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[CONTENTS]

245 **Non-steroidal anti-inflammatory drug supplementation modulates lipid peroxidation and total antioxidant levels in serum of patients with primary dysmenorrhea**
Mustafa Nazıroğlu, Mehmet Güney, Önder Kaplan

252 **Effects of food based yeast supplementation on oxidative stress in rats fed by high cholesterol diet**
Hasan Basri Savaş, Özlem Yüksel, Hatice Şanlıdere Aloğlu, Zubeyde Öner, Ezgi Demir Özer, Fatih Gültekin

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AIM AND SCOPES

Cell Membranes and Free Radical Research is a print and online journal that publishes original research articles, reviews and short reviews on the molecular basis of biophysical, physiological and pharmacological processes that regulate cellular function, and the control or alteration of these processes by the action of receptors, neurotransmitters, second messengers, cation, anions, drugs or disease.

Areas of particular interest are four topics. They are;

A- Ion Channels (Na^+ - K^+ Channels, Cl^- channels, Ca^{2+} channels, ADP-Ribose and metabolism of NAD^+, Patch-Clamp applications)

B- Oxidative Stress (Antioxidant vitamins, antioxidant enzymes, metabolism of nitric oxide, oxidative stress, biophysics, biochemistry and physiology of free oxygen radicals

C- Interaction Between Oxidative Stress and Ion Channels

(Effects of the oxidative stress on the activation of the voltage sensitive cation channels, effect of ADP-Ribose and NAD^+ on activation of the cation channels which are sensitive to voltage, effect of the oxidative stress on activation of the TRP channels)

D- Gene and Oxidative Stress (Gene abnormalities. Interaction between gene and free radicals. Gene anomalies and iron. Role of radiation and cancer on gene polymorphism)

READERSHIP

Biophysics
Biochemistry
Biology
Biomedical Engineering
Pharmacology
Physiology
Genetics
Cardiology
Neurology
Oncology
Psychiatry
Neuroscience

Keywords

Ion channels, cell biochemistry, biophysics, calcium signaling, cellular function, cellular physiology, metabolism, apoptosis, lipid peroxidation, nitric oxide synthase, ageing, antioxidants, neuropathy.

Non-steroidal anti-inflammatory drug supplementation modulates lipid peroxidation and total antioxidant levels in serum of patients with primary dysmenorrhea

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List of abbreviations

COX-2, cyclooxygenase-2
LP, lipid peroxidation
MDA, malondialdehyde
NSAID, non-steroidal anti-inflammatory drug
ROS, reactive oxygen species
SOD, superoxide dismutase
TAS, total antioxidant status

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Abstract

Dysmenorrhea is a common painful gynecological disorder in young women during menstrual cycle. Inflammation and oxidative toxicity were reported in the patients with primary dysmenorrhea although its etiology is still unclear. We investigated effects of non-steroidal anti-inflammatory drug (NSAID) supplementation on lipid peroxidation, total antioxidant status (TAS) and antioxidant vitamin values in serum of patients with primary dysmenorrhea.

We used three groups in this study. First group was used as control. Second group constituted patients (primary dysmenorrhea) group. Third group was daily received oral NSAID for 6 weeks before blood serum taken.

The lipid peroxidation level was higher in patient group than in control although its level was lower in treatment group than in control group. The TAS concentrations were lower in patients group than in control. However, TAS and vitamin C concentrations were higher in treatment group than in patient group. Serum vitamin A, vitamin E and β-carotene concentrations did not differ in the three groups.

In conclusion, we observed that women with primary dysmenorrhea are a consideration with increased oxidative stress in serum. The NSAID supplementation in serum of women may strengthen the antioxidant defense system by decreasing oxidative stress.

Keywords

Oxidative toxicity; antioxidants; primary dysmenorrhea; non-steroidal anti-inflammatory drug; vitamin C.

Introduction

Reactive oxygen species (ROS) directly damage cells, tissues and blood vessels, and stimulate transcription factors such as nuclear factor (NF)-κB. Once activated, NF-κB leads to the upregulation of many genes, including those that result in the production of proinflammatory cytokines IL-1β, IL-6 and tumor necrosis factor-α (TNF-α) (González-Ramos et al 2012). Proinflammatory inflammatory cytokines, interleukins (Yeh et al. 2004) and TNF-α increases, and excessive reactive oxygen species (ROS) production (Yeh et al. 2004; Dikensoy et al. 2008) were reported in the patients with primary dysmenorrhea. Lipid peroxidation (LP) causes injury to cellular and intracellular membranes and may lead to cell injury and subsequently apoptosis and cell death (Nazıroğlu, 2007; Kovacic and Somanathan, 2008). Several antioxidants protect endometrium against oxidative stress (Güney et al. 2007; Güney, 2012). Vitamin E (α-tocopherol) is the most important fat soluble antioxidant in the lipid phase of cells. Vitamin E acts to protect cells against the effects of free oxygen radicals, which are potentially damaging byproducts of the body's metabolism (Nazıroğlu et al. 2004a). Vitamin C (ascorbic acid), as well as being a free radical scavenger, also transforms vitamin E to its active form (Traber, 2007; Talaulikar and Manyonda, 2011). Provitamin A carotenoids such as β-carotene are the major source for retinoids and are involved with signal transduction at cytoplasmic and membrane sites (von Lintig, 2012). It has been previously reported that non steroidal antiinflammatory drug (NSAID), diclofenac in one of the strongest anti-inflammatory agent widely use in primary dysmenorrhea (Moore, 2007). Hence, the NSAID may modulate the primary dysmenorrhea disease through regulation of antioxidant vitamin and lipid peroxidation levels.

Primary dysmenorrhea is painful menstrual cramps without evident pathology to account for them. The disease is very common among young (19-25 years old) women with 40-60% prevalence and it causes severe absenteeism during work and school (Dawood, 2006). Primary dysmenorrhea occurs in only menstrual cycles (Harel, 2012). The dysmenorrhea has been changing women's quality of life, however, most women don't seek a solution they believe it would not make a difference (Dikensoy et al. 2008). The involvement of free radicals in dysmenorrhea is less-known although results of some recent reports been suggested that oxidative stress might play a role in dysmenorrhea development (Dikensoy et al. 2008; Akdemir et al. 2010). ROS are produced within follicle, especially during ovulatory process (Tola et al. 2013). It is believed that oxidative stress may also be a cause of primary dysmenorrhea. The role of ROS and antioxidant in relation to female reproductive function disease such as primary dysmenorrhea and infertility has been a subject of recent interest (Dikensoy et al. 2008; Özkaya and Nazıroğlu, 2010; Özkaya et al. 2011). At this time, the ethological role of oxidative stress and antioxidant vitamins in the patients with primary dysmenorrhea is not fully understood.

The current study was undertaken to investigate (1) the difference on serum lipid peroxidation and antioxidant values between control and patients with dysmenorrhea, (2) to test whether NSAID supplementation improves serum antioxidant capacity.

Material and Methods

Chemicals

All chemicals (α-tocopherol, all-trans retinol, KOH, NaOH, thiobarbutiric acid, 1,1,3,3 tetraethoxy propane, hexane, ethyl alcohol and pyrogallol) were obtained from Sigma-Aldrich Chemical Inc. (St. Louis, MO, USA. All reagents were analytical grade. The reagents were equilibrated at room temperature for half an hour before an analysis was initiated or reagents containers were refilled.

Control and Patients

Twelve patients with primary dysmenorrhea and 6 health controls used in the current study. The study was approved by local ethical committee of Suleyman Demirel University, Medical Faculty and informed consent was obtained from each patients. The study cohort has been selected from an outpatient population of Suleyman Demirel University, Medical Research Center from January 2012 to June 2012.

Main complaint of patients was dysmenorrhea, and thus each patient exposed to detailed gynecologic exams, pelvic ultrasound and laboratory tests. A medical history of the patients was also taken. Each subject was followed for three ovulatory cycles by monitoring pain score for the detecting the disease. Exclusion criteria of the patients were inflammatory diseases (irritable bowel syndrome and inflammatory bowel diseases), fibromyalgia, premature coronary artery disease, diabetes mellitus (both type I and type II) and hypertension. The patients and controls were not taking hormone replacement therapy, vitamin and mineral supplements for 6 months. They were also non-smoker and non-drinking women.

Study groups

We used three groups in this study. First group was used as control (n=6) and they received placebo (candy).

Second group (n=6) constituted primary dysmenorrhea group and blood samples were taken the groups. After 6 weeks daily NSAID (50 mg diclofenac potassium tablet (Dolerex), Abdi Ibrahim Medicine Inc, Istanbul, Turkey) supplementation, blood samples were taken from the patients of third group.

Blood collection and preparation of blood samples

Twelve hours fasting venous blood (5 ml) was taken from the antecubital vein, using a monovette system of blood collection, into non anticoagulated tubes, protected against light. The serum was obtained from the blood samples by centrifugation at 1500 g for 10 min at +4 °C.

The serum samples were stored at -33 °C for < 3 months pending measurements of lipid peroxidation (LP) and total antioxidant status (TAS) values. The remaining serum was used for immediate vitamin concentrations.

Lipid peroxidation (LP) level determinations

Thiobarbituric acid reacts with lipoperoxidation and aldehydes, such as malondialdehyde (MDA), as the most common method to assess LP in biological samples. The LP levels in the serum were measured with the thiobarbituric-acid reaction at 532 nm by the method of Placer et al (1966). as described in a previous study (Nazıroğlu et al. 2011). The values of LP in the serum were expressed as μmol/l.

TAS determinations

The TAS levels were measured calorimetrically using the TAS kit (Mega Tıp Inc, Gaziantep, Turkey) (Erel, 2004). The results in the serum and erythrocytes were expressed in μmol H_2O_2 equivalent/l (μmol H_2O_2 equiv./l).

β-carotene, vitamins A, C and E analyses

Concentrations of vitamin A and vitamin E in the serum samples were determined by spectrofluorometrically (Infinitepro200 Plate reader, Tecan Group Ltd. Männedorf, Switzerland) according to methods of Desai (1984) as described in previous study (Nazıroğlu et al. 2004b). Samples were saponified with sodium hydroxide in the presence of pyrogallol (saturated form in water) as an antioxidant for 30 min at 70 °C. The vitamin A and E were extracted from the serum samples with hexane and the levels were monitored spectrofluorometrically (excitation: 330 nm, emission: 470 nm for vitamin A; excitation: 295 nm, emission: 330 nm for vitamin E). Calibration was performed using standard solutions of all-trans retinol and α-tocopherol in hexane and the results are expressed in μmol/l of serum.

The levels of β-carotene in serum samples were determined according to the method of Suzuki and Katoh (1990). Two milliliters of hexane were mixed with 250 μl serum. The value of β- carotene in hexane was measured at 453 nm in the spectrophotometer.

Serum vitamin C was spectrophotometrically determined by the method of Jagota and Dani (1982) and is expressed in micromoles per liter.

Data analyses

All results are expressed as means ± SD. P-values of less than 0.05 were regarded as significant. Significant values were assessed with Mann Whitney U test. Data was analyzed using the SPSS statistical program (version 17.0 software, SPSS Inc. Chicago, Illinois, USA).

Results

Lipid peroxidation results

The mean serum LP antioxidant values of three groups are shown in Figure 1. Mean LP values as μmol/l in the control, patients and treatment groups were 1.68, 1.95 and 1.73, respectively. The results showed that the serum LP levels were significantly ($p<0.05$) higher in patient group than in the control group although their values were significantly ($p<0.05$) lower in the treatment group than in the patient group.

TAS results

The mean serum TAS antioxidant levels of three groups are shown in Figure 2. Mean TAS values as μmol H_2O_2 equiv/ g prot in the control, patients and treatment groups were 2.17, 1.77 and 2.09, respectively. The results showed that the TAS levels were significantly ($p<0.05$) lower in patients with primary dysmenorrhea than in the control group although their values were significantly ($p<0.05$) higher in treatment group than in patient group.

Vitamin C results

The mean serum vitamin C antioxidant concentrations of three groups are shown in Figure 3. Mean vitamin C values as μmol/l in the control, patients and treatment groups were 75, 74 and 285, respectively. The results showed that the TAS levels did not differ between the patients and control groups. However, NSAID supplementation induced increase of vitamin C concentrations in the treatment groups as compared to control and patients groups ($p<0.001$).

β-carotene, vitamin A and E results

The mean blood serum β-carotene, vitamin A and E concentrations are shown in Table 1. The results showed that there was no statistical significant change on the values in the three groups.

Discussion

We found that LP levels in serum of patients with primary dysmenorrhea were increased although investigated serum TAS values decreased. However, 6 weeks NSAID supplementation caused decrease in serum LP levels but serum TAS and vitamin C concentrations were increased by the supplementations. The dysmenorrhea is characterized by decreased TAS concentrations and increased LP levels in the serum. A limited number of studies of serum of patients with primary dysmenorrhea regarding the effects of antioxidant redox systems and LP levels on the pathogenesis of primary dysmenorrhea have been reported (Yeh et al. 2004; Dikensoy et al. 2008). To the best of our knowledge, the current study is the first to compare the treatment of NSAID with particular reference to oxidative stress and the antioxidant redox systems in serum of patients with primary dysmenorrhea.

Rupture of the follicular wall during ovulation can be modeled as a short inflammatory process (Tola et al. 2013). Near the time of ovulation, an increase in various substances in the follicle which can induce oxidative stress has been measured; these free radical generating agents include histamine, bradykinim, angiotensin, prostaglandins, eicosanoids, proteolytic enzymes, nitric oxide, superoxide (Agarwal et al. 2006). In physiological eumenorrheic women, the uterus well-defined contraction patterns that are influenced by sex steroids and prostaglandins. Of particular interest and relevance to the pathogenesis of primary dysmenorrhea is the uterine contraction pattern during ovarian cycles when the symptoms of dysmenorrhea induce (Dawood, 2006). Ischemia is induced during the uterine contraction by decreasing blood flow to myometrium of uterus (Buhimschi et al. 1995). Ischemia is one of the over

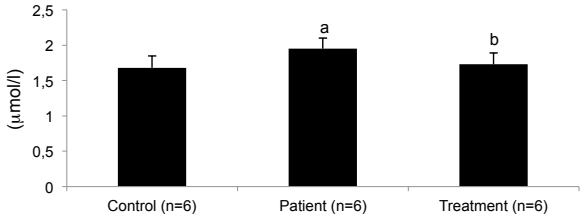

Figure 1. Effects of non-steroid anti-inflammatory drug supplementation on serum lipid peroxidation levels in control and patients with primary dysmenorrhea. (mean±SD). ap<0.05 versus control. bp<0.05 versus patient group.

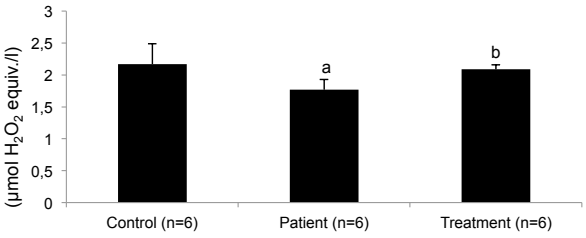

Figure 2. Effects of non-steroid anti-inflammatory drug supplementation on serum total antioxidant status in control and patients with primary dysmenorrhea. (mean±SD). ap<0.05 versus control. bp<0.05 versus patient group.

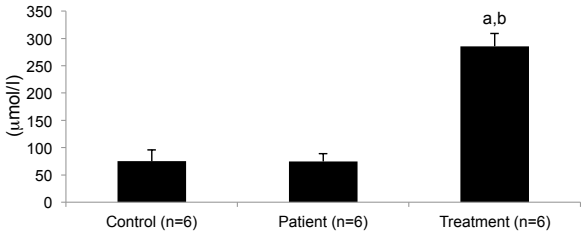

Figure 3. Effects of non-steroid anti-inflammatory drug supplementation on serum vitamin C concentrations in control and patients with primary dysmenorrhea. (mean±SD). ap<0.001 versus control. bp<0.001 versus patient group.

Table 1. Effects of non-steroid anti-inflammatory drug treatment on serum antioxidant vitamin concentrations in control and patients with primary dysmenorrhea. (mean±SD).

Parameters	Control (n=6)	Patients (n=6)	Treatment (n=6)
Vitamin A (µmol/l)	2.74 ± 0.26	2.70 ± 0.37	2.51 ± 0.20
β-carotene (µmol/l)	1.31 ± 0.15	1.34 ± 0.18	1.31 ± 0.17
Vitamin E (µmol/l)	13.66 ± 0.61	15.07 ± 0.84	14.48 ± 0.88

production of ROS in the pathological process (Sirmali et al. 2007). Hence, over production of oxidative stress has been implicated in the pathogenesis of dysmenorrhea (Dikensoy et al. 2008). Current results indicated that LP levels in serum of patients with primary dysmenorrhea increased. Results in the current study indicated that ROS in patients with primary dysmenorrhea is a marker for obligatory minimal metabolic activity within the endometrium.

One define consequences of an excess of ROS in the reproductive system is damage of membrane (Nazıroğlu et al. 2004b), but the question of how this damage affects primary dysmenorrhea remains. Several investigators have studied the involvement of ROS/oxidative stress in the serum and its consequences in women exposing primary dysmenorrhea. Dikensoy et al. (Dikensoy et al. 2008) reported significant role of oxidative stress in etiology of the dysmenorrhea by indicating increase serum levels of lipid peroxidation, nitric oxide and adrenomedullin in patient with primary dysmenorrhea. Similarly, Yeh et al. (2004) reported that lipid peroxidation and interleukin-6 levels increase in serum of patients with primary dysmenorrhea as compared to controls. Recently we observed that the NSAID diclofenac induced a protective effect against oxidative stress and Ca^{2+} entry through modulation of neutrophil voltage gated calcium channels and TRP calcium channels in patients with primary dysmenorrhea (Kaplan et al. 2013).

NSAID may be additional mechanism against oxidative stress by which they exert their anti-inflammatory effects. For example, NSAID destabilize the mRNA of the proinflammatory enzyme cyclooxygenase-2 (COX-2) by inhibiting the activity of p38. It was also reported that NSAIDs with the greatest cytoprotective effect against oxidative stress may exert their effect mainly through the blockade of COX-2 activity (López-Villodres et al. 2012). Lee et al. (2010) reported that NSAID (diclofenac) preserve the endothelium-dependent vasorelaxation against the attack of ROS, in a concentration-related manner. Takayama et al. (1994) reported diclofenac as NSAID inhibited liver injury caused by ischemia-reperfusion through stable radical scavenging and the inhibition of superoxide production in activated phagocytes. In the current study, serum TAS and vitamin C concentrations were increased by the supplementation due to oxidative stress inhibitor properties of the NSAID.

The enzymatic antioxidants such as catalase and glutathione peroxidase and non-enzymatic antioxidants such as vitamins A, vitamin C and vitamin E are important in restoring or maintaining the oxidant-antioxidant balance in blood and tissues (Nazıroğlu, 2012; Nazıroğlu et al. 2012). These antioxidants can provide protection of cells against oxidative stress caused by ROS, which would lead to damage of DNA or other important structures such as proteins and cell membranes. We were not able to measure enzymatic antioxidant values in the current study although they are very important indicators of antioxidant values in ovarian cycles (Agarwal et al. 2006; Özkaya and Nazıroğlu, 2010; Özkaya et al. 2011). The antioxidant vitamins such as vitamin A and E concentrations did not differ in the three groups although LP values are different in the three groups. The LP values may be inhibited by the antioxidant enzymes. In future studies, the enzyme activities should measure in blood of the patients with primary dysmenorrhea.

In conclusion, the role of antioxidants in relation to dysmenorrhea remains unclear. The results of the current study show that primary dysmenorrhea affected oxidative stress related antioxidant levels namely TAS in the blood serum by diminishing the antioxidant levels. However, NSAID supplementation ameliorates the antioxidant changes probably through its free radical scavenging in blood serum of primary dysmenorrhea patients. The results may be help to physicians and on the treatment of primary dysmenorrhea with the NSAID supplementation as well as scientist for clarifying etiology of primary dysmenorrhea. Further studies to clarify their physiological and pathological roles and their relationship to female reproduction should be undertaken, as they could lead to the development of novel strategies for primary dysmenorrhea treatment in the human.

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Declaration of interest

The authors report no financial conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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Effects of food based yeast supplementation on oxidative stress in rats fed by high cholesterol diet

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List of abbreviations
OSI, oxidative stress index
TAS, Total antioxidant capacity
TOS, total oxidant capacity

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Abstract

In living organisms, oxidant and antioxidant systems are in a balance. In the current study, we aimed to study the effects of *Cryptococcus humicola,* which is food based yeast whose cholesterol lowering activity is under investigation, on oxidant and antioxidant systems.

31 adult male, Wistar albino rats weighing 200-250 gr were included in the study. Rats were divided into four groups based on their diets. Rats in Group 1 (Control Group) was fed control diet, Groups 2, 3 and 4 were fed high cholesterol, high cholesterol plus low dose yeast diet (0.1% lyophilysed yeast) and high cholesterol plus high dose yeast diet (2% lyophilized yeast), respectively. After fifty six days, serum samples were obtained from blood of all rats. Total antioxidant capacity (TAS), total oxidant capacity (TOS) and oxidative stress index (OSI) values were determined in the serum samples.

Within the four groups there were no statistical changes on the TAS, TOS and OSI values in the four the groups.

In conclusion, this may lead to questioning the efficiency of the diet. It may be possible to show the antioxidant activity of *Cryptococcus humicola* by increasing the yeast dose, number of subjects and duration of the experiment.

Keywords

Antioxidant, Cholesterol, Yeast, Oxidative stress.

Introduction

Since 3000 B.C., probiotics have been found in many foods such as yoghurt, bread, kefir, koumiss and cheese and used by humans in preparing foods and beverages (Hosona and Nagasawa, 1992). Analysis carried out on the outcomes of an excavation carried out in China revealed that probiotics had been used in bread and beverages since 7000 B.C. (Zhang et al. 1999). In *"Naturalis Historia"*, which is the earliest encyclopaedia of the world, published in A.D. 76-77 by Plinius during ancient Rome, it is stated that milk was acidified and thickened and used in the treatment of many diseases. (Vasile et al. 2012). In the 20th century, the concept of probiotics has been now defined as: "Probiotics are mono- or mixed-culture of live microorganisms which benefits man or animals by improving the properties of the indigenous microflora" (Havenaar R. 1992). Consumption of probiotics has been shown to be effective in strengthening the immune system and preventing tumor formation in gastrointestinal system (Isolauri, 2004). It has been also linked to longer lifespan of intestinal epithelial cells, production of bactericidal materials, maintenance of barrier integrity and improvement of immune response (Boehm et al. 2002; Bruzzese et al. 2006; Benyacoub et al. 2008).

The vegetative reproduction of the yeast strains in *Cryptococcus,* the probiotic used in the present study, has been reported to be either multilateral or in the form of budding at the poles of the cells, not to cause ascospore formation and have no fermentation ability but ability to hydrolyse urea (Kurtzman et al. 2011). *Cryptococcus* is an encapsulated yeast-like fungus. Pathogenic *Cryptococci* are divided into two main species as *Cryptococcus* neoformans and *Cryptococcus gattii.* Non-pathogenic, non-neoformans Cryptococci are present in the air, soil, peagean droppings, and foods such as cheese, milk, beans and wine (Belet, 2011).Yeasts can be isolated as natural contaminants from many cheese types. Yeast species such as *Cryptococcus humicola* have been reported to be transmitted to mozeralla cheese from the hands of workers (Zotolla et al. 2009).

Cryptococcus has been reported be present in raw milk and pasteurized milk as a result of secondary contamination (Esen, 2008). In a study where milk origined yeast strains were identified genetically, Bockelmann et al. (2008) found Cryptococcus humicola strains in raw milk samples. In another study about yeast contamination of different foods, the ratio of Cryptococcus *humicola* has been reported as 5.34% in white cheese (Esen, 2008). Kefir grains, having beneficial effects on human health, contain strains of *Saccharomyces, Candida, Kluyveromyces, Torulopsis* and *Cryptococcus* spp. (Simova et al. 2002)

In living organisms, oxidant and antioxidant systems are in a balance. Reactive products formed continuously by exogeneous and endogeneous sources are rendered harmless by the antioxidant system (Halliwell and Gutteridge, 1984). Oxidative stress is an etiological factor in aging and development of various neurodegenerative diseases (Halliwell and Gutteridge, 1984). For this reason, there has been a trend to consume foods high in antioxidants.

In the present study, our aim was to study the effects of *Cryptococcus humicola,* which is food based yeast whose cholesterol lowering activity is under investigation, on oxidant and antioxidant systems.

Materials and Methods

Animals

Thrity-one male wistar albino rats weighing 200-250 g were used for the experimental procedures. The ambient temperature and relative humidity of the animal room were 21±1°C and 60±7%, respectively. The room was illuminated with artificial light for 12/12 hours dark/light. The animals were allowed free access to standard pelleted food and tap water. All studies were performed with the approval of the ethical committee of Medical Faculty of Suleyman Demirel University.

Experimental Design

The rats were divided into four groups based on their diets.

Group 1 (Control Group) was fed a normal diet.

Group 2 was fed a high cholesterol diet (1% cholesterol).

Group 3 was fed a high cholesterol and low dose yeast diet (1% cholesterol, 0.1% cholic acid and 0.1% lyophilysed yeast).

Group 4 was fed a high cholesterol and high dose yeast diet (1% cholesterol, 0.1% cholic acid and 2% lyophilysed yeast).

Anesthesia and preparation of brain and blood samples

After fifty six days, bloods were taken and rats were sacrificed by 10% Ketamine (Alfamin, Alfasan IBV) and 2% Xylazine Alfazin, Alfasan IBV) anaesthesia Obtained bloods were centrifuged at 3500 rpm for 8 minutes (Rotanta 460 Germany) and serums were collected. Serum samples were transferred to Medical Biochemistry Laboratory and kept at -80 C (Facis SA France) until the analyses were completed.

Total Antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) analyses

For the analysis, serums samples were thawed and mixed with a vortex (Labinco L 46 model, Holland). The serum TAS, TOS and OSI values were measured biochemical autoanalyzer equipment using the TAS and TOS commercial kit (Mega Tıp Inc, Gaziantep, Turkey) (Eren, 2004). The TAS and TOS results in the serum were expressed in μmol troloxEq/L and μmol H_2O_2 equiv/ L, respectively. Oxidative stress index was calculated using the formula (OSI) = TOS/TAS.

Statistical Analysis

Statistical analyses were carried out using SPSS 15.00 package program. Numerical data obtained were expressed as mean+ standard error (SEM). As the number of subject were low in the groups, Kruskall-Wallis test, which is a nonparametric test, was used to compare the average TAS, TOS and OSI values of four groups. <0.05 was regarded as statistically significant.

Results

Effects of food based yeast on total antioxidant status (TAS and μmol troloxEq/L), total oxidant status (TOS and μmol H_2O_2Eq/L) and oxidative stress index (OSI) values in serum of rats (mean± SEM).

Groups	TAS	TOS	OSI
Control diet (n=8)	0.80 ± 0.04	4.53 ± 0.12	5.78 ± 0.7
High cholesterol diet (n=7)	0.81 ± 0.04	5.60 ± 0.49	6.87± 1.07
High cholesterol plus low yeast (n=8)	0.79 ± 0.02	5.24 ± 0.56	6.69 ± 2.08
High cholesterol plus high yeast (n=8)	0.76 ± 0.02	5.02 ± 0.33	6.72 ± 1.62

When TAS, TOS and OSI values were compared by nonparametric Kruskall Wallis test, no significant difference was found among the groups. This may lead to questioning the efficiency of the diet. In rats fed by a diet containing yeast, TOS and OSI values were observed to decrease in line with the yeast added to the diet. However, the differences observed in the statistical analysis were not significant (p>0.05).

Discussion

Hypercholesterolemia has been reported to cause cellular oxidative stress, which increases oxidative stress parameters (Mahfouz et al. 2000). In our study, the total oxidant capacity was increased in the cholesterol group. The said level was observed to decrease to some extent when yeast was added to the diet and decreased more significantly when the level of yeast added was increased. The statistical analysis performed on serum TOS levels did not reveal any significant difference (p>0.05). Increasing the number of rats, the yeast dose and the duration of experiment may yield significant results.

Cholesterol lowering effects of probiotics has been studied recently. In a study conducted on 8 strains of Lactobacillus, Lactobacillus has been shown to reduce total cholesterol significantly (Awaisheh et al. 2013). In another study, cholesterol lowering activity of Lactobacillus probiotics has been shown to range between 40-78%. Yeasts, that are a type of probiotics, has been shown to have positive effects in the treatment of many diseases including hypercholesterolemia (Giorgi, 2009; Vasile et al. 2012). No data has been found in the literature on the cholesterol lowering effect and antioxidant acvivity of *Cryptococcus humicola,* a probiotic yeast used in our study.

The antioxidant activity of probiotics has been studied and it has been reported that yogurt produced using a probiotic culture has an antioxidant effect (Hanie et al. 2011). In a study on *L.casei* and *L. acidophilus,* which are probiotic bacteria belonging to Lactobacillus strain, *L.casei* and *L. acidophilus* have been shown to decrease oxidative stress and have antioxidant and anti-inflammatory properties (Amdekar et al. 2013). In another study conducted on birds to evaluate the anti-oxidant activity of probiotics, a significant anti-oxidant activity has been shown (p<0.01). Proteins have been shown to have an increased antioxidant effect when supplemented with probiotics in bird (Anwar et al. 2012).

In conclusion, the aim of the present study was to show the antioxidant activity of C. humicola, which is a probiotic yeast present in many foods such as kefir, bread, koumiss and cheese. Probiotics have been linked to longer lifespan of intestinal epithelial cells, production of bactericidal materials, maintenance of barrier integrity and improvement of immune response in the gastrointestinal system (Boehm et al. 2002; Bruzzese et al. 2006; Benyacoub et al. 2008). In addition to these beneficial properties, proving the antioxidant activity of C. humicola would show a major benefit of consuming probiotics. Showing an increase in oxidant capacity by

adding *C. humicola* to the diet but having no statistically significant results (Table 1) necessitates new studies to explore this issue further. It may be possible to show the antioxidant activity of c. humicola by increasing the yeast dose, number of subjects and duration of the experiment.

Ethical issue: All of the this study's procedures were approved by the Suleyman Demirel University Head of the Local Ethics Committee of Animal Experiments.

Conflict of interest: The authors declare no conflict of interest.

Additional information: This research was previously presented at the Innovation in Health from the University of Industry "Education Workshop" Izmir, Turkey, as a poster presentation in 2013.

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