Effect of *Allium Cepa* seeds Ethanolic Extract, on Serum Total Antioxidant in Experimental induced Poly cystic ovarian (PCO) rats

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Abstract: Polycystic ovary syndrome (PCOS) is the most frequent cause of female infertility, affecting about 5–10% of women in age of procreation. See antioxidants effects of *Allium* cepa seeds ethanol extract on experimental PCO induced by estradiol-valerat (PPA) in rats. Wistar female rat (n=60) were allocated into three groups, control (n=30), C1: an equal volume of (0.9% NaCl); C2: extract (0.3cc/rat/orally/daily); C3: Sesame oil(0.3cc/rat/orally/daily) and test groups (n=30), that subdivided into groups of 3,one group received extract supplement (0.3cc Sesame oil+ 0.3cc Allium Cepa /rat/orally/daily), second and third groups were induced PCO by single injection of estradiol-valerate (4mg/rat/IM),third group, was received extract supplement, for 60 consequence days. Animals were kept in standard conditions. In last day of study the blood samples of rats in whole groups were removed and prepared to biochemical analysis. level of TAC, Superoxide dismutase and catalase were significantly decreased in pco groups ($p<0.05$), these side effects in groups that received extract significantly increased ($p<0.05$) in comparison to control and PCO groups and level of MDA in PCO groups were significantly increased as compared to control and extract groups ($p<0.05$). Results revealed that administration of *Allium cepa* ethanol extract significantly compensation blood antioxidants level in PCO induces rats.

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1. Introduction

Poly Cystic ovary syndrome (PCOS) is a common endocrine disorder in women during reproductive age (1). PCOS manifestations include metabolic, reproductive, and psychological disorders. Thus, PCOS usually is along with many manifestations including insulin resistance,hyperglycemia, hyperinsulinemia, type 2 diabetes mellitus,cardiovascular disease,hyperandrogenism, ovulatory dysfunction, infertility, increased anxiety,and depression. The phenotype varies depending on life stage, genotype, ethnicity and environmental factors such as life style. The etiology of PCOS remains unclear, it is multifactorial probably. Diagnostic criteria rely on PCOS clinical and biochemical findings. Oxidative stress (OS) may play a role in the pathophysiology of PCOS (1-3).Moreover, in the PCOS women the normal hormonal levels exchange to abnormal levels ; the GnRH level changes indicate LH/FSH level three times more than normal level that can be considered as a diagnostic tool for this disorder(4).The estrogen level exchanges to a high level, but the progesterone level shows the lower level (5).Reactive oxygen species (ROS) are formed in the human body due to metabolism and cellular function; naturally, defensive mechanism of the body is modulating the ROS by antioxidant defences. An antioxidant is a substance that can prevent or delay OS, by scavenging biologically important reactive oxygen species ($O2^{-}$; H2O2; OH; HOCl; ferryl; peroxyl, and alkoxyl) (6).Oxidative stress as a pathological state can be generated when there is an imbalance between pro-oxidant molecules (reactive oxygen and nitrogen species) and antioxidant defences.The role of OS in the pathogenesis of subfertility in both male and female has been shown by studies. The impact of OS on oocytes and reproductive functions remains unclear. The OS would be resulted in pathological diseases in female reproductive tract such as poly cystic ovary syndrome. The environmental pollutants cause OS, so the role of life style is prominent in generating oxidative stress; hence, the antioxidants effect is very important to decrease infertility resulted by oxidative stress (7).Herbs have been used for medicinal purposes since centuries ago, the herbs include hypolipidemic,antiplatelet, antitumor, or immune-stimulating properties that cause to decrease the cardiovascular and cancer risk. Many herbs include antioxidant content alliums are from herbs

group with properties of anti-bacteria, anti-fungi and antioxidant power. *Allium Cepa* (Linn) one of the alliums with beneficial impact upon disease includes antioxidative properties too.(8-10)The aim of this study is to assess the antioxidative effect of *Allium Cepa* seeds ethanol Extract, on Serum Total antioxidant in experimental induced poly cystic ovarian (PCO) rats.

2. Material and methods

2.1. Animals

Sixty adult 8 weeks old Wistar albino female rats of 250±10 grams were obtained from Animal Facility of Pasture Institute of Iran. Rats were housed in temperature controlled rooms (25.C) with constant humidity (40-70%) and 12h/12h light/ dark cycle prior to use in experimental protocols. All animals were treated in accordance to the Principles of Laboratory Animal Care [NIH]. The experimental protocol was approved by the Animal Ethical Committee in accordance with the guide for the care and use of laboratory animals prepared by Tabriz medical University. All rats were fed a standard diet and water. The daily intake of animal water was monitored at least one week prior to start of treatments in order to determine the amount of water needed per experimental animal. Wistar female rat were allocated into three groups, control (n=30),C1: an equal volume of (0.9% NaCl);C2: extract (1cc/rat/orally/daily);C3: Sesame oil(0/3cc/rat/orally/daily) and test groups (n=30), that subdivided into groups of 3,one group received extract supplement (0.5cc Sesame oil+ 0.5cc Allium Cepa /rat/orally/daily),second and third groups were induced PCO by single injection of estradiol-valerate (4mg/rat/IM),third group, was received extract supplement, for 60 consequence day.in 60th day of study,5cc blood samples of Rats in whole groups were removed and prepared to biochemical pathological analysis. Animals were kept in standard conditions.

2.2. Measurement of Serum Total Antioxidant capacity (TAC)

TAC was measured in serum by means of a commercial kit (Randox Co-England). The assay is based on the incubation of 2, 2'-azino-di-(3-ethylbenzthiazoline sulphonate) (ABTS) with a peroxidase (methmyoglobin) and hydrogen peroxide to produce the radical cation ABTS+, which has a relatively stable blue-green color, measured at 600 nm. The suppression of the color is compared with that of the Trolox, which is widely used as a traditional standard for TAS measurement assays, and the assay results are expressed as Trolox equivalent (mmol/L).

2.3. Measurement of Serum MDA

Tissue MDA levels were determined by the thiobarbituric acid (TBA) method and expressed as nmol MDA formed/mL.Plasma MDA concentrations were determined with spectrophotometer. A calibration curve was prepared by using 1,1',3,3'-tetramethoxypropane as the standard.

2.4. Glutathione peroxidase (GPX) activity measurement in serum

GPx activity was quantified by following the decrease in absorbance at 365 nm induced by 0.25 mM H2O2 in the presence of reduced glutathione (10 mM), NADPH, (4 mM), and 1 U enzymatic activity of GR.

2.5. Super oxide dismutase (SOD) activity measurement in serum

The activity of superoxide dismutase (SOD) was measured by following the method of Beyer and Fridovich.

2.5. Catalase (CAT) activity measurement in serum

Serum catalase activity was determined according to the method of Beers and Sizer as described by (Usoh etal.,2005) by measuring the decrease in absorbance at 240nm due to the decomposition of H20 in a UV recording spectrophotometer. The reaction mixture (3 ml) contained 0.1 ml of serum in phosphate buffer (50mM, pH 7.0) and 2.9ml of 30mM H_2O_2 in phosphate buffer pH 7.0. An extinction coefficient for H2O2 cm-1 was used for calculation. The specific activity of catalase was expressed as moles of H2 reduced per minute per mg protein. at 240nm of 40.0M-1 cm-1 was used for calculation. The specific activity of catalase was expressed as moles of H2O2 reduced per minute per mg protein.

2.6. Statistical analysis

Statistical analysis was done using the ANOVA and test for comparison of data in the control Group with the experimental groups. The results were expressed as mean ± S.E.M (standard Error of means). P-value less than 0.05 were considered significant and are written in the Parentheses.

2.7. Preparation of extracts

The Allium Cepa seeds were first of all washed and dried on laboratory tables at room temperature (27 ± 2°C). They were later pulverized using the crusher machine in Pharmacognosy department in Tabriz University of medical sciences, Iran. 100 g each of the pulverized seeds were macerated separately in distilled water and 50% ethanol, for 72 h, with periodic stirring. Each extract was filtered repeatedly using a sterile muslin cloth, cotton wool and filter paper. After preparation ethanol extract was keep in refrigerator (0±4°C).

2.8. Preparing daily using supplement

For using daily ethanol extract of 0.3 cc of Allium cepa, it was puddled with same volume of Sesame oil and provided supplement was prescribed by gavage methods in treatment groups.

3. Results

3.1. Results of Serum Total Antioxidant capacity (TAC)

Administration of 1cc/rat/orally/daily Allium Cepa seeds ethanol Extract for sixty consecutive days significantly increased Serum Total Antioxidant capacity (TAC) concentration in experimental group as compared with the control group (P< 0.05), (Table I).

3.2. Results of malondialdehyde (MDA) concentration in serum

Administration of 1cc/rat/orally/daily Allium Cepa seeds ethanol Extract for sixty consecutive days significantly decreased malondialdehyde (MDA) concentration in experimental group as compared with the control group (P< 0.05), (Table I).

3.3. Results of Super oxide dismutase (SOD) concentration in Serum

Administration of 1cc/rat/orally/daily Allium Cepa seeds ethanol Extract for sixty consecutive days significantly increased Super oxide dismutase (SOD) concentration in experimental group as compared with the control group(P< 0.05), (Table I).

3.4. Results of Glutathione peroxidase (GPX) activity in serum

Administration of 1cc/rat/orally/daily Allium Cepa seeds ethanol Extract for sixty consecutive days significantly increased Glutathione peroxidase (GPX) concentration in experimental group as compared with the control group(P< 0.05),(Table I).

3.5. Results of Catalase (CAT) activity in serum

Administration of 1cc/rat/orally/daily Allium Cepa seeds ethanol Extract for sixty consecutive days significantly increased of Catalase (CAT) concentration in experimental group as compared with the control group (P< 0.05),(Table I).

Table 1- *effects of Allium Cepa seeds ethanol Extract, on TAC, MDA, CAT, SOD, GPX in PCO rats.*

Groups / parameters	PCO+ (0.3cc Sesame oil+ 0.3cc Allium Cepa ethanolic extract /rat/orally/daily)	PCO estradiol-valerate (4mg/rat/IM)	Sesame oil(0.3cc/rat/orally/daily)+ Allium Cepa seeds ethanolic Extract	Sesame oil (0.3cc/rat/orally/ daily)	Allium Cepa seeds ethanolic extract 0.3cc/rat/orally/daily)	Control 0.9% NaCl
Catalase(u/mg Hb)	298.1±0.05*	106.4±0.05**	445.4±0.05*	346.4±1.05*	500.4±3.05**	306.4±4.05
Superoxide dismutase (SOD), (u/g Hb)	981±0.55*	877±0.55*	1965±0.55	1112±0.55	1875±0.55*	1000±0.55
GPX, (u/mg Hb)	111±0.5*	100±0.5*	165±0.5*	115±0.5	175±0.5*	125±0.5
TAC (mmol/ml)	1.0 ±0.05*	0.6 ±0.05*	1.9 ±0.05	1.6 ±0.05	3.6 ±0.05*	1.8 ±0.05
MDA (mmol/ml)	4.1 ±0.05*	7.1 ±0.05*	2.8 ±0.05*	2.1 ±0.05*	3.1 ±0.05*	5.1 ±0.05

Data are presented as mean ± SE.

*Significant different at P< 0.05 level, (compared with the control group).

**Significant different at P< 0.01 level, (compared with the control group).

4. Discussion

Reactive oxidative species (ROS) and antioxidants are in balance in a healthy body. Active oxygen species is a phrase used to describe a variety of molecules and free radicals (chemical species with one unpaired electron) derived from molecular oxygen. In female reproductive tract, it effects on oocyte maturation to fertilization, embryo development and pregnancy (11,12). In the reproductive tissue, the elevated level of ROS is followed by active metabolism and steroidogenesis and may cause oocyte and DNA damage; besides, the ROS play a physiological role during ovulation that is similar in some responses to inflammation. ROS may play a role in the regulation of growth of ovarian mesenchyme for example in a pathological condition like PCOS,excessive oxidative stress may contribute to ovarian mesenchyme hyperplasia.One of protective mechanisms of the body against the ROS effects is anti-oxidative enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPX) for eliminating the ROS. ROS can activate scavenging system, a Redox system, that can repair oxidized and damaged molecules by using NADPH as an original electron source (12,13). The ROS cause lipid peroxidation, so it can damage DNA and/or change in cell signalling and cellular function. The oxidative stress (OS) lead to damage to DNA of ovarian epithelium or cell apoptosis; however, oxidative status of the cell modulates follicular growth, corpus luteum formation endometrial differentiation and embryonic growth. Oxidative stress may also be the cause of preeclampcia, abortion, endometriosis, PCOS, infertility, mole hidatyform, radical-induced birth defects; hence, evaluating and protecting against OS is very important in reproductive science. Recent findings illustrated the positive influence of oxygen radicals and ROS in many physiological states like development of germ cells, the uterine environment, oocyte maturation, ovulation, and corpus luteum function and regression. Oxidative stress (OS) may play a role in the pathophysiology of PCOS. OS may affect Insulin resistance (IR) that is common in young non-obese PCOS women (12,14). Antioxidants as defensive mechanism of the body modulate the reactive oxygen species effect, and they also prevent

oxidative stress. Enzymatic antioxidant defences include superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase (CAT). Non-enzymatic antioxidants that can be represented by ascorbic acid (Vitamin C), α-tocopherol (Vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants. There is a balance between both the activities and the intracellular levels of these antioxidants normally that is essential for the organism's health (15). Superoxide dismutase (SOD) produced in cumulus oophorus cells is closely associated with oocyte maturation and also GPx and catalase are significant for reproduction health ;On the other hand, GSH as a redox system is in large amount in the oocytes(13,16,17). Despite oxidative stress has many physiological roles, the higher production of these agents may lead to an increased risk of ovarian pathology that would probably be exacerbated under conditions of reduced antioxidant status. (18) The studies indicated that oxygen radicals may function as intracellular regulators of steroidogenesis in the corpus luteum (19). Oxidative stress and depletion of the antioxidant glutathione (GSH) cause apoptosis in many systems. Previous work showed that antioxidants prevented apoptosis effectively (20). Apoptosis, a type of physiological or active cell death, has been implicated as a mechanism underlying regression of the corpus luteum (CL) in the rat, bovine, rabbit and ovine ovary. Reactive oxygen species play an important role in luteolysis in the rodent ovary (21). Paraoxonase 1 (PON1) as an enzymatic antioxidant prevents lipid peroxidation. According to studies there is a direct relation between increased malondialdehyde (MDA) and decreased antioxidant (PON1) activity and total antioxidant capacity (TAC). Since PON1 activity is lower in the PCOS women, dyslipidemia is common in these women; it may cause lower-density-lipoprotein (LDL) oxidation in arterial walls, so atherogenesis is also common in the PCOS women (22). Herbs are suggested as rich sources of antioxidant compounds including a wide variety of active phytochemicals, including the flavonoids, terpenoids, lignans, sulfides, polyphenolics, carotenoids, coumarins, saponins, plant sterols, curcumins, and phthalides. These compounds may protect LDL cholesterol from oxidation, inhibit cyclooxygenase and lipoxygenase enzymes, inhibit lipid peroxidation, or have antiviral or antitumor activity. According to studies there are many evidences indicating the pro-oxidation and antioxidation properties of Alliums by protecting against free radicals.The various species of Alliums have wide antioxidative activity due to inclusion of antioxidative enzymes (catalase, peroxidase, superoxide-dismutase, glutathione-peroxidase), non-enzymic antioxidants (reduced glutathione and total flavonoids), content of soluble proteins, vitamin C, carotenoids, chlorophylls a and b, as well as the quantities of malonyldialdehyde and •OH and O2• – radicals and very low concentrations of toxic oxygen radicals (8,23). According to USDA nutrient database the nutritional value of *Allium Cepa* seed includes antioxidants such as polyunsaturated fatty acids, monounsaturated fatty acid, vitamin C, vitamin E, vitamin A, zinc,folate, niacin. Since erythrocyte lipid peroxidation products (MDA), glutathione (GSH), ascorbic acid, plasma vitamin E and activities of antioxidant enzymes super oxide dismutase (SOD), glutathione peroxidase (GPX), catalase in erythrocyte, plasma glutathione-S-transferase (GST) and serum homocysteine levels exchange to abnormal levels in polycystic ovary syndrome, in this study we assessed the impact of *Allium Cepa* antioxidants on enzymatic antioxidant levels associated to PCOS, we subdivided the rats into 3 experimental groups,and 1 control group, one experimentl group just received *Allium Cepa* extract, second group was received *Allium Cepa* extract while the PCOS was induced by estradiol valerate before; in third group, PCOS also induced without receiving of *Allium Cepa* extract ; after 60 days results indicated the higher level of antioxidants such as CAT, SOD, GPx in first experimental group than control group and the improvement of antioxidant levels in The PCOS group that received *Allium Cepa* extract while these antioxidant levels in forth group was lower significantly. According to this study the polyunsaturated fatty acids may cause to increase the blood antioxidant levels involving in pathophysiology of PCOS (24). This study is a confirmation of the other our research on the effect of polyunsaturated fatty acids (PUFAS) as antioxidant that had indicated reduction of lipid peroxidation products in rats with PCOS (25). Since, PUFAS reduce lipid peroxidation products, the risk of atherosclerosis and cardiovascular would decrease after supplementation with PUFA. (26) PUFA is necessary in many reproductive processes including prostaglandin production and steroid metabolism; they also play a significant role in fertilization of spermatozoa. Recently, the positive effect of these antioxidants in reproductive tract has demonstrated that PCOS also might be modulated (27,28). The antioxidative effect of vitamin E in the body depends on active pathways of hydroperoxides, aldehydes, and other oxidation products. The protective effect of vitamin E against oxidative stress has been shown in the improvement of cardiovascular diseases by decreasing of lipid peroxidation, superoxide (O2-) production by impairing the assembly of nicotinamide adenine dinucleotide phosphate (reduced form) oxidase as well as by decreasing the expression of scavenger receptors (SR-A and CD36) (23,29,30).

The studies indicated the effect of Vitamin E on improvement of oxidative stress in impaired sperms (31). Vitamin A and carotenoids can accept and donate electrons. Carotenoids such as beta-carotene exert antioxidant functions in lipid phases by quenching O2 or free radicals (32,33). Antioxidants may protect the ovaries from oxidative stress; according to studies dietary vitamin A and beta-carotene are associated with reduced risk of ovarian cancer (34). Folate and zinc existing in *Allium Cepa* have role in spermatogenesis. In female reproductive tract, folate is important for oocyte quality and maturation, implantation, placentation, fetal growth and organ development. Zinc has also been implicated in testicular development, sperm maturation and testosterone synthesis. In females, zinc plays a role in sexual development, ovulation and the menstrual cycle; both folate and zinc have antioxidant properties that counteract reactive oxygen species (ROS) (35). Ascorbate postulates to be an effective antioxidant ; it impose antioxidation activity directly and indirectly by reaction with aqueous peroxyl radicals, and restoring the antioxidant properties of fat-soluble vitamin E,respectively.Thus, the antioxidation activity of ascorbate is in prevention of lipid peroxidation in the membranes of intracellular organelles, so it can protect non-lipid nuclear material from free radicals; moreover, Vitamin C can act the extracellular antioxidant in plasma and fluids surrounding the lung, lens and retina (36-38). The lipid modification by niacin was also reported in the patients with diabetes; on the other hand, the alteration in lipoprotein metabolism was reported in researches (39,40). studies on monounsaturated fatty acid that is the other antioxidant of *Allium Cepa* illustrated the lower level of total cholesterol and lower level of low-density-lipoprotein (LDL) with this antioxidant, but this effect about PUFA is significantly higher (41). According to antioxidation activity of these antioxidants as mentioned *Allium Cepa* is a rich source of antioxidants.

5. Conclusion

The results demonstrated after treating with *Allium Cepa* ethanol extract in the rats induced PCOS, antioxidant levels (CAT, SOD, GPX, TAC) would increase in this group while the rats that didn't receive *Allium Cepa* these antioxidants were low ; on the other hand, MDA as an oxidative species also lowered in treated PCOS group. According to this study and the other studies PUFAS is important as an antioxidant in order to improve and modulate the PCOS outcomes.

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