Anti-inflammatory Effect of Silymarin on Ovarian Immunohistochemical Localization of TNF-\(\alpha\) Associated with Systemic Inflammation in Polycystic Ovarian Syndrome

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SUMMARY: Tumor necrosis factor alpha (TNF-\(\alpha\)) and interleukin (IL)-6, are prominent mediators of inflammation and have been confirmed to be elevated in at least a subgroup of women with polycystic ovary syndrome (PCOS). In this study, the effects of Silymarin (SLM) on the expression TNF-\(\alpha\), IL-6, CRP and symptoms of PCOS were studied. In this research, PCOS was induced by injection of Estradiol Valerate. PCOS rats were divided into control and experimental groups received intraperitoneal injection SLM extract daily. After syndrome induction, ovaries were collected for histological and immunohistochemical evaluations. Serum IL-6 was detected by the ELISA kit. The results indicated the significant reduction in inflammatory markers and significant changes follicular layers thickness in the treatment group as compared with control. It can be concluded that having anti-inflammatory substances, Silymarin is effective in symptoms of this syndrome and metabolic syndrome.

KEY WORDS: Immunohistochemistry; Inflammation; Silybum Marianum extract.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disease that affects 10% of women of reproductive age (Baillargeon et al., 2005; Allahbadiaa & Merchant, 2011) and lead to anovulation (Pasquali et al., 2006; Nabiuni et al., 2011). This syndrome was first described by Ashlyn & Leventhal (Deligeoroglou et al. 2009; Skritic et al., 2011). Ovarian function in PCOS, detected in adulthood, but is rooted in childhood or even during fetal life and leads to irregularities in the adrenal axis - pituitary – hypothalamus (Ehrmann et al., 2005; Saadati et al., 2012). In these patients, obesity increases the risk of diabetes and Cardiovascular Disease (Akbarzadeh et al., 2011).

With the increase of LH to FSH ratio in these patients, the ovaries increase androgen synthesis (Marx & Mehta, 2003). The serum levels of TNF-\(\alpha\) and increased expression in adipose tissue in human and rodents with Polycystic, showed role of TNF factor in the regulation of normal ovaries normal activity (Spaczynski et al., 1999).

TNF-\(\alpha\) is a necrotic factor that plays an important role in the regulation of normal ovarian activity and in the rat, causing apoptosis of granulosa in antral follicles. This factor is expressed in oocytes, granulosa cells of healthy and atretic follicles, and locally by lutein granulosa cells. In fact, TNF-\(\alpha\) is an angiogenic factor that cause stimulates, proliferation and steroidogenesis follicular sheath cells (Son et al., 2007).

The high expression of TNF-\(\alpha\) in rats, In addition induction of apoptosis in granulosa cells of antral follicles, mitogenic theca interstitial cells stimulating activities and increasing the number of steroidogenic cells, cause progress of PCOS. So, in women with polycystic ovary syndrome granulosa layer decrease and increase in follicular sheath thickness is observed (Chang et al., 2007; Son et al.).

Shown that hyperglycemia cause increases ROS production from peripheral blood mononuclear cells from these patients. ROS induce oxidative stress in the cells and

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NF-κB activation pathway leading to increased transcription of TNF-α and insulin resistance. This Insulin resistance is one of the causes of ovarian cysts due to changes in LH activity and insulin with abnormal rise in androgen plays a role in this syndrome (González et al., 2006).

Cytokines play a major role in response to the inflammatory stimuli and tissue damages. Interleukin-6 expression is basically regulated by Beta Kappa core factor (NF-κβ) and interleukin (IL-6) causes stimulus, regulates synthesis of acute phase protein, activates axis of hypothalamus-pituitary and disorder in signal transduction of glucose through changing activity of serine-threonine kinases. IL-6 plays a key role in the pathogenesis of chronic inflammation, insulin resistance (Pedersen et al., 2006).

Several studies have reported diurnal variations in various cytokines, including IL-6, IL-12 or TNFα, as well as in the leukocyte subset cells that are responsible for their production. The change in inflammatory cytokines, such as IL-6, possibly induced by the enhanced release of the stress mediators, cortisol and catecholamine, is one potential pathway occurring in post sleep deprivation (Brice et al., 2015).

CRP is a member of pentraxins family. As the most sensitive inflammatory indices, CRP plays an important role in immune response. The CRP gene, located on the short arm of chromosome 1, contains only one intron, which separates the region encoding the signal peptide from that encoding the mature protein. Induction of CRP in hepatocytes is principally regulated at the transcriptional level by the cytokine IL-6, an effect which can be enhanced by IL-1β. Both IL-6 and IL-1 control expression of many acute phase protein genes through activation of the transcription factors STAT3, C/EBP family members, and Rel proteins (NF-κβ) (Black et al., 2004; Ehrmann et al.; Pedersen et al.).

SLM as the most effective material Milk Thistle with scientific name Silybum Marianum is composed of a group of chemicals called flavonolignan (Roghani et al., 2012). Milk Thistle of the Asteraceae family and is originally native to the Mediterranean region (Kren & Walterová, 2005). This material has effects at the cellular level and metabolic regulation (Taghvaei et al., 2013). SLM suppresses inflammatory cytokines (TNF-α and IL-1) and is a potent inhibitor of NF-κβ activation in response to TNF. This effect was mediated through the inhibition of phosphorylation and degradation of IκB, an inhibitor of NF-κβ. NF-κβ plays a critical role in inflammation (Agarwal et al., 2006).

Recently, it was shown that the pleiotropic anti-angiogenic effects of silybin on HUVEC and human microvascular endothelial cells (HMVEC-dermal origin) involve growth inhibition, cell cycle arrest, apoptosis induction, inhibition of capillary tube organization, reduced invasion and migration of human endothelial cells. One of the molecular pathways involved in these effects, including down-regulation of survive in and inhibition of Akt and NF-κβ signaling, and inhibition of matrix metalloproteinase-2 (MMP-2) secretion (Gazák et al., 2007).

Given the anti-inflammatory, antioxidant and anticancer effects of SLM, in this experiment, the effect of SLM in reducing inflammatory symptoms of polycystic ovary syndrome was studied.

MATERIAL AND METHOD

In this study of 144 adult female Wistar rats weighing 160±20 gr were used from the animal house of the Kharazmi University, Tehran, Iran, in a central animal care facility were kept, housed in plastic cages (30 x 19 x 13 cm) under a 12-hour light, 12-hour dark cycle (lights on from 6:00 to 20:00). Humidity and temperature were set at 55 ± 15 % and 20 to 24 °C, respectively, and free access to water and commercial food (Behparvar Com, Iran) was provided. All procedures were carried out according to the Guidelines for the Care and Use of Laboratory Animals (National Research Council, 1996). In this experiment, adult female Wistar rats with a 2-3 regular estrous cycle period within a twelve- to fourteen-day period were used. PCOS rats were selected on the basis of displaying a minimum of two continuous estrous cycles. Rats were in the estrous stage of their reproduction cycle.

For the induction PCOS phenotype there are induced hormonal and non-hormonal methods including testosterone, estradiol valerate (EV), dehydroepiandrosteron (DHA), adrenocorticotropic (ACTH) and long-term Light. in this study of hormonal induction with estradiol valerate to rats were used. Therefore, the injection site was sterilized with 70% alcohol and 2 mg BW estradiol valerate subcutaneous injection. To sham rats, the same amount of sesame oil (estradiol solvent) injections were administered. All animals were under vaginal smear analysis for a period of 60 days until the appearance of persistent vaginal cornification (PVC), a sign of follicular cysts in the ovary. In addition to, other approvals for the induction of polycystic ovary syndrome, three rats were killed and histological and serological tests confirmed the induction of rats compared to controls. After verifying the induction of PCOS, the PCOS group was divided into two subgroups, PCOS and PCOS + SLM. PCOS + SLM received 100 and 200 mg/kg BW SLM intraperitoneal injection (IP) for 14 consecutive days, while sham group received DMSO (Di methyl sulfoxide) as SLM solvent.
After 14 consecutive days of treatment with SLM, rats were anesthetized with CO₂, trunk blood was collected, and serum samples were separated by centrifugation at 6,000 rpm for five minutes. Samples were kept at -40 °C for later serological experiments. Ovaries were separated from the twisted oviduct tubes. Ovarian samples for histological and immunohistochemical experiments were fixed in Bouin and formalin, respectively.

**Histological analysis:** After fixation the samples for 14 hours in Bouin’s fixative, Samples with solutions of increasing alcohol levels of 20 % to 100 % for 45 minutes to an hour each were dehydrated and after clearing with toluene (2 h) were paraffin embedding. The samples were cut to a thickness of 6-7 mm by microtome and sections were placed on gelatin-coated slides. To study the histologic sections after paraffin removal and discharge by decreasing levels of alcohol solution, were stained with hematoxylin and acid - alcohol differentiated and stained with eosin and were examined by light microscopy.

**Immunohistochemical analysis:** After deparaffinization, sections were boiled in citrate buffer (10 mM) to pH=6 for 20 minutes in a microwave oven to reveal antigens. Nonspecific binding sites by placing sections in primary antibody albumin bovine serum (Sigma) 4 % in PBS for one hour at room temperature was carried out. Then the slices with TNF-α polyclonal antibody diluted (abcam) (1:50) 4 % PBS-BSA for 24 h at 4 °C and incubated in a humidified chamber. Endogenous peroxidase was quenched with 3 % (v/v) hydrogen peroxide in methanol (ten minutes at room temperature). The slides were washed and secondary antibody for one hour at room temperature in a humidified chamber. Endogenous peroxidase was quenched with 3 % (v/v) hydrogen peroxide in methanol (ten minutes at room temperature). The slides were washed and secondary antibody for one hour at room temperature in a humidified chamber was incubated. Detected by the marker, Di-amino-benzidine (DAB) was performed with a brown precipitate. Then, the sections were analyzed by light microscopy (Zeiss, Germany). All statistical analyses were performed with image J, one way ANOVA and INSTAT software version 3. P-Values of 0.05 or less were considered statistically significant. Corresponding graphs were plotted using EXCEL program.

### Results

**Hormone concentrations.** Control and estradiol valerate-induced animals were weighted. It was shown that in addition to a significant increase in body weight (P <0.001) in the induction group compared to the control group, the increase in abdominal fat was observed macroscopically. After intraperitoneal injection of SLM for 14 days reduced body weight was observed (P <0.01) had significantly and abdominal fat was also reduced compared to the PCOS group. In this study, no significant differences were observed between the results of the control group and the sham group. Therefore, publishing the results of the sham group was not included in the results (Table I).

By a scale-sensitive ovarian weight (SARTORIUS-Germany) it was shown that the mean weight of the ovaries in the PCOS group increased significantly compared to controls (P <0.01), however, the mean ovarian weight in samples treated almost as much as the control (P <0.01) showed (Table I).

Chemiluminescent immunoassay (CLIA) showed a significant increase (P<0.05) in the levels of testosterone, estradiol and LH in the PCOS group and significant decrease (P<0.05) in the levels of progesterone and FSH versus control group. In this study, administration of different doses of SLM in PCOS rats during 14 consecutive days to achieve positive results. Different doses of SLM caused a significant decrease in the levels of estradiol, testosterone, LH and significant increase in the levels of progesterone and FSH, Due to the appearance of corpus luteum in the SLM-treated ovaries (Table I).

**Different groups of follicles.** To determine changes in follicular development, Follicles were divided into six group including primordial follicles (PMF), primary follicles (PF), preantral follicles (PAF), antral follicles (AF), cystic follicles (CF) and corpus luteum (CL). A significant decrease was observed in the number of primary follicles, antral follicles,

### Table I. Hormone concentrations, ovarian and body weight of rats in the control, PCOS and treated with SLM groups.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Control</th>
<th>PCOS</th>
<th>SLM 100 mg/kg BW</th>
<th>SLM 200 mg/kg BW</th>
</tr>
</thead>
<tbody>
<tr>
<td>P4 (ng/ml)</td>
<td>71.035 ± 2.705</td>
<td>29.38 ± 0.5</td>
<td>48.75 ± 0.25</td>
<td>**65 ± 0.32</td>
</tr>
<tr>
<td>T (ng/ml)</td>
<td>0.65 ± 0.04</td>
<td>0.96 ± 0.01</td>
<td>0.76 ± 0.02</td>
<td>**0.695 ± 0.005</td>
</tr>
<tr>
<td>E2 (ng/ml)</td>
<td>0.029 ± 0.001</td>
<td>0.052 ± 0.003</td>
<td>0.032 ± 0.001</td>
<td>**0.028 ± 0.001</td>
</tr>
<tr>
<td>FSH (ng/ml)</td>
<td>2640.29 ± 55.66</td>
<td>617 ± 99</td>
<td>1312.655 ± 54.9</td>
<td>**1584.23 ± 15.72</td>
</tr>
<tr>
<td>LH (ng/ml)</td>
<td>3 ± 0.15</td>
<td>5.565 ± 0.245</td>
<td>2.885 ± 0.085</td>
<td>*2.71 ± 0.09</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>160 ± 6.57</td>
<td>***227.5 ± 6.67</td>
<td>***210 ± 16.25</td>
<td>**180.55 ± 6.72</td>
</tr>
<tr>
<td>Ovarian weight (mg)</td>
<td>12.7 ± 0.3</td>
<td>***21 ± 0.39</td>
<td>***17 ± 0.39</td>
<td>**15 ± 0.23</td>
</tr>
</tbody>
</table>

Mean hormone concentrations ± Standard deviation (SD) ***P<0.0001, ** P<0.001, *P<0.05.
corpora lutea, primordial follicles and preantral follicles in PCOS ovaries. In addition, some large cystic follicles with a thin granulosa of 2–3 cell layers were observed. In PCOS group, no corpus luteum was detected. In the control group, ovaries contained no cyst but several follicles at different stages of development, and also corpus luteum, were distinguishable. These results were indicative of a complete induction of the PCOS phenotype. Thus, according to our observations, after 60 days of treatment with EV leading to cysts, follicles arrest and consequently, the cause of anovulation.

In morphological studies in rats treated with SLM, an increase in the number of primordial and preantral follicles and corpus luteum was observed, and a significant decrease the number and size of cysts compared to the PCOS group. Injection of high doses of SLM (200 mg / kg BW) showed almost no cysts were found. Also, the present number of corpus luteum in this group shows their ovulation (Table II).

In addition to, the various morphometric studies in the follicular sheath cell layers of graded ocular and subjective eye 10 and 40 were used for light microscopy. Follicular sheath thickness measurements in the PCOS group according to previous reports and present research showed a significant increase that has been induction of PCOS in this group of animals. In the group treated with SLM follicular sheath layer measured by graded ocular done and the results were compared with the control and PCOS groups. This comparison showed that SLM caused a significant decrease in the thickness of this layer in the ovaries of animals and causes release of oocyte complex – Cumulus, probably due to the anti-inflammatory effect of SLM that reduces inflammation in the follicular sheath and ultimately reduces the thickness of this layer (Table II).

IL-6 assay. In this study, PCOS induction lead to a significant rise in IL-6 inflammatory index (P< 0.001 vs. control rats). The effect of SLM (100, 200 mg/kg BW) on the level of IL-6 in PCOS rats was examined for 14 days after complete induction of PCOS. Our study indicated that SLM reduces IL-6 content in the SLM-treated groups as compared to the PCOS group. It also indicated that SLM with dose of 200 mg/kg BW SLM is more effective than the dose of 100 mg/kg BW (Fig. 1).

Evaluation of changes in CRP. In addition to inflammatory changes CRP levels were measured in rats with PCOS. CRP is a member of pentraxins family. As the most sensitive inflammatory indices, CRP plays an important role in immune response. PCOS induction lead to a significant raise in this systemic inflammatory index, and its reduction in rats treated with SLM was significant. injection of SLM significant decrease (P<0.05) in the levels of CRP in the SLM-treated rats (Fig. 2).

Histological analysis. In this study, a histological analysis was performed. Polycystic ovary (C) the number of large cystic follicles with thin granulosa layer and thick follicular sheath. Ovaries treated with high doses of SLM (200 mg/kg BW) (B) that is large Graafian follicle with thin follicular sheath that ovulation signs were normal. Figure 3A shows
normal ovaries full of corpus luteum. Secondary row shows comparing the granulosa layer and follicular sheath of antral follicles in polycystic ovary (a), treated with SLM (b) and normal ovarian (c) (Fig. 3).

**Immunohistochemistry analysis.** Considering the importance of expression of TNF-α in angiogenesis and the ovulation process. TNF-induced increased expression in ovarian pathology. In this study we examined the expression levels of TNF-α, this factor was expressed in pre-antral follicles, granulosa and cumulus layer of antral follicles in normal ovaries. The intensity of TNF-α was significantly increased in these cells versus control group. In addition, the final antral follicle granulosa layer of cystic follicles in ovaries with PCOS. TNF-α was expressed at very low levels in follicular theca layer. In cellular layers follicles the ovaries of treated with SLM decreased the expression levels of TNF-α. Granulosa layers of pre-antral and antral follicles and the cells surrounding the oocyte expressed less TNF-α (Fig. 4).

![Graph showing CRP levels](image)

**Fig. 2.** PCOS induction led to a significant raise in CRP (P < 0.001) vs. control rats. SLM treatment effects on levels of CRP production (pg/mL) in polycystic ovarian syndrome (PCOS). Baseline parameters of PCOS rats (n = 8), control (n = 8) and SLM-treated rats (n = 8). ***P< 0.001: PCOS vs. Control, + P < 0.05: SLM200 vs. PCOS.

**Fig. 3.** Histological analysis of normal ovaries (A, a) compared with PCOS (C, c) and ovaries treated with SLM (B, b). The morphological changes of the rats’ ovarian tissues were stained with hematoxylin and eosin, as described in the Materials and Methods section. A, a) A representative rat’s ovarian tissue section from the control group, which had normal appearance (A×100,40), (Scale bar, 50, 5 mm). C, c) A representative rat’s ovarian tissue section from the PCOS group showed thickening surface albuginea, under which there were many follicles in different phases (including atretic follicles and cystic dilating follicles), as well as fewer layers of granular cells, disappeared oocytes and corona radiating within the follicles (B×100,40), (Scale bar, 50, 20 mm). B, b) A representative rat’s ovarian tissue section from treatment with Sylimarin, which showed increased granular cell layers, and some ovulation phenomena (C×100,40) (Scale bar, 50,5 mm). AF: atretic follicle, CF: cystic follicle (*), CL: corpus luteum, GCL: granular cell layer (→).
Fig. 4. Photo micrographs of immunohistochemical expression of TNF-α in the primary and secondary follicles, infolded layers and follicular liquid of Graafian follicles and cysts. A,a: normal expression of TNF-α in pre-antral and antral follicle in normal ovaries show. Row B,b and C,c: increased expression of TNF-α in pre-antral follicle in the ovaries of PCOS. Reduction in TNF-α expression in primary and pre-antral follicles in the ovaries treated with low dose of SLM in Row (D,d), (E,e) and high dose of SLM in F are shown. Original magnification, x100 (a-d) and x400 (e-f), (Scale bar, 25 mm). granular cell layer (→), follicular liquid of Graafian follicle (Δ).
**H-score.** The H-score system was based on the proportion and intensity of brown staining cells. For H-score assessment, ten fields were chosen at random at 400× magnification and the staining intensity of each slide was scored as 0, 1, 2 or 3 corresponding to the presence of negative, weak, intermediate or strong brown staining, respectively. The total number of cells in each field and the number of cells stained in each intensity were counted. The average percentage positive was calculated and the following formula was applied in each group:

\[
\text{H-Score} = \frac{(\% \text{ of cells stained at intensity 0} \times 0) + (\% \text{ of cells stained at intensity 1} \times 1) + (\% \text{ of cells stained at intensity 2} \times 2) + (\% \text{ of cells stained at intensity 3} \times 3)}{100}
\]

H-score between 0 and 400 was obtained where 400 was equal to 100% of the cells stained strongly (Kalantari et al., 2012).

In PCOS group presented strong immunoreactivity to TNF-α was observed in ovarian stroma (P<0.001), preantral follicles (P<0.001), granulosa layer (P<0.001). Diffuse, usually weak TNF-α expression was seen in theca layer compared with the control group. TNF-α expression in stromal cell in the SLM-treated PCOS group showed Significant decrease (P<0.001) compared with PCOS. Also immunostaining in the theca layer in SLM-treated PCOS group was less intense than in PCOS group. In the SLM-treated PCOS group compared with PCOS, TNF-α presented low expression in granulosa (P<0.001) and preantral follicles (P<0.05) (Fig. 5).

**DISCUSSION**

TNF-α is an inflammatory cytokine that is produced by lymphoid cells, mast cells, endothelial cells, heart myocytes, adipose tissue, and nervous tissue fibroblasts. Its abnormal expression and hyperactivity can lead to pathological conditions such as inflammation and polycystic syndrome (Park et al., 2008).

The results of this study showed the increased follicular sheath thickness, decreased granulosa cells thickness in PCOS group, decreased follicular sheath thickness, and increased granulosa cells in the PCOS treated with SLM groups. TNF-α in rat induced apoptosis in granulosa cells of the antral follicles (Spaczynski et al.) probably due to the overexpression of TNF in polycystic ovaries leading to a thin layer of granulosa cells and promotion of this syndrome.

Spaczynski, reported changes in expression of TNF-α can lead to increased follicular sheath layer thickness and decreased granulosa cells in the middle antral follicles and graafian follicle in this syndrome. The results of this study is consistent with Spaczynski study that TNF-alpha causes proliferation and differentiation of theca cells and increased steroidogenesis of the follicular layer cells.

SLM may cause decreased follicular sheath thickness and increased ovulating through inflammation reduction and

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![Fig. 5](image-url) The mean of H-score in the control group, PCOS and treated with low and high dose of silymarin in stroma, preantral follicles, granulose and theca layers of antral follicles. 

**P<0.001, **P<0.01, *P<0.05. *** P< 0.001: PCOS vs. Control, +++ P < 0.001, ++ P < 0.01 and + P < 0.05 SLM groups vs. PCOS.
the reduced expression of TNF-α. Furthermore, the increased follicular sheath layer in the syndrome could be due to increased expression of TGF-β. As a result, due to the increased collagen, the excessive collagen production in this layer during ovulation can prevent the oocyte release (Raja-Khan et al., 2014).

Chris Kelly reported that the CRP levels in women with PCOS are significantly higher than normal subjects. The increased BMI in the samples can increase CRP levels. He concluded that the expression of adipocyte-derived cytokines in patients with high BMI like IL-6 and TNF-α plays an important role to cause inflammation (Kelly et al., 2001).

In 1990, Watson some evidence that proved IL-6 secretion by ovary (Kraakman et al., 2013). Basic production of IL-6 occurs in culture of primary ovarian tumors and ovarian cancer cell. In addition, IL-6 is produced from numerous granulosa cells of many species. IL-6 production in granulosa cells was shown in various studies on rats, cows, rabbits, and humans (Watson et al., 1990).

In 1998, Keck et al., proved that human granulosa cells produce IL-6 and have IL-6 receptors. Therefore, IL-6 is able to affect the activity of granulosa cells and specifically the steroidogenesis of these cells through the autocrine mechanism. Interfering IL-6 in regulating production of granulosa steroids in animals was proved (Gorospe & Spangelo, 1993).

According to the literature, it was proved that PCOS has a close relationship with metabolic syndrome and inflammation, cytokine increase, disorder of hormones level of hypothalamus - pituitary - ovarian axis, and insulin resistance. Therefore, it is probable that treating the rats with SLM would lead to adjusting these syndromes to improve PCOS.

Senthil Kumaran et al. (2009) showed that a high-fat diet in mice by oral administration of EGCG (the main catechin in green tea) caused a significant decrease in CRP. The green tea extract can reduce CRP levels (Namita et al., 2012). The increased CRP level can be observed in type II diabetes and PCOS associated with cardiovascular disease. Morin-Papunen et al. (2003) showed that metformin reduced serum CRP levels in patients with PCOS and SLM.

The immunohistochemical study of polycystic ovaries treated with SLM showed a significant decrease in TNF-α level. Over-expression of the COX-2 enzyme and TNF in vascular tissue and peripheral nerves can increase the production of PGE-2, the increased activity of NF-κB and lipoxygenase, IL-1, TNF and inflammation (Baluchnejadmojarad et al., 2010).

Manna et al. (1999) studied the effect of SLM on NFκB activation induced by various inflammatory agents. SLM blocked TNF-α-induced activation of the NF-κB in a dose- and time-dependent manner. It was mediated through inhibition of phosphorylation and degradation of IκBα inhibitory protein. NF-κB-dependent reporter gene transcription was also suppressed by SLM (Manna et al.).

As SLM is able to inhibit 5-lipo-oxygenase enzyme production and Leukotrienes, so it can prevent inflammation in polycystic ovary syndrome (Farideh et al., 2010).

Spritzer et al., found that the basic decrease and response-stimulating of the luteinizing hormone in obese patients with PCOS may be associated with resistance to the leptin and also leads to increases in the luteinizing hormone secretion. In other words, the increased leptin can resulted in increased LH. In patients with PCOS, LH / FSH ratio and insulin resistance are higher than the control group. Zafari et al., in 2010 have shown that chamomile extract in polycystic ovary significantly decreased the levels of estradiol and LH and FSH due to the symptoms of the syndrome (Welschen et al., 1973). In this study, the prevalence of the syndrome reduced by inhibitory effects of SLM.

Welschen showed the increase of LH is required for the final stages of follicular growth in the PMSG-treated (Pregnant mar’s serum gonadotrophin) rats (Attarzadeh et al., 2012). But it is the main complication of this syndrome, since releasing the oocyte requires of the increase of LH. Welschen reported that the secondary follicles (between 28 to 56 days left to come) can increase the LH concentrations, and the expansion and consolidation of the cysts. In the present study, due to the presence of cysts, the number of these follicles were increased which is consistent with the Welschen study. It can be concluded that the LH concentrations in the treated group significantly reduced compared with the PCOS group. The number of follicles reduction by SLM is one of the important factors in reducing the symptoms of ovarian cysts and this syndrome.

Serum concentrations of testosterone and androstenedione in women with PCOS compared with normal women 50 to 150 % increased (David et al., 2005). Increasing the production of androgen precursors in the follicular sheath cells leads to the increased production of androstenedione, which is converted to form testosterone by the β17-hydroxy steroid dehydrogenase (β17) or by the aromatase enzyme into the estrone forms (Mohseni et al., 2010). In this study, the testosterone levels in the study group in higher doses of the SLM intraperitoneally injected were significant (P < 0.001). The anti-angiogenic effects of the SLM in the Nabiuni study (Karimzadeh et al., 2013), possibly is
referred to the decreased proliferation and follicular sheath thickness and thus reducing the production of testosterone.

Nicola Doldi et al. (1998) showed that progesterone and estradiol production of granulosa cells from women with PCOS is not normal. This suggests that patients with PCOS compared with healthy subjects show a different reaction to the gonadotropins. They stated that the serum concentrations of estradiol and progesterone in PCOS subjects is significantly increased and decreased, respectively (Doldi et al.). Although it is consistent with our results, but in this study, SLM intraperitoneally injected for 14 days, so, the results were contrary and respectively cause decreases and increases in estradiol and progesterone hormones which were statistically significant, perhaps the increase in the progesterone hormone rate can be due to an increase in corpus luteum in the samples treated with SLM.

Kouchesfahani et al. reported that in the ovaries of PCOS group using the bee venom in rats the primary and primitordial follicles increased. Our results showed that the use of higher doses of SLM could significantly affect the follicular growth rate and increased follicular types. In fact, the ovaries treated with SLM significantly increased primordial and primitive follicles were according to research conducted by Kouchesfahani and colleagues it could be due to activation of the ovary.

In the alloxan or streptozotocin-induced diabetic rats, SLM affects the kinetics of glucose 6-phosphatase and gluconeogenesis leading to decreased blood glucose (Senthil Kumaran et al.). Metformin is one of the most effective drugs for treatment of PCOS which can reduce the amount of glucose; as a result, we can state that the same path SLM may reduce symptoms of dumping syndrome.

Baluchnejadmoharad et al. showed one of the effects of lowering blood glucose by SLM is reducing oxidative stress. On the other hand, the inflammatory processes plays an important role in the pathogenesis of diabetes. Currently, the role of inflammatory factors such as COX-2 and TNF-α inhibitors in alleviating complications of diabetes has been considered (Baluchnejadmoharad et al.).

CONCLUSIONS

The results of the present study is consistent with the previous studies indicating that SLM reduction effects of PCOS on ovarian, such as follicular sheath diameter increases, the increase in ovarian weight and increased TNF-α expression in ovarian tissue.

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