording of biochemical pregnancy.

Laboratory Methods

For all of the subjects, Fasting Plasma Glucose, triglyceride, total cholesterol, LDL and HDL were measured by auto-analyzer (Hitachi 47 automatic analyzer; Hitachi, Tokyo, Japan). Synthetic paraoxon used as substrate for measuring the activity of PON1. The initial rate of substrate hydrolysis to p-nitrophenol was calculated as a PON1 activity and was expressed as U/mL. Serum MDA levels, an index of lipid peroxidation were measured according to the method described by Draper and Hadley (1990), which is based on the determination of the concentration of thiobarbituric acid reactive products. MDA concentration was expressed as μg/mL. The values of CRP were measured by the colorimetric method in all subjects (19; 20; 21).

Statistical Analysis

All statistical analyses were performed using the statistical software package SPSS, version 20 (SPSS Inc, Chicago, IL). Comparisons of two means were tested by t-test between different groups. The chi square test was used to compare discrete variables. The results are expressed as Mean±SD and the P-values <0.05 were considered statically significant.

Results

The basic clinical information of patients is presented in Table 1. This table shows that there is no significant difference in age, body mass index, blood glucose, lipid profile, the duration of infertility C-reactive protein (CRP), MDA, and PON1 activity between groups.

The mean number of Oocytes retrieved was significantly higher in Silymarin than placebo groups (12±5.34 Vs 8.13±4.34) (P=0.003). Also, the mean number of MII oocytes in Silymarin group were 2.20±1.50, while this index was 6±3.42 in placebo group (P=0.0001).

In table 2, we have shown that the mean number of transferred embryos and pregnancy rate were significantly higher in Silymarin group than placebo group. Other IVF parameters between both groups have had no significant differences.

From base line to end of intervention, biochemical and inflammatory parameters were measured in patients. CRP level was significantly decreased in Silymarin group (Figure 1). Also, the activity of PON1 activity significantly increased from 32±7to 60±10 in Silymarin group ($p<0.0001$) (Figure 2). Indeed, HDL was significantly increased in Silymarin group but other laboratory parameters haven't significant change during treatment (Table 3).

Discussion

Since the positive effect of decreasing in oxidative stress in IVF outcome and as a regards the strong antioxidant activity of Silymarin, we investigated for the first the effect of Silymarin on IVF outcome in PCOS women. In this study we have shown that the Oocytes retrieved, transferred embryos and finally pregnancy rate in PCOS women who received Silymarin were significantly better than placebo group.

There have few studies have investigated the effect of Silymarin on IVF outcome. Hazim Ismaeel.AL-Ahmed in a study has investigated the effect of Silybum marianum (L.) Geartn extract on male and female factor of productivity and IVF outcome in mice. They have shown that semen quality and IVF outcome in mice that treated with Silybum marianum (L.) Geartn were improved, too(22). In another animal study, Jang et al have demonstrated that Silymarin have increase embryo development rate in bovine which underwent IVF due to increasing in antioxidant capacity(23). Moosavifar and their colleague in a clinical trial investigated 40 women who underwent IVF because of male factor that the case group received Silymarin 70 mg three times a day. Although the number of 15-18 mm follicles, number of oocytes, and endometrial thickness did not differ between the two groups but the apoptosis of granulosa cells which have negative effect on follicle development and oocyte quality was decreased in the treatment group (24) .

In this study we have shown that the Silymarin could significantly increase serum levels of HDL-C, and caused a significant increase in the activity of PON1. Also, Silymarin has shown anti-inflammatory action with significantly decreasing in CRP values in patients with PCOS.

Growing body of evidences has shown that in patient with PCOS the ratio of central obesity, type 2 diabetes mellitus and dyslipidemia is increased(25; 26). Also, it was well established in women with PCOS, imbalance between the levels of oxidants and antioxidant in serum and follicular fluid will be occurred (9, 5;27; 6). The presence of antioxidant system in vital reproductive events such as the completion of meiosis in dominant oocyte, LH Surge, and ovulation is important. Oxidative stress also causes undesirable effects such as spontaneous abortion, recurrent miscarriage, preeclampsia and IUGR, and even leads to poor results in IVF especially in women with PCOS (27; 28; 7). previous studies have shown that In one hand, PCOS patients have high oxidative stress and in the other hand, possible benefits of antioxidant supplementation of ART culture media to increase the likelihood for ART was assessed (13; 29). Thus this issue has focused the attenuation of oxidative stress on the use of antioxidants in IVF procedure in infertile women with PCOS.

Milk thistle herb (Silybum marianum) has long been known as an antioxidant and anti-inflammatory medication (14;15). In a study performed on diabetic rats received intraperitoneally Silymarin, after 8 weeks, glucose, LDL, TG, and MDA levels decreased and serum levels of superoxide dismutase (SOD) which is a component of the antioxidant system increased(14). Taher and colleagues, in a study in which women with PCOS treated with metformin, Silymarin or both of them for three months, observed that simultaneous use of metformin and Silymarin improves the status is disturbed hormones and increases the ovulation rate(6). This medication with few side effects and high tolerability has many benefits in liver diseases, reducing total cholesterol, reducing insulin resistance in patients with DM and proved effects in cirrhosis. Even there are many articles about its use in patients with various cancers (30; 31; 32).

Mohamadin et al have shown that Paraoxonase 1 activity decreased in PCOS women(27).Paraoxonase 1 is an enzyme which has antioxidant activity and its activity has inverse relationship with oxidative stress. In this study we have shown that the activity of Paraoxonase 1 in PCOS women who treated with Silymarin significantly increased. Previous studies have shown that reduced in activity of this enzyme correlated with resistance to insulin, cardiovascular disease, dyslipidemia and diabetes (33; 34). Since, oxidative stress correlated with poor IVF outcome, and Silymarin has strong antioxidant agent, we speculated that improving IVF outcomes after administration of Silymarin may due to decrease in oxidative stress.

The result of our study has been indicated that due to consumption of Silymarin the level of HDL increased and we can discuss that this increase may result of increase in PON1 activity. Previous studies have demonstrated that treatment strategies that improve endocrine and reproductive function in PCOS women were associated with increase in HDL. In agreement with ths, Orio and colleagues used a combination of Silymarin plus Berberin 2 times a day for 3 months in PCOS patients (28). They observed a significant reduction in waist circumference, waist-to-hip ratio, TG, and LDL, and an increase in HDL and SHBG. Also ovulation in the case group improved. So we can say increase in serum HDL is an indirect indicator of improvement of PCOS.

PCOS women usually have overweighed and in this condition the level of inflammation markers such as CRP would be increased. Also, growing body of evidences indicated that in these women we have chronic low inflammation due to possible role increased oxidative stress (35; 36). In this study we have shown that Silymarin can significantly decrease the serum level of CRP. Previous study demonstrated that CRP level have correlation with body weight (37; 38). Since, in this study we treated patient in short time, and we did not have significant decreases in BMI (unpublished data), we can say decrease in CRP may due to decrease in oxidative stress.

One of the important causes of poor embryonic development and oocyte maturation in IVF procedure is increasing in oxidative stress (39; 40). Luddi et al in a study have demonstrated that when we use a combination of Vitamin A,E and folate, that all of them have strong antioxidant activity, can significantly decreases not suitable oocytes in treated group. Also, in their study there have no difference in the IVF out come between treated and non-treated group(41) .Velthut et al in their study have shown that antioxidant status have a positive correlation with IVF/ICSI outcome(7.). In another study which have been done by Sharami et al, have been demonstrated that when vitamin C level increases in FF we may have better embryo quality(42.). All of the mentioned studies indicated that decreasing oxidative stress could improve IVF outcome in women with/without underlying pathogenesis.

Conclusion

In this study we have demonstrated that PCOS women who receive Silymarin have greater activity of Paraoxonase 1 activity and lower level of CRP. Also, we have shown that Oocytes retrieved, Transferred embryos and finally pregnancy rate were significantly increased in these women. So, based on our finding we suggested that Silymarin can improve IVF outcome in women with PCOS probably via decreasing in oxidative stress and inflammation. Since, Silymarin is using widely and this drug is pharmacologically safe and well tolerated with patients and has strong antioxidant activity, we suggested that patient with PCOS especially whom underwent IVF can use it.

Conflict of interest

The authors have no conflict of interest

Acknowledgment

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Figures

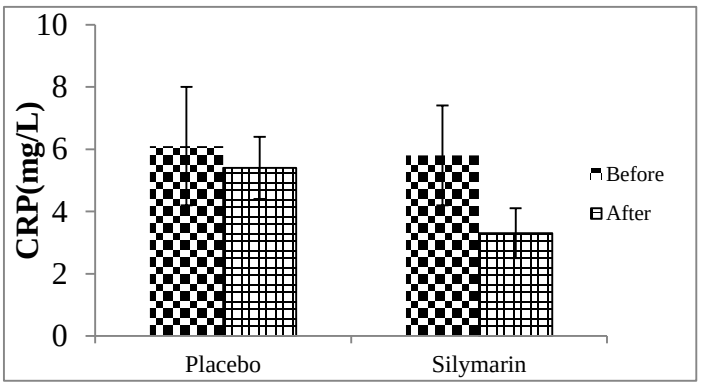

Figure 1. Comparison the serum level of CRP in both groups between before and after trial
Serum level of CRP was dramatically decreased after administration of Silymarin

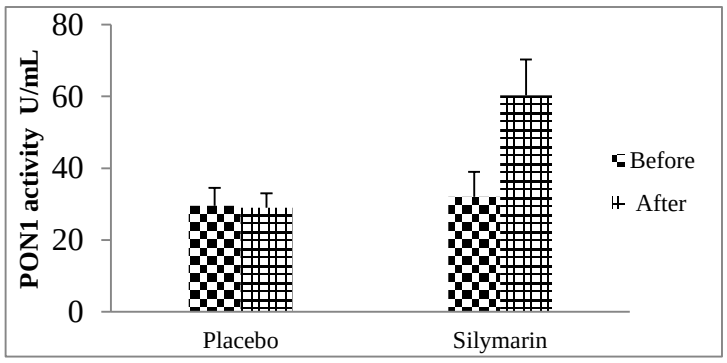

Figure 2. Comparison the PON1 activity in both groups between before and after trial
The activity of PON1 increased after administration of Silymarin

Table 1. Baseline clinical and laboratory profile in Silymarin and placebo groups.

	Silymarin (n=20)	Placebo (n=20)	zP-value
Age (years)	34.3±0.7	32.9±0.5	0.05<
BMI (kg/m^2)	23.9±0.6	22.9±0.3	0.05<
FPG(mg/dL)	91.3±7.2	93.1±6.4	0.05<
TG (mg/dL)	192.7±21.2	186.8±24.3	0.05<
Total Cholesterol (mg/dL)	181.6±34.9	176.6±36.1	0.05<
HDL (mg/dL)	40.1±9.5	39.7±9.2	0.05<
LDL (mg/dL)	84.7±18.3	81.4±16.3	0.05<
CRP (mg/L)	5.8±1.6	6.1±1.9	0.05<
PON1 activity	32±7	29.5±5	0.05<
MDA (micgr/mL)	0.38±0.18	0.34±0.13	0.05<
Duration of infertility (years)	4.3±0.6	3.9±0.5	0.05<

Data are expressed as Means±SEM. FPG, fasting plasma glucose; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein, CRP, C-reactive protein; PON1, Paraoxonase 1; MDA, Malondialdehyde.

Table 2. Comparison of IVF outcomes in Silymarin and placebo groups.

	Silymarin (n=20)	Placebo (n=20)	P-value
number of MI oocytes	2.20±1.50	3.00±1.57	0.081
Transferred embryos	5.5±1.14	4.2±2.49	0.012
E2 level (hCG day) (pg/mL)	3965.16±2968.80	2700.10±1467.07	0.054
Endometrial thickness (mm)	8.96±1.18	8.53±1.35	0.246
Pregnancy rate	13 (65%)	7 (35%)	0.016

Table 3. Final clinical profile in Silymarin and placebo groups.

	Silymarin (n=20)	Placebo (n=20)	P-value
FPG (mg/dL)	87.7±6.8	85.1±6.1	0.05<
TG (mg/dL)	190.2±22.5	185.3±21.3	0.05<
Total Cholesterol (mg/dL)	172.6±31.4	172.1±33.8	0.05<
HDL-C (mg/dL)	53.4±9.8	40.3±9.6	0.026
LDL-C (mg/dL)	78.4±.4	80.5±13.2	0.05<
CRP (mg/L)	3.3 ±0.8	5.4±1.0	0.013
PON1 activity(U/mL)	60±10	0.75±0.30	<0.0001
MDA (micgr/mL)	0.6727±0.395	29±4	0.05<

Data are expressed as Means±SEM. FPG, fasting plasma glucose; TG, triglycerides; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CRP, C-reactive protein; PON1, Paraoxonase 1; MDA, Malondialdehyde.

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