

IMPACT OF NUTS CONSUMPTION ON ANTIOXIDANT STATUS AND PRO-OXIDANT PARAMETERS IN HEALTHY HUMAN VOLUNTEERS

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Abstract:

The aim of the current research was to study the effect of consumption of a mixture of almond and pistachio on antioxidants and pro-oxidants levels in healthy human volunteers. This dietary intervention study was carried out during the period from February to March, 2013 on a total of 48 apparently healthy males students from Military Academy / Zakho, Kurdistan Region- Iraq. Blood samples were withdrawn from all volunteer who were living in a controlled environment and analyzed for the determination of serum antioxidants, pro-oxidants. Parameters were obtained at baseline, 3 and 6 weeks after daily consumption of 50 gm. of a mixture of almond and pistachio.

The results of the current study demonstrated that daily consumption of 50 g of almond and pistachio mixture for 3-6 weeks, significantly increased ($P < 0.05-0.005$) the levels of total antioxidant, Ceruloplasmin, Superoxide dismutase and Glutathione reductase. On the other hand, the levels of the pro-oxidants Malonaldehyde and Peroxy-nitrite were significantly decreased ($P < 0.005$) after 6 weeks of nuts consumption compared to the control. From this dietary intervention trial, it can be concluded that almonds and pistachio mixture improved anti-oxidants and pro-oxidants status compared with those of the healthy volunteers.

Key words: Anti-oxidants, Pro-oxidants, Human volunteers, Almond and Pistachio

Introduction:

Oxidative stress is a condition originating an imbalance between oxidants and antioxidants leading to the production of excess reactive oxygen species (ROS) (Huang *et al*, 2005). Reactive oxygen species are produced as natural by products of normal metabolism and play important roles in cellular signaling and homeostasis (Carter *et al*, 2007). Oxidative stress contributes to the general decline in optimum bodily functions (Naito *et al*, 2010). The from its related disorders need their neutralization and immunity boosting via the consumption of antioxidants phytonutrients, other dietary ingredients or by endogenous protection systems (Kumari, 2011).

Normally, cells are protected against ROS damage by enzymes, vitamins, uric acid and glutathione, as well as, by using free radical scavengers such as polyphenol antioxidants (Tomaino *et al*, 2010). The three major antioxidant enzymes are superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx or GSH-Px) (Halliwell, 2007). Antioxidants and their roles in the development of cardiovascular diseases have become one of the main research target (Kocyigit *et al*, 2006).

Some studies showed that the consumption of some antioxidants could reduce the oxidative stress and lower the risk of cancer (Megan *et al*, 2009). Nuts have been evaluated for their potential antioxidant scavenging free radical abilities (Chiavaroli, 2010). Studies have shown that a diet low in saturated fatty acids (SFAs) and cholesterol tends to decrease the risk of heart disease (Vadivel *et al*, 2012). Foods, such as nuts, rich in monounsaturated (MUFAs) or polyunsaturated fatty acid (PUFAs) have been recommended as substitutes for high SFAs food to reduce the risk of coronary heart disease by favorably altering cholesterol levels in the body (Srinath, 2003).

Epidemiologic studies showed that frequent nut consumption decreases the risk of coronary heart disease (CHD) (López-Uriarte *et al*, 2010). Compared with people who consumed nuts less than once per week, people who eat nuts at least five times per week showed 50% reduction in (CHD) risks. Furtherer more, nuts are also a good source of dietary fibers, vitamins, micronutrients, antioxidants, and amino acids, such as arginine (Sari *et al*, 2010).

Nuts are high in arginine, a precursor of nitric oxide (NO), and phenolic compounds which

have excellent antioxidant activities, acting as hydrogen donors, reducing agents and radical scavengers. The inhibition of free radicals accumulation is important in the reduction of the risk of chronic diseases like (CVD) and cancer (Teresinha *et al*,2011).

Nuts such as almonds and pistachios are rich in several other beneficial compound, such as omega-3- fatty acids, which has been proposed that the bioactive compounds in nuts may help lowering the risk factors for CVD by improving endothelial function and regulating BP, as well as lowering oxidative stress and inflammation (Soliman, 2012). Oxidants and antioxidants play an important role in maintaining a balance between free radicals production by metabolism or derived from environmental sources and the antioxidant system of the body (Ravindra *et al*, 2004).

Subjects and Methods: Subjects:

Forty-eight male student volunteers, mean age 22 years between 18 and 36y old, from Military Academy / Zakho, Kurdistan Region of Iraq, who were living in the same place, under controlled environment, and having the same diet were involved in the current study. They also had regular wake/sleep hours with the same daily activity. They were not provided with any additional food other than the recommended in the dietary protocol, with free access to water.

The enrolled subjects were healthy and free of acute or chronic medical disorders with no family history (father and / or mother) of heart diseases with normal bodies. Furthermore, all subjects underwent a detailed physical examination by a physician and the detailed medical history for each was recorded. Exclusion criteria involved smoking, consumption of alcohol, history of eating nuts frequently (more than once a week), a history of food or nut allergy, and regular use of any medications including vitamin supplements. The volunteers were informed about the nature of the study and a written consent was obtained from each subject.

The study design included three controlled-feeding periods. The first group, a run-in period preceded the test diet to establish a baseline for regular meals prepared in the Academy kitchen. These included all major food groups but did not include nuts. The amount of food was standardized for each volunteer. The second and third groups,

included the addition of 50 gm of a mixture of almond and pistachios (25gm of each) consumed with the same controlled diet as the first group for three and six weeks, respectively. The students were instructed to eat their daily ration of nuts in the morning with or after breakfast.

Methods:

A pre-tested questionnaire was designed to obtain information on age, anthropometric measurements, smoking, alcohol and nut consumption, type of diet (vegetarian or mixed), family history of diseases, past medical history and any medication if available. Antioxidants and pro-oxidants were assayed.

Collection of Blood Samples:

Venous blood samples (10 ml) were collected between 7.00- 9.00 a.m after 12-14 hour fasting using disposable syringe and placed in sterile capped disposable tubes. Two ml of blood was placed in EDTA containing tube to prepare whole blood for SOD assay and the remaining 8ml was placed in plain plastic tubes. All blood samples were transferred in a cooling ice bag to Zakho Hospital. The blood samples were then centrifuged (HITASHI model O5P-21) at 3000 rpm for 10 minutes to separate serum from the clot. Serum samples were divided into 5 parts in eppendorf capped tubes frozen at -28 °C until the time of analysis.

Estimation of Antioxidant Markers:

Ceruloplasmin level was measured spectrophotometrically by modified Menden method (Menden *et al*. 1977). Superoxide dismutase activity by method described by Woolliams *et al*. (1983). Glutathione reductase was measured by the method described by Goldberg and Spooner (1983) and Total antioxidant status was measured by the method described by Miller *et al*. (1993).

Estimation of Pro- oxidant Products:

Serum Malondialdehyde Level was measured by method of Buege and Aust, using thiobarbituric acid and serum peroxynitrite level was measured spectrophotometrically.

Statistical Analysis:

All data were analyzed using the statistical package for social sciences SPSS version 20

software for windows 7. The results were expressed as mean \pm standard error of mean (mean \pm SEM). One way ANOVA-test was used to compare parameters in different studied groups. P-values ($P \leq 0.05$) were considered statistically significant.

RESULTS

The results of the effect of consumption of a mixture of almond and pistachio for zero, 3 and 6 weeks on serum antioxidants and pro-oxidants are shown in Table (1-3) and Figures (1- 3) . As the results indicate, the levels of antioxidants (CP, SOD, GR and TAS) were increased, but not to the same extent, since the level of TAS was significantly increased during the first 3 weeks of nut's consumption (Table1), whereas the levels of CP, SOD and GR were significantly ($P < 0.05$ to 0.001) increased after 6 weeks of nut's consumption, as compared with the control group (Table 3).

On the other hand, the levels of pro-oxidants parameters, namely, MDA and Peroxy nitrite after 3 and 6 weeks of nut's consumption were inversely decreased with increasing the duration of nut's consumption. Accordingly, the levels of the studied pro-oxidants were decreased at significant levels (P-values between 0.03 to 0.05 after) after 3 weeks of nut's consumption, whereas after 6 weeks on nut's consumption the levels of the above pro-oxidants were further decreased at much higher significant levels as compared with the control group.

As indicated in comparison Figures (1 to 3), high percent of increases in antioxidants parameters and decreases in pro-oxidants were observed after 6 weeks of nut's consumption, in which the percent's of change in both antioxidants and pro-oxidants parameters were highly significant ($P < 0.001$) as compared with the control.

Table 1: Mean \pm SEM of antioxidant and pro-oxidant levels in group1 and 2:

Variables	Group 1 N=48 Mean \pm SEM	Group 2 N=48 Mean \pm SEM	Sig.	Percent change %
CP (mg/ml)	27.08 \pm 0.588	27.8 \pm 0.580	0.198	+2.6
SOD (U/mol)	168.49 \pm 2.42	176.13 \pm 2.27	0.18	+4.5
GR (U/L)	50.29 \pm 1.17	53.04 \pm 1.23	0.26	+5.5
TAS (mmol/L)	1.32 \pm 0.019	1.41 \pm 0.02	0.027	+6.8
MDA (nmol/L)	0.87 \pm 0.013	0.79 \pm 0.017	0.05	-9.2
Peroxy nitrite (mmol/L)	1.50 \pm 0.035	1.39 \pm 0.033	0.03	-7.9

*= significant according to one way ANOVAs test.

Table 2 : Mean \pm SEM of antioxidant and pro-oxidant levels in group 2 and 3:

Variables	Group 2 N=48 Mean \pmSEM	Group 3 N=48 Mean \pm SEM	Sig.	Percent change %
CP (mg/ml)	27.8 \pm 0.580	29.22 \pm 0.58	0.66	+5.1
SOD (U/mol)	176.13 \pm 2.27	183.47 \pm 2.34	0.02	+4.2
GR (U/L)	53.04 \pm 1.23	57.76 \pm 1.25	0.22	+8.9
TAS (mmol/L)	1.41 \pm 0.02	1.48 \pm 0.025	0.031	+4.9
MDA (nmol/L)	0.79 \pm 0.017	0.71 \pm 0.018	0.001	-10.1
Peroxy nitrite (mmol/L)	1.39 \pm 0.033	1.31 \pm 0.033	0.06	-5.7

Table 3. Effect of consumption of a mixture of almond and pistachio for baseline, 3 weeks and 6 weeks on Antioxidant and Pro-oxidant levels.

Variables	Baseline N=48 Mean \pmSEM	3 weeks N=48 Mean \pm SEM	6 weeks N=48 Mean \pm SE	Sig.
Ceruloplasmin (CP)(mg/ml)	27.08\pm0.588	27.8\pm0.58	29.22\pm0.583	0.028*
Superoxide Dismutase (SOD) (U/mol)	168.49\pm2.34	176.13\pm2.27	183.47\pm2.42	0.001*
Glutathione Reductase (GR) (U/L)	50.29\pm1.25	53.04\pm1.23	57.76\pm1.17	0.004*
Total Antioxidant Status (TAS) (mmol/L)	1.32\pm0.025	1.41\pm0.021	1.48\pm0.019	0.001*
Malondialdehyde (MDA) (nmol/L)	0.87\pm0.018	0.79\pm0.017	0.71\pm0.013	0.004*
Peroxy nitrite (mmol/L)	1.50\pm0.033	1.39\pm0.033	1.31\pm0.02	0.032*

* Means the presence of a significant difference (one way ANOVA).

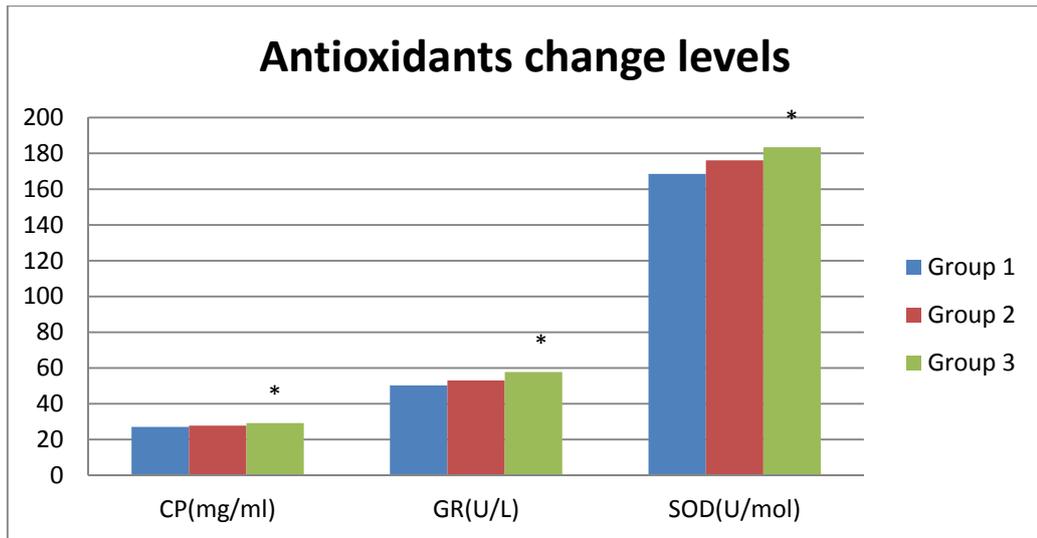


Figure 1: Antioxidants level in three studied groups
 * Indicates the presence of a significant difference (one way ANOVA).

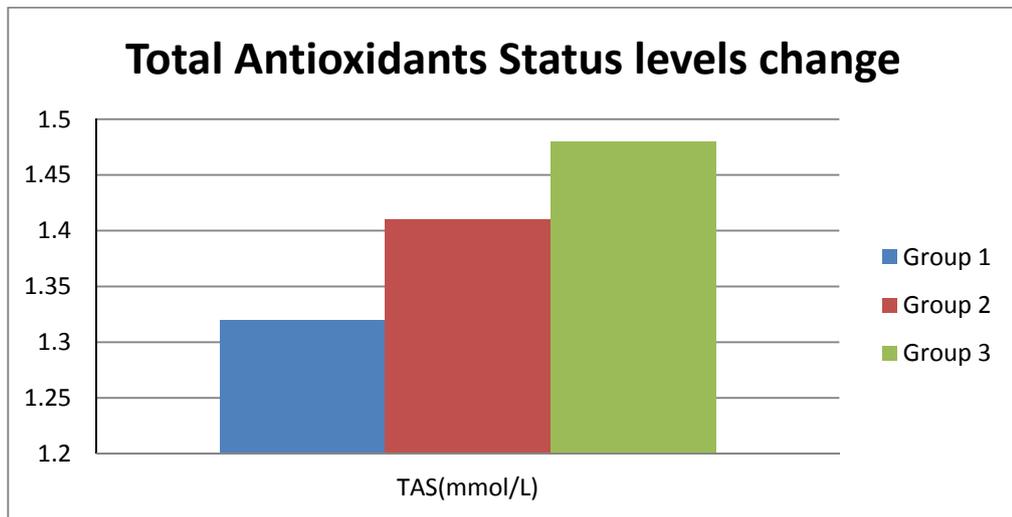


Figure 2: Total Antioxidants status level in three studied groups
 * Indicates the presence of a significant difference (one way ANOVA).

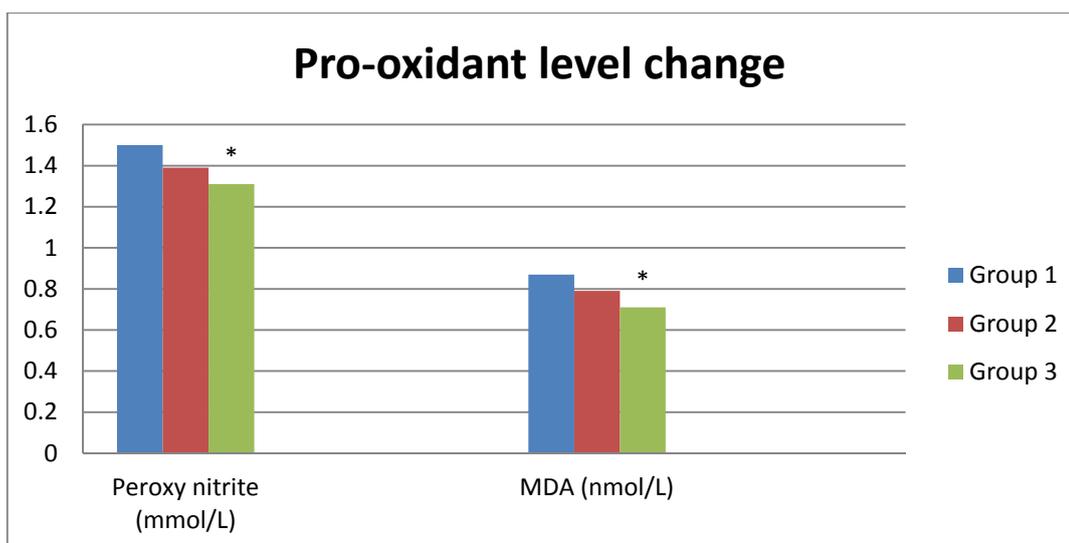


Figure 3: Pro-oxidants level in three studied groups

* Indicates the presence of a significant difference (one way ANOVA).

DISCUSSION

Recent nutritional research had focused on nut's consumption, including almond and pistachio, as a part of healthy diet due to its protecting effect by reducing the risk of heart disease and coronary risk factor (Griel and Kris-Etherton, 2006). It seems possible that the antioxidants present in nuts could work with other important nut constituents in an additive and synergistic way to protect cell organelles against oxidative stress (Salas-Salvadó *et al*,2011).

The results of the present study show that the inclusion of a mixture of almond and pistachio in the diet significantly increase the levels of the antioxidants CP, SOD, GR and TAS. These results are in agreement with these of other studies which indicated that consumption of nuts enhances antioxidant status (Jenkins *et al*, 2006; Couillard *et al*,2006). Furthermore, it is worthwhile to mention that TAS was the first parameters significantly increased after only 3 weeks of nuts consumption by 6.8% and after 6 weeks, it was increased by 10.8%. This was followed by other antioxidant parameters (CP, GR and DOS) which exert their effect and increased significantly after 6 weeks of nut's consumption by 8.9 %. From the above results, it can be concluded that TAS was affected faster, whereas, GR was affected to a greater extent and all parameters were gradually proportionally increased with time.

Increased antioxidant capacity and subsequent protection of body tissues from oxidative stress after consumption of nuts have been reported by other workers (Kocyigit *et al*, 2006;Torabian *et al*,2009; López-Uriarte *et al*, 2010; Chiavaroli,2010). Furthermore, Canales *et al*,(2007) reported enhanced superoxide dismutase and glutathione peroxidase activities when subjects consumed almond-powder supplement diet. Also it has been shown that consuming pistachio rich meals had high anti-oxidative effects on the body tissues, based on its high antioxidant content (Dreher *et al*,2012). Since pistachio nut's are rich in monounsaturated fatty acids, antioxidants such as vitamin E, lutein, b-carotene, and proanthocyanidins, offer protection against oxidative stress in body tissues (Baer *et al*,2012;Wang *et al*,2012).

Serum ceruloplasmin, which is a copper-carrying protein, used as an indicator for the overall level of oxidative stress in the body (Lee *et al*,2012). Ceruloplasmin protects polyunsaturated fatty acids in the red blood cell membranes from active oxygen radicals (Chauhan *et al*,2004). Superoxide, since it is the first reduction product of molecular oxygen, is considered as an important source of hydroperoxides which are deleterious free radicals (Chauhan *et al*,2004).

In addition to the protection role of these antioxidants in cellular integrity against ROS mediated injury (Dreher,2012); it has been also demonstrated that SOD could decrease cell death

and enhance the recovery of contractile function (Wang and Zweier,1996). Glutathione was also reported to protect the cells against oxidative damage and play a role in preventing the transformation of hemoglobin into methemoglobin due to its oxidation (Ulku *et al*,2009).

Free radicals injure biological membranes by lipid peroxidation (Jenkins *et al*,2008). Stable degradation products of such as MDA may, therefore, can be used as a marker for peroxidation of polyunsaturated fatty acids (Dreher, 2012). In the present study, consumption of a mixture of almond and pistachio along with the diet for six weeks, caused significant decrease in the levels of MDA and peroxy nitrite. Furthermore, the effect on MDA was more as compared with peroxy nitrite, since after six weeks of treatment, their levels were decreased by 18.4% and 12.6%, respectively; compared with the control group.

Nuts also contain tocopherols and several phenolic compounds with remarkable antioxidant potential, may counteract the pro-oxidant effects of PUFA on LDL oxidation and decreases DNA damage (Salas-Salvadó *et al*,2011). Peroxynitrite has been shown to readily react with most biological molecules (lipids, amino acids, and DNA), and consequently cause extensive cell damage (Christen *et al*,1997). An important etiological role for oxidants such as peroxy nitrite in the cardiovascular diseases is suggested by the observed inverse correlation between dietary antioxidant consumption and incidence of disease (Shimizu *et al*,2007). It can be concluded from the results of the current study that the use of a mixture of both almond and pistachio provides a better protection against oxidative status than consuming each one alone.

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پوخته:

نامانج ژ فه کولینی ئەوه بو خاندنا کارتی کرنا ب کارئینانا تیکه لی باو و فستق لسه ر دژه ئوکسید و پروئوکسید بو ئاستی مروفتی خوبه خشین ساخلم ئەه خاندنا دهستیوهر دانا خوراکی هاتیه کرن دناؤه بهینا کانونا دووی و ئاداری لسه ر 48 قوتابین کور یین ساخلم ژ ئە کادیمی سهر بازی زاخو ههریما کوردستان 2013 سامپلیت خوینی هاتینه وهر گرتن ژهه می خوبه خشین د ژینگه هه کا کونترول کریدا بو شیکردنی و دیار کرنا ناو خوین دژه ئوکسید و پروئوکسید. هو کار هاتینه وهر گرتن ژسه رتا وپشتی 3 تا 6 ههفتیا ژخارنا بهرده وام 50 گرام ژ تیکه له یی باو و فستقا ده رئه نجامی قی خاندنی دیار کر کو خارنا بهرده وام 50 گرام ژ تیکه له یی باو و فستقا سیگنیفیکانتلی بلند کرل نیک دژه ئوکسید و سیرولوبلازمین سوپه رئوکسیده یز ديسميوتته یز و گلو تیلین ژلایه کی دیفه پروئوکسیده یز و مالونالدهاید سیگنیفیکانتلی کیمکر پشتی شهش هفتیا ژ خارنی ل دو ما هیا قی دهستیوهر داندل خارنی کو ئەه تیکه له دژه ئوکسید و پروئوکسید زیده کریه خارنی بهراورد دگهل کونترولی بی ساخلم ($P < 0.05 - 0.005$).

ملخص

وكان الهدف من البحث الحالي إلى دراسة تأثير استهلاك مزيج من اللوز والفسق على مضادات الأكسدة ومستويات الموالية للتأكسد على متطوعين من البشر الأصحاء. وقد أجريت هذه الدراسة تدخل الغذائية خلال الفترة من فبراير إلى مارس، 2013 على ما مجموعه 48 طالبا من الذكور اصحاء من الأكاديمية العسكرية / زاخو في كردستان العراق كل إقليم. تم سحب عينات دم من جميع المتطوعين الذين كانوا يعيشون في بيئة تسيطر عليها وتحليلها لتحديد المواد المضادة للاكسدة في مصل الدم، الموالية للتأكسد. تم الحصول على المعلومات في الأساس، وبعد 3 و 6 أسابيع الاستهلاك اليومي من 50 جم. من مزيج من اللوز والفسق.

أظهرت نتائج هذه الدراسة أن الاستهلاك اليومي من 50 غراما من اللوز والفسق الخليط لمدة 3-6 أسابيع، زاد

معنويا

مستويات من إجمالي مضادات الأكسدة، سیرولوبلازمین، فوق الأكسید الفائق واختزال الجلوتاثیون ($P < 0.005$) من ناحية أخرى، فإن مستويات Malonaldehyde الموالية للتأكسد وبيروكسي النتريت وانخفضت معنويا ($P < 0.005$) بعد 6 أسابيع من استهلاك المكسرات مقارنة بالمجموعة الضابطة. من هذا تدخل الغذائي، فإنه يمكن استنتاج أن اللوز والفسق خليط تحسن المضادة للتأكسد ووضع الموالية للتأكسد مقارنة مع تلك من المتطوعين ال