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Research Article

The Effect of Green Tea on IL-6 and CRP level in Model of Polycystic Ovary Syndrome as an Inflammation State

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Abstract

Background: Having low-grade chronic inflammation state symptoms such as elevated C-reactive protein and interleukin-6 play a crucial role in polycystic ovary syndrome (PCOS). Green tea has anti-inflammatory properties. This research evaluates the effect of green tea on inflammatory indexes. Adult Wistar rats were exposed to subcutaneous administration of estradiol valerate for PCOS induction. PCOS rats were divided into control and experimental groups received intraperitoneal injection green tea extract daily. Animals were anesthesia with chloroform. Ovary and serum were taken to measure the inflammatory markes using ELISA kits and Histomorphometric studies. The data were analyzed using One-Way ANOVA with significance level $P<0.05$.

Results: The results indicated the significant reduction in inflammatory markers and significant changes follicular layers thickness in the treatment group as compared with control.

Disscoution: it can be concluded that having anti-inflammatory substances, green tea is effective in symptoms of this syndrome and metabolic syndrome.

Keywords: Polycystic ovary syndrome, Wistar rat, green tea extract, inflammation, ELISA.

1 Background

Cytokines play a major role in response to the inflammatory stimuli and tissue damages. Interleukin (IL) -6 (20-30 kD aglycoprotein) is a pleiotropic cytokine, which is mainly produced by immune system and different types of adipose tissue, hepatocytes, and ovarian follicular granulosa [1]. Interleukin receptors are made of two sets, including GP-130 and il-6r. Interleukin-6 is on 7p21 chromosome. Interleukin-6

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expression is basically regulated by Beta Kappa core factor (NK-kb) 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. Interleukin-6 plays a key role in the pathogenesis of chronic inflammation, insulin resistance, and CV diseases [2].

CRP is a member of pentraxinsfamily. As the most sensitive inflammatory indices, CRP plays an important role in immune response. In human, CRP gene lies on 1q21 to 1q23 chromosomes. CRP protein is produced by liver and it is almost the strongest inflammatory marker, as its increase is responsible for inflammation. Appearance of CRP is considered as a strong predicator for metabolic abnormality. This substance is produced in liver and intimal of vessels. Despite CRP's inflammatory role, using different mechanisms, such as producing nitric oxide (NO), increasing molecules adhesion, and changing absorption of low-density lipoprotein (LDL) by macrophages, it is able to damage vessels. The increase of this protein (as an independent predicator of CV risk) increases damages to CV system 2 - 5 times. Its content is mainly regulated by some cytokines, especially 1L6 [2].

PCOS is a reproduction abnormality together with a metabolic disorder. It is seen among 5-10 percent of women during the age of fertility and it is the major factor of infertility [3-4]. Women with PCOS are chronically with anovulation; however, ovulation may occur spontaneously and pregnancy may sometimes occur. Irregular menstruation, which is one of the signs of PCOS syndrome, is usually continued since menarche, as the menstruation pattern never occurs [5]. Biochemical disorders indicating PCOS, which are seen in patients, include increased serum concentration of the androgenic hormones such as testosterone, androstenedione, dehydroandrosterone, dehydroandrosterone sulfate, hydroxyl progesterone-17 produced in ovary [6], hirsutism, oligomenorrhoea, amenorrhea [7-8], abnormality in releasing gonadotropin together with the increase of LH secretion in proportion to FSH [5] In this syndrome, obesity and increase in body fat (accompanied by a central lipid distribution pattern, which is described as visceral obesity [9] and some disorders including sweet diabetes type-II, cardiovascular abnormalities and endometrial, breast cancer seem to be common [10]. Moreover, it is followed by insulin resistance, increased insulin, and blood sugar drop [11].

Green tea is a product made by leaves and sprouts of *Camellia sinensis*, which is almost the commonest drink after water. Green tea is a non-fermented tea [12]. Flavonoids of green tea account for 36 percent of the dry weight of tea and it is the major part of theflavonoids is flavan-3-ols, which is called catechin. Two-hundred and thirty seven milliliters of green tea contains 30-130 mg epigallates. Catechins are strong *in-vivo* and *in-vitro* antioxidants. It also contains some minerals and vitamins that increase antioxidant potential of this type of tea [13]. The biological effects of green tea that is based on the epigallate effects include cardiovascular diseases and some cancers, anti-hypertension effect, body weight control (weight reduction), antiviral and antibacterial activities, participation in anti-diabetic processes (through controlling saliva and intestine amylase that prevents starch breakdown). [14] Other effects include anti-mutagenic and anti-inflammatory properties, protecting against ultraviolet rays, increasing density of bone minerals and anti-fibrotic properties, reducing resistance against cholesterol and triglycerides, and reducing resistance against insulin [13, 15].

PCOS can be considered as a metabolic disorder accompanied by endocrinal disorder, as women with such syndrome suffer cardiovascular problems. Syndrome of insulin resistance was also seen in these patients. The correlation between pre-inflammatory cytokines and insulin resistance is a pathophysiological relationship and a guiding path for insulin resistance and cardiovascular illness followed by PCOS [16, 17 and 18].

Moreover, prevalence of the illness among the obese people exceeds the thin people. Obese women show higher il-6-generating levels as compared with thin women. These data show that adipose tissue is a great source of IL-6 in women. In addition, polymorphism c-174g exists inside IL-6 promoter. It is shown in vitro that this gene is related to the increase of IL-6 level and hyperandrogenismin thin women. Therefore,

IL-6 can establish a relationship between anthropometric and syndrome of metabolic and hyperandrogenismin people with PCOS [19, 20, 21, 22 and 23].
On the causes of PCOS, it can be stated that PCOS is an endocrine abnormality and many markers involve in its prevalence, including mutation in the genes encoding gonadotropin and/or mutation in inflammatory markers [24]. We study the amount of inflammatory markers, as the recent studies extensively show that there is a close correlation between inflammation and chronic illnesses, especially insulin resistance and PCOS. There are many inflammatory factors involved in PCOS. The present study examines the effect of green tea extract as an inflammatory marker on il-6 and CRP, as many studies show that these factors are strong managers of inflammation system [24, 25 and 26].

2 Methods

This study was conducted on 96 Wistar rats with the average weight 200±20 gr. The rats were kept under the approximate temperature of 22˚C, 12:12 dark-light. Before drug administration, the rats were kept under the above conditions at least for 7 days so that they become accustomed to the environment completely. During this period, the animals consumed normal dish and sufficient water.
The rats selected for induction had two successive regular periods of estrous cycle after preparing *vaginal smear* daily test for 12-14 days [16]. Hormonal induction using estradiol valerate was applied in this project. For this purpose, 2 mg/kg estradiol valerate is injected subcutaneously. After injection, *vaginal smear* test is prepared daily. The test is continued as long as the changes of estrus cycle and its irregularity are appeared and we reach the stage of Persistent Vaginal Cornification (PVC), which is usually 60 days after injecting estradiol valerate. To confirm induction of syndrome, 2 ml blood sample is taken from a rat's heart. histomorphometric studies by the hematoxylin and eosin technique was performed. Ovaries were placed in bouin fixative for histological analysis; fixed samples were kept in alcohol solutions of 20 to 100% for a period of 45 min to dehydrate, and later in alcohol/xylen (50:50) and xylen (3 times) for clearing, and finally blocked in paraffin. Samples were sliced to sections of 7 micron thickness using a microtome, and these sections were placed on slides, previously coated with gelatin, prior to staining with hematoxylin-eosin for histological observations.
After syndrome confirmation using the measurement of hormone levels and the histomorphometrical studies on part of an inducted group, the remaining rats were divided into five experimental groups: The PCOS rats that received no injections (PCOS control),The rats that received saline (sham), The PCOS rats that received of 50 mg/kg of green tea extract (GT-Treated-1), The rats that were received intraperitoneal injection 100 mg/kg of green tea extract (GT-Treated-2), The rats that were received intraperitoneal injection 200 mg/kg of green tea extract (GT-Treated-3) for 14 days, consequently.

2.1. Green Tea Extraction

The green tea was collected in Lahijan city located in Gilan province. The collected samples were dried in the shade and its leaves were removed from the plants' stems. Then, they were pulverized to a powder form by mechanical means. To prepare hydro-alcoholic extract, 200 gram of the powder of the dried green tea leaves was soaked with 1500 ml of ethyl alcohol for 48 hours. The obtained solution was evaporated after it was filtrated in the constant flow of the air. Then, it was completely dried. Thereafter, dried extract was collected. Then, the hydro alcoholic extract of the green tea was used to prepare the desired dose of the treatment [16].

2.2. CRP Measurement

After taking blood samples from hearts of the rats and preparing blood serum using anELISA kit (Millipore's MILLIPLEX® MAP Rat/Mouse CRP Single Plex USA), CRP contents were measured.

Serological analysis was carried out to measure IL-6 serum level and hormonal changes. An ELISA kit rat IL-6 platinum ELISA/ (Bender Medsystems, Vienna, Austria) was used to diagnose IL-6 serum level as per the manufacturer's instructions. This method was iterated 3 times. The results expressed as (pg/ml) MEAN±SD were used for individual rats. Sensitivity of this method for IL-6 was 12 pg/ml. First, all the wells washed using buffer solution 1%. Then add 100 cc assay buffer to the blank wells, 50 cc assay buffer and 50 cc serum to sample wells. Then 50 cc biotin was added to all wells and put them under the room temperature (RT) for 2 hours. Next, wash the wells using buffer solution 6 times and add 100 cc HRP and leave them for 1 hour RT. After washing, add 100 cc TMB to the wells for 10 minutes RT. Then add 100 cc Stop solution to them and read the stripe using an Elisa Reader Machine at a wavelength of 450 nm.

3 Results

3.1. IL-6 assay

In this study, PCOS induction led to a significant rise in IL-6 inflammatory index ($p < 0.001$ vs. control rats). The effect of green tea (100mg/kg) on the level of IL-6 in PCOS rats was examined for 14 days after complete induction of PCOS. As shown in Figure 1, the respective IL-6 levels in control, PCOS and Green tea-treated PCOS rats were 50, 100 and 200 pg/mL. Our study indicated that green tea reduces IL-6 content in the green tea-treated groups as compared to the control group. It also indicated that green tea with dose of 100 mg/kg BW is more effective than other doses and such effectiveness is significant as compared to 50 and 200 mg dosage (Fig1).

CRP is an exquisitely sensitive systemic marker of inflammation and tissue damage. The cute-phase response comprises the nonspecific physiological and biochemical responses of endothermic animals to most forms of tissue damage, infection and inflammation. Plasma CRP is produced only by hepatocytes, predominantly under transcriptional control by the cytokine IL-6, although other sites of local CRP synthesis and possibly secretion have been suggested. PCOS induction led to a significant raise in this systemic inflammatory index, and its reduction in rats treated with green tea was significant (Fig 2).

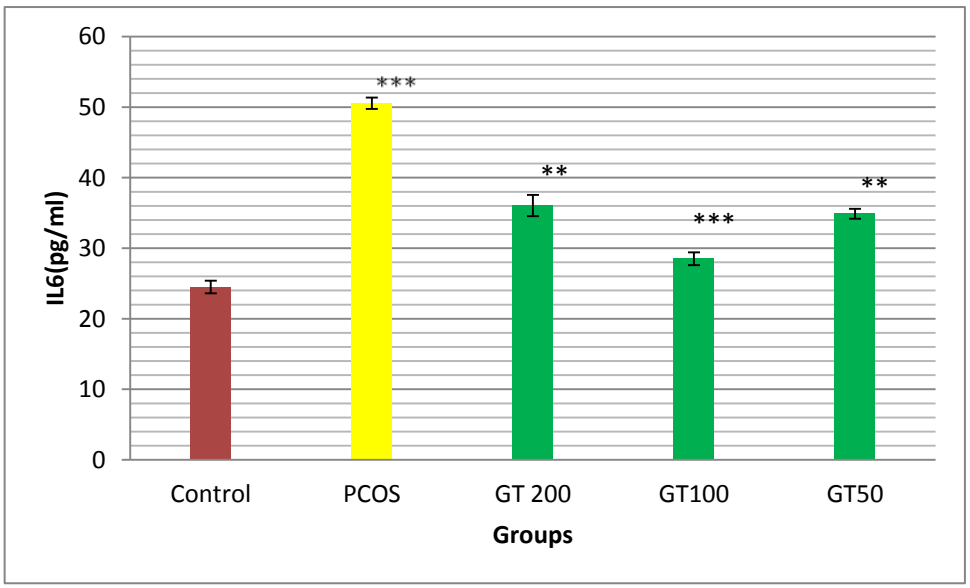


Figure 1: PCOS induction led to a significant rise in IL-6 ($p < 0.001$) vs. control rats. Green tea treatment effects on levels of IL-6 production (pg/mL) in polycystic ovarian syndrome (PCOS). Baseline parameters of PCOS rats (n = 8), control (n = 8) and Green tea-treated rats (n = 8). *** $p< 0.001$ ** $p < 0.01$ vs. control.

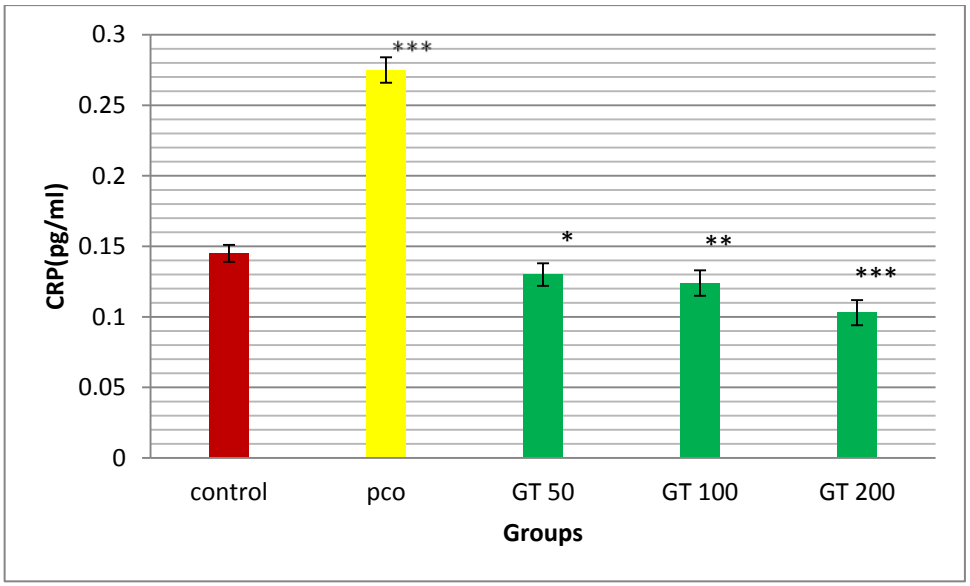


Figure 2: PCOS induction led to a significant raise in CRP ($p < 0.001$) vs. control rats. green tea treatment effects on levels of CRP production (pg/mL) in polycystic ovarian syndrome (PCOS). Baseline parameters of PCOS rats (n = 8), control (n = 8) and Green tea-treated rats (n = 8). *** $p< 0.001$ ** $p < 0.01$ *$p<0.05$ vs. control.

3.2. Histological Finding

Examining morphology of the tissue sections prepared from ovaries in the experimental group of follicles at different stages including graph showed that the follicles had thick granular layer and thin theca compared to the PCOS control group. Moreover, a significant number of corpus luteum, indicators of ovulation, was observed. However, greater number of cystic follicles was observed in the PCOS control group. They had thin granular layer and thick theca. Moreover, low number of corpus luteum was observed which indicated that injection of estradiol valerate induced polycystic ovaries reduced the number of active follicles as well as ovulation (Fig3).

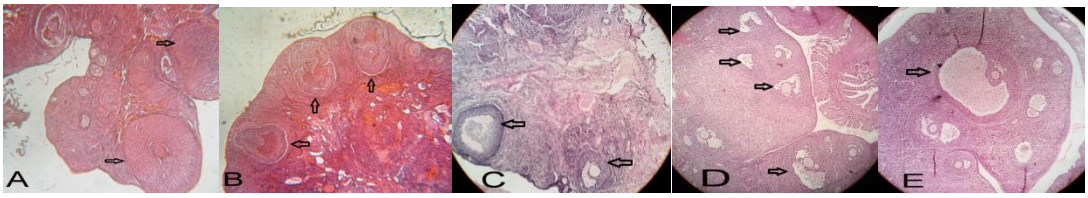


Figure 3: Histological analysis showing normal (a),PCOS(b) and green tea extract -treated PCOS (c,d,e) ovaries. Ovarian sections were stained with hematoxylin and eosin. An increased in the number of cysts (arrow), lack of corpus luteum (arrow head), and increased levels of ovarian stroma (star) in b is compared to a. In part (c,d,e), a decrease in the number of cysts and the ovarian stroma level, and the appearance of a corpus luteum can be detected in the experimental group receiving 50,100,200mg/kg of green tea respectively. X400.

4 Discussion

In the present study, we investigated the inflammation as the main feature of PCOS and the therapeutic effect of Green tea on **PCOS.**

PCOS is a complex and diverse abnormality indicating one or many abnormalities within a phenotype. One spectrum of these abnormalities can be studied in the field of metabolic processes, as it is seen the patients with PCOS who are usually obese, insulin resistance, hyperinsulinemia, and diabetes type II (27, 28). The following reasons indicate that they play a crucial role in creation and intensification of PCOS symptoms with the following reasons:

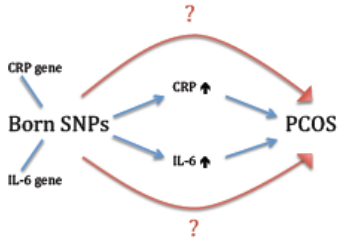
First, CRP is one of the major proteins of inflammation, which is secreted by liver in response to the increase of inflammatory cytokines such as Interleukin (IL)-1 beta, IL-18, IL-6 [29]. High concentration of CRP, which is related to the risk of CV diseases, is seen in women with PCOS. According to the study of Nabiuni *et al*, the rate of this protein in the polycystic rats induced by estradiol was significantly high [31-30].

Second, the increase of c-174g factor expression, which is in the Il-6 gene promoter area and regulates the activity of promoter, due to the increase of il-6 level, intensifies insulin resistance, obesity, and hyperandrogenism. These are the symptoms common between PCOS and metabolic syndrome (MBS) [19, 20, 21, 22 and 23].

Third, free fatty acids are the basic ligands for toll like receptors that are central regulators of innate immune receptors. By increasing lipolysis process and consequently increasing free fatty acids, androgens cause inflammation. Therefore, free fatty acids act as a direct connection between hyperandrogenism (well-known factor of PCOS) and inflammation (one of the features of metabolic syndrome) [32].

Regarding the close correlation between MBS and PCOS, it seems that we may reduce fertility, CV, and inflammatory problems by controlling the factors involving creation and development of these two syndromes. The major factors involving in both syndromes include interleukin-6 and CRP. Various studies indicate that CRP and IL6 in women increase PCOS [33, 34]. A group realized that they increase in women with PCOS and others believe that they increase in women with PCOS suffering from obesity and insulin resistance [35].

The genetic studies indicate that single nucleotide polymorphisms (SNPs) are in il-6 and CRP genes. With the plasma levels of il-6 and CRP increasing, they increase [36].



In PCOS model, blood mononuclear cells (PBMCs) migrate into a developing adipose tissue. They are then differentiated between the macrophages secreting cytokines such as TNF [37]. In addition, in vascular smooth muscle cells and CRP adipocytes cells (that increase in PCOS), TNF and IL6 are produced by activating signaling path of p38 MAPK-TLR4. According to the studies of Spaczynski*et al*., tumor necrosis factor alpha (TNF-α), which is produced from these two paths, causes proliferation and differentiation of single cells and increase of follicular cell layer, which is seen in PCOS [38].

The IL-6, which was released from adipose tissues, increases expressions of glucose transporter GLUT4, insulin recept*or* substrate 1 (IRS-1), TNF-a, and suppressor of cytokine signalling-3 *(*SOCS3*)* in hepatocytes and induces insulin resistance through decreasing lipogenesis and inhibiting glycogen synthesis. Moreover, the adipose tissue itself releases Nonesterified fatty acids (NEFAs) that increases capacity of muscle, liver and pancreatic beta cells. In the presence of lipid, NEFA also increases insulin resistance. Ability to create inflammatory states by IL6 can be discussed from two perspectives: Releasing IL-6 and TNF-α and free fatty acids excites JNK and IKK-B/NF-KB path. Therefore, inflammatory mediators develop incremental adjustments. White adipose tissue (WAT) releases chemokine ligand (CCL) 5, CCL2, and CCL8 through LY6C monocytes that increase classically activated macrophages (CAMs) and inflammatory phenotype. It is interesting that item increases insulin resistance and item reduces sensitivity to insulin [39].

In 1990, Watson some evidence that proved IL-6 secretion by ovary [40]. 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. IL-6 production in granulosa cells is excited by FSH, IL-1, and lipopolysaccharide (LPS) [41]. 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 [42]. Relying on these studies, we can create a strong link between syndrome symptoms, including imbalance in releasing pituitary and ovarian hormones and changing at il6 level that result in intensification. The changes were proved by the results of this study and the studies of the earlier authors of the present research.

According to the literature, it was proved that PCOS has a close relationship with metabolic syndrome and inflammation (cardiovascular disorders (CVD), cytokine increase, disorder of hormones level of hypothalamus - pituitary – ovarian axis, and insulin resistance). Therefore, it is probable that treating the rats with green tea would lead to adjusting these syndromes to improve PCOS. The relationship between mortality caused by CVD and consumption of green tea was highly impressive during last decade. In this regard, reduction of consuming10 green tea cups a day in a Japanese society caused 42 percent of the mortality caused by CVD in men. This was more effective in women. In addition, a reduction of 37 percent of mortality caused by infarction and myocardial infarction was observed. The studies show that the use of green tea extracts capsules for 12 weeks reduces blood CRP in human studies [43]. In the study of Sentle *et al*; EGCG oral consumption, the major catechin of tea, considerably reduced CRP in the rats with high-fat diet [44, 45].

Due to inhibiting NF-kB and ap1, epigallates inhibits TNF expression. Fellaini's study showed that after treating the transgenic rats with high expression of TNF in their lungs with green tea, TNF and interleukin reduced expression for 70 percent and 80 percent, respectively both at mRNA level and protein level [46].

Oxidative Stress activates MARKs p38 and JNK, which plays a key role in regulating production of inflammatory cytokine. EGCG of green tea inhibits both JNK and p38 through inhibiting transcription factors of Ap-1 and NF-kB. It also inhibits p38 inducing interleukins and JNK activity and changes in binding affinity of AP-1 to DNA. In addition, EGCG can retard cytokines expression through affecting down-regulate MRNA [47, 48, 49].

Epigallates inhibit proliferation of adipocytes and their differentiation in 3t3-l1 cells. It also inhibits lipid oxidation, increases glut-4 in animal models, improves energy in human, and inhibits induction of cytokine-induced beta cells. Anti-obesity effects of epigallates are due to increasing adiponectin expression. Catechin gallate decreases level of blood glucose through affecting alimentary canal and blocking glucose received from tissues; the latter partially increases glucose contents. Epigallate (the major compound of green tea) increases sensitivity to insulin and reduce hepatic glucose [50].

5 Conclusion

In the present study, reduction of cytokine level of the suffering rats indicates the effectiveness of epigallate on the female rats, which had a collection of a serious symptom, including hormonal imbalance and histological and metabolic abnormalities. With a view to the earlier study of the same author, whose data have not been released yet, some significant changes indicating histomorphometric and hormonal are obvious. It is expected that green tea is a method with the minimum side effects for pregnancy of those with PCOS and lifestyle-related illnesses.

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References

[1] B.L. Spangelo, P. C. Isakson, R. M. MacLeod, Production of interleukin-6 by anterior pituitary cells is stimulated by increased intracellular adenosine 3',5'-monophosphate and vasoactive intestinal peptide, Endocrinol, 127 (1990) 403-9.
http://dx.doi.org/10.1210/endo-127-1-403

[2] B. K. Pedersen, The anti-inflammatory effect of exercise: its role in diabetes and cardiovascular disease control, Essays in Biochemistry, 42 (2006) 105-117.
http://dx.doi.org/10.1042/bse0420105

[3] R. Azziz, K. S. Woods, R. Reyna, T. J. key, E. S. knochenhauer, B. O. Yildiz, The prevalence and features of the polycystic ovary syndrome in an unselected population, Journal of clinical Endocrinology and Metabolism, 89 (2004) 2745-2749.
http://dx.doi.org/10.1210/jc.2003-032046

[4] D. A. Ehrmann, Polycystic ovary syndrome, The New England journal of Nedicine, 352 (2005) 1223-1236.
http://dx.doi.org/10.1056/NEJMra041536

[5] Nanette K. Wenger, Leon Speroff, Barbara Packard, Cardiovascular health and disease in women, New England Journal of Medicine, 329 (1993) 247-256.
http://dx.doi.org/10.1056/NEJM199307223290406

[6] William, Famillal association in women with polycystic ovary syndrome, Fertil Steril, 80 (2003) 145-193.

[7] T. Sabuncu, M. Harme, M. Harma, Y. Nazligul, F. Kilic, Sibutramine has a positive effect on clinical and metabolic parameters in obese patients with polycystic ovary syndrome, Ferthil Steril, 5 (2003) 1199-1203.
http://dx.doi.org/10.1016/S0015-0282(03)02162-9

[8] F. L. Teixeira, E. Chadabracata, Hoolee, Aberrant expression of growth differentiation Factor 9 in oocytes of women with polycystic ovary syndrome, ClinEndocrinolMetab, 87 (3) (2000) 1337-1399.
http://dx.doi.org/10.1210/jcem.87.3.8316

[9] A. Dunaif, K. R. Segal, W. Futterweit, A. Dobrjansky, Profound peripheral insulin resistance, independent of obesity in polycystic ovary syndrome, Diabetes, (1989) 1165-1174.
http://dx.doi.org/10.2337/diab.38.9.1165

[10] M. Hunter, Polycystic ovary syndrome: its not just infertility, Copyright by American Academy of Family physicians, (2000).

[11] B. Yildirim, N.Sabir, B.Kaleli, Relation of intra-abdominal fat distribution to metabolic disorders in nonobese patients with polycystic ovary syndrome, Fertil Steril, 79 (2003) 1358-1364.
http://dx.doi.org/10.1016/S0015-0282(03)00265-6

[12] L. M. Costa, S. T. Gouveia, J. A. Nobrega, Comparison of heating extraction procedures for AI, Ca, Mg and Mn in tea samples, Ann Sci, 18 (2002) 313-318.
http://www.ncbi.nlm.nih.gov/pubmed/11918191

[13] D. Wu, G. X. Wei, Tea as a functional food for oral health, Health Nutrition, 18 (2002) 443-444.
http://www.ncbi.nlm.nih.gov/pubmed/11985958

[14] J. Mann, A. S. Truswell, Essentials of Human Nutrition, New York: Oxford university press, (1998).

[15] M. C. Sabu, R. Kuttan, Anti–diabtic activity of medicinal plants and its relationship with their antioxidant property, J Ethnopharmacol, 81 (2002) 155-160.
http://dx.doi.org/10.1016/S0378-8741(02)00034-X

[16] P. M. Ridker, C. H. Hennekens, J. E. Burging, N. Rifai, C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women, New England J Med, 342 (2000) 836-43.
http://dx.doi.org/10.1056/NEJM200003233421202

[17] S. Benson, O. Janssen, S. Hahn, S. Tan, T. Dietz, K. Mann, et al; Obesity, depression, and chronic low-grade inflammation in women with polycystic ovary syndrome, Brain BehavImmun, 22 (2008) 177-84.
http://dx.doi.org/10.1016/j.bbi.2007.07.003

[18] C. C. Kelly, H. Lyall, J. R. Petrie, G. N. Gould, J. M. Connell, N. Sattar, Low grade chronic inflammation in women with polycystic ovarian syndrome, J ClinEndocrinolMetab, 86 (2001) 2453-5.
http://dx.doi.org/10.1210/jcem.86.6.7580

[19] G. Villuendas, J. L. San Millan, J. Sancho, H. F. Escobar-Morreale, The −597 G→A and −174 G→C Polymorphisms in the Promoter of the IL-6 Gene Are Associated with Hyperandrogenism, Journal of Clinical Endocrinology and Metabolism, 87 (2002) 1134-1141.
http://dx.doi.org/10.1210/jcem.87.3.8309

[20] K. G. Jones, D. J. Brull, L. C. Brown, M. Sian, R. M. Greenhalgh, S. E. Humphries et al; Interleukin-6 (IL-6) and the prognosis of abdominal aortic aneurysms, Circulation, 103 (2001) 2260-2265.
http://dx.doi.org/10.1161/01.CIR.103.18.2260

[21] J. Hulkkonen, M. Pertovaara, J. Antonen, A Pasternack and M Hurme,Elevated interleukin-6 plasma levels are regulated by the promoter region polymorphism of the IL-6 gene in primary Sjogren's syndrome and correlate with the clinical manifestations of thedisease, Rheumatology, 40 (2001) 656-661.
http://dx.doi.org/10.1093/rheumatology/40.6.656

[22] A. Kubaszek, J. Pihlajamaki, K. Punnonen, P. Karhapaa, I. Vauhkonen, M. Laakso, The C-174G promoter polymorphism of the IL-6 gene affects energy expenditure and insulin sensitivity, Diabetes, 52 (2003) 558-561.
http://dx.doi.org/10.2337/diabetes.52.2.558

[23] J. M. Fernandez-Real, M. Broch, J. Vendrell, C. Gutierrez, R. Casamitjana, M. Pugeat et al; Interleukin-6 gene polymorphism and insulin sensitivity, Diabetes, 49 (2000) 517-520.
http://dx.doi.org/10.2337/diabetes.49.3.517

[24] L. Ramírez, M. José Luis San Millán, F. Héctor, Escobar-Morreale, Genomic Variants in Polycystic OvarySyndrome, ClinicaChimicaActa, (2006) 14-26.
http://dx.doi.org/10.1016/j.cca.2005.10.017

[25] P. Póvoa, C-reactive Protein, a Valuable Marker of Sepsis, Intensive Care Medicine, (2002) 235-243.
http://www.ncbi.nlm.nih.gov/pubmed/11904651

[26] B. B. Duncan, M. I. Schmidt, J. S. Pankow, C. M. Ballantyne, D. Couper, A. Vigo, R. Hoogeveen, A. R. Folsom, G. Heiss, Low-Grade Systemic Inflammation and the Development of Type 2Diabetes The Atherosclerosis Risk in Communities Study, Diabetes, (2003) 1799-1805.
http://dx.doi.org/10.2337/diabetes.52.7.1799

[27] A. Dunaif, Insulin resistance and the polycystic ovary syndrome:mechanism and implications for pathogenesis, Endocrine Reviews, 18 (1997) 774-800.
http://dx.doi.org/10.1210/edrv.18.6.0318

[28] S. Franks, Polycystic ovary syndrome, New England Journal of Medicine, 333 (1995) 853-861.
http://dx.doi.org/10.1056/NEJM199509283331307

[29] P. Calabró, J. Willerson, E. Yeh, Inflammatory cytokines stimulated Creactive protein production by humancoronary artery smooth muscle cells, Circulation, 108 (2003) 1930-2.
http://dx.doi.org/10.1161/01.CIR.0000096055.62724.C5

[30] L. Karimzadeh, M. Nabiuni, H. MohseniKouchesfehani, H. Adham, A. Bagheri, A. Sheikholeslami, Effect of bee venom on IL-6, COX-2 and VEGF levels in polycystic ovarian syndrome induced in Wistar rats by estradiol valerate, Journal of Venomous Animals and Toxins including Tropical Diseases, 19 (2013) 32.
http://dx.doi.org/10.1186/1678-9199-19-32

[31] S. Verma, S. Devaraj, I. Jialal, CReactive protein promotes atherothrombosis, Circulation, 113 (2006) 2135-50.

[32] T. Sathyapalan, L. Atkin, Mediators of Inflammation in polycystic ovary syndrome in relation to adiposity, Mediators Inflamm, 75 (2010) 86-56.

[33] N. Boulman, Y. Levy, R. Leiba, S. Shachar, R. Linn, O. Zinder, et al; Increased C - reactive protein levels in the polycystic ovary syndrome: a marker of cardiovascular disease, J ClinEndocrinolMetab, 89 (2004) 2160-5.
http://dx.doi.org/10.1210/jc.2003-031096

[34] P. M. Ridker, C. H. Hennekens, J. E. Burging, N. Rifai, C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women, New England J Med, 342 (2000) 836-43.
http://dx.doi.org/10.1056/NEJM200003233421202

[35] A. Vgontzas, D. Papanicolaous, E. Bixler, et al; Elevation of plasma cytokines in disorders of excessive daytime sleepiness: role of sleep disturbance and obesity, J Chin EndocrinolMetab, 85 (2000) 1151-8.
http://dx.doi.org/10.1210/jcem.85.3.6484

[36] H. F. Escobar-Morreale, M. Luque-Ramirez, F. Gonzalez, Circulating in flammato markers in polycystic ovary syndrome: a systematic review and meta-analysis, Fertility and Sterility, (2011) 1048-58.
http://dx.doi.org/10.1016/j.fertnstert.2010.11.036

[37] F. Gonzalez, N. Rote, J. Minium, J. Kirwan, Increased activation of nuclear factor kappa B triggers inflammation and insulin resistance in polycystic ovary syndrome, J ClinEndocrinolMetab, 91 (2006) 1508-1512.
http://dx.doi.org/10.1210/jc.2005-2327

[38] N. Liu, J. Liu, Y. Ji, P. Lu, C. Wang, F. Guo, C-reactive protein induces TNF-α secretion by p38 mapk-tlr4 signal pathway in rat vascular smooth muscle cells, Inflammation, 34 (2011) 283-90.
http://dx.doi.org/10.1007/s10753-010-9234-z

[39] R. Z. Spaczynski, A. Arici, A. Duleba, Tumor Necrosis Factor-alpha stimulates proliferation of rat ovarian theca-interstitial cells, biology of reproduction, 61 (1999) 993-8.
http://www.ncbi.nlm.nih.gov/pubmed/10491635

[40] M. J. Kraakman, T. L. Allen, M. Whitham, P. Iliades, H. L. Kammoun, E. Estevez, G. I. Lancaster, M. A. Febbraio, Targeting Gp130 to Prevent Inflammation and Promote Insulin Action, Diabetes, Obesity and Metabolism, (2013) 170-175.
http://dx.doi.org/10.1111/dom.12170

[41] J. M. Watson, J. L. Sensintaffar, J. S. Berek, et al; Constitutive production of interleukin 6 by ovarian cancer cell lines and by primary ovarian tumor cultures, Cancer Res, 50 (1990) 6959-65.
http://www.ncbi.nlm.nih.gov/pubmed/2208162

[42] W. C. Gorospe, B. L. Spangelo, Interleukin-6 production by rat granulosa cells in vitro: effects of cytokines, follicle-stimulating hormone, and cyclic 3',5'-adenosine monophosphate, BiolReprod, 48 (1993) 538-43.
http://dx.doi.org/10.1095/biolreprod48.3.538

[43] C. Keck, Z. Rajabi, K. Pfeifer, et al; Expression of interleukin-6 and interleukin-6 receptors in human granulosa lutein cells, Mol Hum Reprod, 4 (1998) 1071-6.
http://dx.doi.org/10.1093/molehr/4.11.1071

[44] D. A. Vignali, Multiplexed particle-based flow cytometric assays, J Immunol Methods, 243 (2000) 243-55.
http://dx.doi.org/10.1016/S0022-1759(00)00238-6

[45] E. Morgan, R. Varro, H. Sepulveda, J. A. Ember, J. Apgar, J. Wilson, L. Lowe, R. Chen, L. Shivraj, A. Agadir, R. Campos, D. Ernst, A. Gaur, Cytometric bead array: a multiplexed assay platform with applications in various areas of biology, Clinical Immunology, 110 (2004) 252-66.
http://dx.doi.org/10.1016/j.clim.2003.11.017

[46] G. L. Johnson, R. Lapadat, Mitogen-activated protein kinase pathways mediated by ERK, JNK, and p38 protein kinase, Science, 298 (2002) 1911-2.
http://dx.doi.org/10.1126/science.1072682

[47] S. Narayanan, A. Glasser, Y. S. Hu, A. M. McDermott, The effect of interleukin-1 on cytokine gene expression by human corneal epithelial cells, Exp Eye Res, 80 (2005) 175-83.
http://dx.doi.org/10.1016/j.exer.2004.08.027

[48] S. K. Dower, E. E. Qwarnstrom, Signalling networks, inflammation and innate immunity, BiochemSoc Trans, 31 (2003) 1462-71.
http://dx.doi.org/10.1042/BST0311462

[49] M. E. Cavet, K. L. Harrington, K. W. Ward, J. Z. Zhang, Mapracorat, a novel selective glucocorticoid receptor agonist, inhibits hyperosmolar-induced cytokine release and MAPK pathways in human corneal epithelial cells, Mol Vis, 16 (2010) 1791-800.
http://www.ncbi.nlm.nih.gov/pubmed/20824100

[50] E. Shaulian, M. Karin, AP-1 as a regulator of cell life and death, Nat Cell Biol, 4 (2002) 131-6.
http://dx.doi.org/10.1038/ncb0502-e131