J. Agric. Sci. Mansoura Univ., 28(4): 2819 - 2834, 2003

THE HYPOCHOLESTEROLEMIC EFFECT OF SELECTED NATURAL ANTIOXIDANTS ON RATS

Fayad, Abeer S. * and Aida R. Mowafy**

* Nutrition and Food Science Dept., Faculty of Home Economics, Helwan University.

** Nutrition Institute- Cairo- Egypt.

ABSTRACT

This study was undertaken to study the influence of hypercholesterolemic diet on some parameters and to study the effect of adding antioxidants [Lutein (L), vitamin C and vitamin E] as a single, double and in combination on hypercholesterolemic rats. The experiment was carried out on (54) male albino rats, distributed equally in (9) group. One group was kept on the normal basal diet throughout the experimental period (8 weeks) and referred to as normal control. All other groups were fed on hypercholesterolemic diet for (3) weeks. One group was maintained on this diet for (5) weeks without antioxidants supplementation and referred to as positive control. The other groups were supplemented with antioxidants lutein, vit. C, vit. E, (L+C), (L+E), (C+E), (E+C+L). Weekly body weight gain, food intake and food efficiency ratio were calculated. At the end of the experimental period, animals were sacrified. Relative weight of some organs were calculated. Blood samples were collected to determine total cholesterol (TC), triglyceride (TG), HDL-c, LDL-c, liver function enzymes (GOT and GPT), hemoglobin (Hb) and hematocrit (Hct).

Results showed that the administration of hypercholesterolemic diet caused insignificant elevation in TC, TG, LDL-c, GOT, GPT levels. On the other hand significant reduction in HDL-c, Hb and Hct was detected affect ingestion of hypercholesterolemic diet.

All treatments without vit. E showed significant decrease in TC level. Each of vit. C, (C+L) and (E+C+L) caused significant increase in HDL-c level while all treatments showed no effect on TG level. Vit. C, L, (C+L) led to significant decrease in GOT level. Upon vit. E and C treatments there were significant elevation in Hb and Hct levels. These results showed association between antioxidant intake and reduced risk of hypercholesterolemia disease. Moreover the combination between two or three of these antioxidant may lead to improve the efficiency of these antioxidants, as hypocholesterolemic agents. More study need to be done to determine the possibility of using these combination of antioxidants in food products as a dietary treatment for hypercholesterolemic individuals.

INTRODUCTION

Some studies have shown that several diseases are inversely correlated with a high consumption of fruit and vegetables. The protective factors in these foods may be vitamins A, C and E because of their antioxidant properties (Eichholzer *et al.*, 2001 and Tyssandier *et al.*, 2002). Evidence from clinical trials and epidemiologic studies suggests that antioxidants supplementation is associated with reduced risk for some forms of cancer, diabetes, cataract and hypercholesterolemic (Meydani *et al.*, 1998).

Carotenoids which are considered plant pigment also found to be naturally occurring antioxidants nutrients. Carotenoid as antioxidant can

inactivates harmful free radicals through their singlet oxygen quenching capacity (Van den Berg *et al.*, 2000).

Lutein and its structural isomer zeaxanthin have recently been added to the list of potentially beneficial nutrients and phytochemicals provided by leafy greens. The body of evidence that lutein and zeaxanthin might contribute to health and the delay of chronic disease was the topic of a 2001 (Mares- Perlman and Erdman 2002).

Lutein and zeaxanthin belong to the large class of plant pigments referred to as carotenoids. They are more polar than many other carotenoids, due to the presence of hydroxyl groups on the cyclic ring structure. Unlike the provitamin A carotenoids (α- and β- carotene and cryptoxanthin), they can not be converted to vitamin A. Their presence in tissues is due entirely to ingestion of plant sources; they are not synthesized by animal tissues. However, a variety of metabolites may be found in animal sources, and several exist in human blood and milk (Khachik *et al.*, 1997).

Lutein are present in a wide variety of fruits and vegetables and impart a yellow color to the plants they are found in, such plant as corn. Their concentration is particularly high in leafy green vegetables such as spinach, collards, kale, pumpkin, peas, beans and corn (Mares-Perlman *et al.*, 2002). Also lutein can be present in some animal products such as egg yolks due to plant products eaten by animals (Bailey and Chen, 1989).

Supplementation with ascorbic acid was associated with decrease LDL-c and triglycerides, modest decrease in total cholesterol and increase HDL-c (Bagchi and Puri, 1998). Also other studies found a significant association between elevated plasma vit. C. concentrations and increased concentration of HDL- c and reduced concentrations of LDL-c (Ness, *et al.*, 1996).

Several studies reported that high level of plasma vit. C was associated with significantly reduced in cardiovascular disease incidence and/or mortality (Brubacher *et al.*, 1994).

The evidence suggests that of the three major antioxidants (vit. E, beta-carotene and vit. C.), vit. E had the strongest and most independent inverse association with coronary heart disease (CHD). It is not only lipid-soluble but also the most abundant antioxidant in LDL-c and this suggests that it plays a role in the protection against atherogensis (Paul *et al.*, 2001). Other research reported that vit. E intake plays a critical and beneficial role in the pathogenesis of CHD (Biesalski, 1999).

The present study was designated to investigate the hypocholesterolemic effects of selected natural antioxidants (Lutein, α-tocopherol and Ascorbic acid) individually and in combinations on rats.

MATERIALS AND METHODS

Test material:

Vitamin E (α-tocopherol), Vitamin C (Ascorbic acid) and Lutein (L) were obtained from the Nutrition Institute.

Experimental design:

Forty-five male albino rats Sprague Dawely strain weighing between 120-130 gm. The rats were included into nine groups, each cage contained

six rats. The first group was kept as normal control. The other groups were given hypercholesterolemic diet for 21 days after that blood samples were taken from each rat. After this period of time one group was maintained on this diet for (5) weeks without antioxidant (positive control). Other groups were fed on hypercholesterolemic diet and antioxidants for (5) weeks.

The antioxidant nutrients were added at level of 800 mg/Kg diet for vitamin E according to Kappus and Diplock (1992), 1000 mg/Kg diet for vitamin C according to Diplock, (1995) and 300 mg/Kg for lutein according to Jewell and O' Brien (1999).

Rats were divided into (9) groups according to food intake:

Group (1): fed on basal diet (negative control).
Group (2): fed on hypercholesterolemic diet (positive control).
Group (3): fed on hypercholesterolemic diet + vitamin E.
Group (4): fed on hypercholesterolemic diet + vitamin C.
Group (5): fed on hypercholesterolemic diet + lutein (L).
Group (6): fed on hypercholesterolemic diet + vitamin E + C.
Group (7): fed on hypercholesterolemic diet + vitamin E + L.
Group (8): fed on hypercholesterolemic diet + vitamin C + L.
Group (9): fed on hypercholesterolemic diet + vitamin E + C + L.

Animals were weighted twice a week and food intake was calculated , at the end of the experimental periods animals were scarified . relative weight of some organs were calculated .

Experimental Diets:

A- Basal diet:

The basal diet used consisted of 15% casein, 10% cotton seed oil, 4% salt mixture, 1 vitamins mixture, 5 % cellulose and 65% corn starch.

B- Hypercholesterolemic diet:

The hypercholesterolemic diet consisted of 10% casein, 1% cholesterol, 4% salt mixture, 1% vitamins mixture, 5% cellulose, 10% cotton seed oil and 69% corn starch (Shinnick *et al.*, 1990).

The salt mixture used was prepared according to method of Hegsted *et al.*, (1941) and vitamin mixture used was prepared according to the method of Campbell (1961).

Blood Analysis:

At the end of the experiment, the rats were fasted over night, lightly anesthetized (under ether). Approximately 2- ml blood was withdrawn from hepatic portal vein into clean dry centrifuge plastic tubes. Heparinized micro tubes were also used for the estimation of hemoglobin and hematocrit. Blood samples were then centrifuged and serum obtained to determine hemoglobin according to Drabkin, (1949), hematocrit according to Mc-Inory (1954), total cholesterol according to Finely (1978), triglycerides according to Fossati and Principe (1982), LDL-c according to Friedewald *et al.*, (1972), HDL-c according to Burstein (1970), GOT and GPT according to Reitman Frankel (1957).

Statistical Analysis:

Statistical analysis of the obtained data was performed by calculating the mean, standard error, T-test and (ANOVA) were used to represent the quantitative data (SAS, 1996).

RESULT AND DISCUSSION

Results showed the biological effect of different antioxidants (vitamin E, C and lutein) individually and in combination (E and C) or (E and L) or (C and L) or (E, C and L).

Data in Table (1) show the mean values of body weight gain, food intake and food efficiency ratio of control and hyperlipidemic rats. Group fed a cholesterol-supplemented diet had low values compared to the group fed on basal diet. These observations agree with the results of other investigators who reported that food intake, food efficiency ratio and body weight gain were reduced upon feeding high cholesterol diet as compared to control group (Kahlon *et al.*, 1997, and Salem, 1999).

Table (1): Daily food intake , Body weight gain and Food efficiency ratio of rats fed basal or hyperlipidemic diet. (M±SE).

Parameters	Control		Prob>(T)
	Negative	Positive	
Body weight gain (gm)	3.86 ± 0.36	2.07 ± 0.28 **	0.0046
Food intake (gm)	67.58 ± 4.56	58.84 ± 6.39	0.2981
F.E.R	0.058 ± 0.013	0.034 ± 0.010	0.1899

In other study Schouten *et al.*, (1985) found that body weight gain was significantly increased in animals fed the high cholesterol diet, whereas food intake was not significantly increased. The investigators reported that the increase in body weight gain may be referred to the higher fat content and thus higher energy density of the high- cholesterol diet.

Table (2) shows relative weight of some organs of control hyperlipidemic rats. Relative weight of liver in rats fed the hyperlipidemic diet (4.67 gm) was significantly higher than those fed on the normal diet (6.29 gm). While relative weight of spleen was significantly decreased (0.31 gm) as compared to the control (0.52 gm). Relative weight of kidney and heart were not affected by the hyperlipidemic diet. Kahlon *et al.*, (1997) found an increase in relative weight of liver in rats fed hyperlipidemic diet. And this increase may be referred to fat deposition mainly in liver, since the main effect of the high fat-cholesterol diet was a severe liver steatosis with an accumulation of both cholesterol and triglycerides (Robins *et al.*, 1994).

Table (2): Relative organs weight of rats fed basal or hyperlipidemic diet.(M±SE).

Organs	Control		Prob > (T)
	Negative	Positive	
Liver	4.67 ± 0.19	6.29 ± 0.28 **	0.0015
Kidney	1.08 ± 0.07	1.04 ± 0.02	0.6091
Spleen	0.52 ± 0.04	0.31 ± 0.02 **	0.0053
Heart	0.38 ± 0.03	0.41 ± 0.03	0.4779

Results in Table (3) showed that the levels of total cholesterol and LDL-cholesterol concentrations were increased significantly ($p < 0.001$) and this results agree with Turley *et al.*, (1999) and Salem (1999). On the other hand HDL-cholesterol concentration was significantly ($p< 0.001$) decreased as compared to control group and these results are in agreement with Salem (1999).

Table (3) also presents mean values for serum GOT and GPT of groups fed normal diet compared to hypercholesterolemic diet. Results show significant increase in GOT and GPT enzymes for rat groups fed hypercholesterolemic diet (79.81 IU/L and 37.63 IU/L) respectively as compared to control group (54.13, 24.53) respectively. This results agree with the results obtained by Sies and Stahl (1995).

With regard to hemoglobin and hematocrit levels results showed significant decrease in both parameters for rats fed of hyperlipidemic diet (11.86 gm/dl and 35.56 gm/dl) than the control groups (13.61 gm/dl and 39.31 gm/dl).

These results agree with Sies and Stahl (1995) and Kahlon *et al.*, (1997) who attributed these effect to poor utilization and defects in metabolism that occur by feeding the animal diet containing high cholesterol and saturated fat.

Table(3): Serum lipid profile , liver functhion enzemes and hemoglobin, hematocrit levels of rats bed a basal or hyperlipidemic diet (M ± SE).

Parameters	Control		Prob > (T)
	Negative	Positive	
Total Cholesteol (mg/dl)	73.32 ± 1.37	140.32 ± 2.07 ***	0.0000
Triglyceride (mg/dl)	86.18 ± 1.52	94.37 ± 3.68	0.0740
HDL-c (mg/dl)	48.47 ± 0.70	30.32 ± 2.16 ***	0.0006
LDI-c (mg/dl)	15.88 ± 0.61	75.94 ± 2.21 ***	0.0001
GOT (IU/L)	54.13 ± 1.07	79.81 ± 2.60 ***	0.0000
GPT (IU/L)	24.53 ± 1.03	37.63 ± 2.46 **	0.0012
Hemoglobin (g/dl)	13.61 ± 0.32	11.86 ± 0.32 **	0.0054
Hematocrit (g/dl)	39.61 ± 0.35	35.56 ± 1.18 *	0.0317

As shown in Table (4) antioxidants had no effect on food intake or food efficiency ratio. This is in line with Yamamoto *et al.*, (1995) and Rein *et al.*, (1998). And these results found that the differences in body weight gain among the groups supplemented with vitamin E or lutein or combinations of (vitamin E + Lutein) or (vitamin C+ lutein) or (vitamin E+C+ lutein) were not statistically significant. This results coincide with Rein et al ., (1998). On the other hand supplementation with vitamin C and a combination of (vitamin E+C) significantly increased the body weight gain (3.95 gm and 5.05 gm,) compared to the positive control group (2.07gm). These results agree with Mohfouz *et al.*, (1997) who reported that the growth rate after feeding combination of (E+C) supplemented hypercholesterolemic diet was greater than that without antioxidant supplementation. The present results showed that supplementation with vitamin E and vitamin C improve the body weight gain from (3.95 gm/week) for vitamin C group to (5.05 gm/week) for vitamins (C+E) group.

Table (4): Body weight gain, food intake and food efficiency ratio (FER) of hypercholesterolemic rats fed antioxidants for (5) weeks.

Parameters	Control positive	E	C	L	E+C	E+L	C+L	E+C+L	Pr>F
Body weight gain (g/week)	2.07 c ± 0.28	2.64 bc ± 0.34	3.95 ba ± 0.42	2.81 bc ± 0.24	5.05 a ± 0.50	2.41 bc ± 0.18	3.38 bc ± 0.28	1.89 c ± 0.38	F=9.37 Sig 0.0001 **
Food intake (g/week)	58.84 a ± 6.39	55.67 a ± 6.14	64.13 a ± 9.90	69.35 a ± 3.25	70.04 a ± 5.79	55.73 a ± 2.57	60.38 a ± 3.41	56.79 a ± 6.87	F=0.95 Sig 0.4806
F.E.R	0.034 a ± 0.010	0.046 a ± 0.012	0.059 a ± 0.008	0.039 a ± 0.013	0.070 a ± 0.010	0.053 a ± 0.010	0.055 a ± 0.008	0.033 a ± 0.005	F=1.56 Sig 0.1823

Means with the same letter are not significantly different. (M±SE).

Data in Table (5) revealed that the values of organs weight as a percentage of body weight indicated that supplementation of the diet with vitamins had little or no significant effect on liver weight. This results agreed with those reported by Diplock (1995) and Sulli, *et al.*, (1998) who reported that there was slight increase in liver weight for rats fed on vitamin E (2gm/kg diet). However, the result disagreed with Weldon *et al.*, (1983) who reported that treatment with vitamin E caused an increase in relative liver weight. On the other hand supplementation of rats diet with combination of vitamin (E+C) significantly decreased liver weight than supplementation of rats diet with vitamin E individually. This results similar to other investigators (Sulli *et al.*, 1998) who reported that the liver weight of hypercholesterolemic rabbits treated with combination of B-carotein and vitamin E was significantly deceased than after treatment with vitamin E individually. The mean organs weight (Kidney, spleen and heart) as a percent of body weight for all groups which fed on diet contained antioxidants were not significantly changed compared to that of the positive control group. These results agree with Sulli *et al.*, (1998). On the other hand Brown *et al.*, (1997) illustrated that vitamin E at high or prolonged intake may have a pro-oxident activity that may affect various tissues and organs. Other study which investigated the effect of - β carotene, α-tocopherol and α tocopherol+β-carotene reported that no significant differences were observed in triceps and heart muscle weights among the different treatment groups (Sulli, *et al.*, 1998).

Table (5): Relative organs weight (g/100g B.wt) of hypercholesterolemic rats fed antioxidants for (5) weeks.

Parameters	Control positive	E	C	L	E+C	E+L	C+L	E+C+L	Pr>F
Liver	6.29 ba ± 0.28	7.88 a ± 0.33	6.00 ba ± 0.35	7.45 ba ± 0.36	5.68 b ± 0.49	7.39 ba ± 0.49	6.09 ba ± 0.25	5.97 ba ± 0.84	F=3.30** Sig 0.0093
Kidney	1.04 a ± 0.02	1.14 a ± 0.07	1.02 a ± 0.08	1.03 a ± 0.07	1.05 a ± 0.09	1.05 a ± 0.06	1.07 a ± 0.06	1.11 a ± 0.14	F=0.24 Sig 0.9723
Spleen	0.31 a ± 0.02	0.45 a ± 0.06	0.39 a ± 0.05	0.38 a ± 0.02	0.47 a ± 0.03	0.41 a ± 0.03	0.46 a ± 0.04	0.49 a ± 0.05	F=1.85 Sig 0.1119
Heart	0.41 a ± 0.03	0.46 a ± 0.04	0.41 a ± 0.04	0.45 a ± 0.03	0.39 a ± 0.03	0.43 a ± 0.03	0.39 a ± 0.04	0.38 a ± 0.02	F=0.73 sig 0.6444

Means with the same letter are not significantly different. (M±SE).

Table (6) indicated the mean values of serum cholesterol of different groups of rats. There were significant decrease in serum total cholesterol upon feeding diet supplemented with vit. C, lutein, vit. (E+C), (E+L), (C+L) and (E+C+L) groups (P<0.001) as compared to the positive control group. This results agree with Simon *et al.*, (1993) who reported that increased vit. C intake could play an important role in the cholesterol homeostasis of females with elevated total cholesterol level.

Table (6): Serum lipid pattern (mg/dl), of hypercholesterolemic rats fed antioxidants for (5) weeks.

Parameters	Control positive	E	C	L	E+C	E+L	C+L	E+C+L	Pr>F
Total Cholesterol	140.32 a ± 2.07	144.41 a ± 5.73	110.22 bc ± 4.32	112.06 bc ± 3.25	108.31 bc ± 4.54	105.84 c ± 4.61	111.04 bc ± 5.57	102.61 c ± 6.00	F=10.74 Sig0.001 **
HDL-c	30.32 a ± 2.16	35.93 a ± 1.87	48.10 b ± 1.43	37.23 ca ± 2.24	32.56 a ± 0.98	36.48 a ± 1.15	46.45 cb ± 1.12	48.73 b ± 1.98	F=27.38 Sig0.000 ***
LDL-c	75.94 a ± 2.21	77.45 a ± 2.80	49.61 b ± 1.70	61.46 cd ± 2.51	56.03 cdb ± 1.98	54.31 cb ± 1.88	53.65 cb ± 2.42	47.32 b ± 1.98	F=21.29 Sig0.000 ***
Triglyceride	94.37 a ± 3.68	99.31 a ± 6.47	84.32 a ± 5.18	90.23 a ± 5.90	81.41 a ± 5.58	87.32 a ± 4.22	86.58 a ± 5.11	80.27 a ± 6.34	F=1.81 Sig 0.1196

Means with the same letter are not significantly different. (M±SE).

Recent studies reported that hypercholesterolemic diet supplemented with vitamin C lowered significantly serum cholesterol (Hamilton, *et al.*, 2000). Anderson, *et al.*, (1997) added that consumption of one gm vit. C /day for 4 weeks caused reduction in total serum cholesterol.

Results of lutein supplementation agree with Alberto, *et al.*, (1999) who reported that lutein was the only carotenoid that had a significant inverse association with risk for stroke. Rowley *et al.*, (2001) found that significant increase in plasma concentrations of lutein was correlated with significant reduction in the prevalence of hypercholesterolemia.

On the other hand the group fed on diet containing vit. E (144.41 mg/dl) were not differed significantly than that of the positive control group (140.32 mg/dl).

These results agree with Yamamoto *et al.*, (1995) who reported that α-tocopherol had no significant effect on cholesterol level. Same results were obtained by Sun *et al.*, (1997) and Rein *et al.*, (1998). On the other hand other investigators reported the ability of vit. E supplements to prevent hypercholesterolemia (Kaikkonen et al ., 2001). In other study, animal fed a cholesterol rich diet supplemented with α- tocopherol have a lower total plasma cholesterol than animals consumed low level of dietary α-tocopherol (Brandes et al, 2000 and Schwenke *et al.*, 2002).

The present results agree with Sharma *et al.*, (1999) who stated that alpha-tocopherol offers the best hope, but the question is how much of it

should be administered for the prevention of atherosclerosis. At the same trend Sahin *et al.*, (2002) found that greater dietary vit. E and C lead to reduce serum cholesterol concentration (P=0.001). The results also demonstrated that the combinations of two or three antioxidants caused a highly significant decrease in total cholesterol when compared with positive control group. This results agree with Mietus-Snyder and Malloy (1998) and Sulli *et al.*,(1998) .

Other study on rabbits indicated that diet supplementation with mega dose of vit. C and E significantly reduced the plasma cholesterol (Mahfouz *et al.*, 1997). This could be due to the fact that vit. C may regenerate vit. E and scavenge free radicals in the cytoplasm (Biesalski 1999). Other research found that increased intake of vit. C, could lead to improve vit. E status and that combined supplementation with vitamins C and E is more active than supplementation with either vitamin alone in healthy adults (Jeng *et al.*, 1996 and Hamilton *et al.*, 2000).

The present study showed that combination of vit. E,C and L recorded the lowest decreased level, when compared with positive control, this results agree with Oldham and Bowen (1998) who reported that the addition of different types of antioxidants to hypercholesterolemic diet caused a highly significant reduction in cholesterol level and may be due to the synergistic effect of the combined antioxidants.

Data in Table (6) indicated also the mean value of serum-high-density lipoprotein for different groups of rats. The high-density lipoprotein has been shown to play anti-atherogenic roles and reduce the risk of hypercholesterolemic disease (Lichtenstein *et al.*, 1998). The mean value of serum HDL of the positive control group was 30.32 $\pm$ 2.16 mg/dl. Supplementation with vitamin E, (E+C), (E+L) had no significant influence on HDL-c level. These results agree with Rein *et al.*, (1998). Those investigators reported that the addition of vit. E had no significant effect on HDL-c levels of hypercholesterolemic rats, which was in agreement with Schwenk *et al.*, (2002).

Supplementation with vit. (E+C) in the present study had no significant effect on HDL-c. This result agree with (Mahfouz *et al.*, 1997). However the present results contrasted with the result reported by Niki *et al.*, (1995).

On the other hand significant changes were noticed as a results of the addition of vit. C, (C+L) and (C+E+L). The results of vit. C consisted with those reported by Carr and Balz., (1999).

From the results of the present study it was appeared that the best effects was obtained after supplementation with (E+C+L). This observation may be due to the synergistic effect of combination of fat soluble and water soluble antioxidants.

Supplementation with antioxidant caused significant decrease in LDL-c level as compared with positive control group except with regard to vit. E fed group. This result agree with Sun *et al.*, (1997) and Rein *et al.*, (1998) who reported no significant effect in vit. E supplemented group regarding LDL-c level as compared with the positive control group. In the same respect other investigaters established that vit. E protects membrane and LDL

against oxidative stress (Sardar *et al.*, 1996). In addition the present results showed that supplementation with other parameter caused significant reduction in LDL-c when compared with positive control group. The same observation indicated by Mosinger (1999) who found that the administration of antioxidants might have a protective effect against increased LDL cholesterol. In the same respect Das' et al ., (1997) reported that vit. C treatment caused a reduction in LDL-c. Also the protective effect of vit. C against coronary vascular disease [CVD] was associated with a decrease in serum LDL-c in hypercholesterolemic animals. And other research found that combination of vit. E and vit. C decrease LDL-c (Mietus-Snyder and Malloy, 1998).

The results of the present study indicated that combination of two or three types of antioxidants supplementation may lead to more protective effective than supplementation with one type of antioxidants. LDL-c concentration of group fed (E+C+L) supplementation was (47.32 mg/dl) which was considered more reduction than positive control group (75.94 mg/dl).

The present findings agree with Oldham and Bowen study , (1998) who reported that the addition of β-carotein +E+C to hypercholesterolemic diet caused a highly significant reduction in LDL-c concentration and that this could be due to the synergistic effect of the combined antioxidants.

The results of the present study show also the effect of supplementation with the three antioxidants (E, C and lutein) as individual or in combinations on the level of triglycerides in hypercholesterolemic rats. No significant difference at 0.05 level between the treated group and the positive control group with regard to their effects on triglycerides.

These results agree with Bierenbaum *et al.*, (1985) which reported that triglyceride levels were not affected by administration of 400 mg/dl to 2000 mg/dl vitamin E. Similar results obtained by Xiao *et al.*, (1997). Other study reported that increasing the dietary α -tocopherol supplementation did not change plasma triglyceride level (Schwenke et al 2002). However other studies (Rowley *et al.*, 2001 and Sahin et al ., 2002) found that high intake of dietary vit. C and (C+E) resulted in decreased concentration of serum triglycerides significantly. The reasons of this difference may be related to duration of the study and concentration of the antioxidants. Conversly Das *et al.*, (1997) reported that triglyceride was increased in rats fed on diet containing 25 mg vit. C /100 gm body weight.

Results in Table (7) indicated significant decrease in GOT enzymes for rat groups fed hypercholesterolemic diet supplemented with vit. C and (C+L). On the other hand the other studied antioxidant did not has an effect on GOT level. These results agree with Yamamoto *et al.*, (1995) and Meydani *et al.*, (1998) who stated that vit. E supplementation did not affect the GOT activity. On the other hand other research found significant increase in GOT and GPT enzymes for rat groups fed diets supplemented with vit. E (Diplack, 1995). These differences may be related to the dose concentration and the time introduced the supplementation to the animal under study.

From the same table it is clear that addition of vit. C to hypercholesterolemic rats significantly decreased GOT activity in comparison to positive control (61.06 IU/L and 79.81 IU/L, respectively). These results

agree with Liu and Lee, (1998). In contrast results reported by other investigators showed that vit. C did not has an effect on GOT level (Diplock, 1995 and Sahin *et al.*, 2002). The combination between vit. (E+C)(E+L),(C+L) and (E+C+L) caused a slight reduction in GOT level but the difference was not significant.

Table (7): Effect of antioxidants on liver function enzymes (IU/L), hemagobin and hematocrit (g/dl) of hypercholesterolemic rats fed antioxidants for (5) weeks.

Parameters	Control positive	E	C	L	E+C	E+L	C+L	E+C+L	Pr>F
GOT	79.81 a ± 2.60	79.03 a ± 1.67	61.06 c ± 2.814	77.60 ba ± 3.45	68.23 bac ± 3.60	77.51 ba ± 3.72	65.03 bc ± 2.77	70.32 bac ± 2.18	F=5.93 Sig0.0002 ***
GPT	37.63 bac ± 2.46	44.62 a ± 2.12	31.04 c ± 1.40	41.31 ba ± 2.14	32.14 bc ± 2.43	35.42 bac ± 2.70	31.63 c ± 1.05	35.42 bac ± 1.61	F=5.77 Sig0.0002 ***
Hemoglobin	11.86 c ± 0.32	14.01 ba ± 0.08	15.03 a ± 0.21	13.05 bc ± 0.48	12.76 bc ± 0.57	12.30 bc ± 0.24	12.03 c ± 0.35	11.26 c ± 0.63	F=9.24 Sig0.0001 ***
Hematocrit	35.56 dc ± 1.18	42.04 ba ± 1.11	45.07 a ± 0.91	39.12 bc ± 0.80	38.22 bc ± 0.93	36.06 dc ± 0.84	36.07 dc ± 0.38	33.80 d ± 1.04	F=16.12 Sig0.0001 ***

Means with the same letter are not significantly different. (M±SE).

This table indicates that the addition of antioxidants did not cause significant effect on the values of GPT as compared with positive control groups. This results agree with Hathcock (1997) who found vit. C have no effect on GPT enzyme and Yamamoto *et al.*, (1995) who desided that vit. E did not have effect on GPT enzyme.

Data in Table (7) clearly showed that supplementation with either vit. E and C significantly increased the hemoglobin and hematocrit values, while other treatments with antioxidants showed no effect when compared with positive control group. This results agree with Bender, (1997) who attributed this effect to the fact that vit. C enhances iron absorption from gastrointestinal tract. In addition other study stated that vit. E protects red blood cells against oxidation (Meydani, 1995).

These results showed association between dietary intake of natural antioxidants which frequently present in fruit and vegetables and a reduced risk resulted from elevated blood lipids. Further studies need to be done to determine the possibility of using combination from the investigated antioxidants in food products as dietary treatments for hypercholesterolemic individuals.

REFERENCES

Alberto, A.; B.R. Eric; A.H. Miguel; G.Edward; K. Ichiro; J.S. Meir and C.W.waiter (1999): Relation of consumption of vitamin E, vitamin C, and carotendoids to risk for stroke among men in the United States. Annals of Internal Medicine: Article. June 15:130 (12),1-9.

Anderson, D.; B. Phillips; A. Edwards; R. Ayesh and K. Butterwoeth (1997): The effect of vitamin C supplementation on biomarders of oxygen radical generated damagein human volunteers with low or high cholesterols level. Environment, Mol. Mutagen, 30:161-174.

Bagchi, K. and S. Puri (1998): Free Radicals and Antioxidants in Health and Disease La Revude de Sante de la Mediterrance Oriental.4(2):350-360.

Bailey, C.A. and B.H. Chen (1989). Chromato-graphic analyses of xanthophylls in egg yolks from laying hens fed turf Bermuda grass (cynodon dactylon) meal. J. Food Sci., 54:584-586,592.

Bender, D.A. (1997): Introduction to nutrition and metabolism. Second Edition, P.139-140. Taylor and Francis, London.

Bierenbaum, M.; F. Noonan and L. Machilin (1985). The effect of supplemental vitamin E on serum parameters in diabetics, post coronary and normal subjects. Nutr. Rep, Int., 31:117-118.

Biesalski, H.K. (1999). The role of antioxidative vitamins in primary and secondary prevention of coronary heart disease. Int. J. vitam. Nutr. Res. 69:179-186.

Brandes, R.P.; S. Brandes; R.H. Boger; S.M. Bode-Boger and A. Mugge (2000). L-arginine supplementation in hypercholesterolemic rabbits normalizes leukocyte adhesion to non-endothelial matrix Life Sci., 66:1519-1524.

Brown, K.M.; P.C. Morrice and G.G. Duthie (1997), Erythrocyte vitamin E and plasma ascorbate concentrations in relation to erythrocyte peroxidation in smokers and non-smokers: dose response to vitamin E supplementation. Am. J. Clin. Nutr., 65:496-502.

Brubacher, D.; P. Jordan; U. Moser; H. Stahelin and K.F. Gey (1994).17-year follow-up of the basle prospective study. 2nd congress on antioxidant vitamins and beta-carotene. Berlin. October 10-12. Abstract #24.

Burstein, M.(1970).HDL cholesterol/ phospholipids Separation of high density lipoproteins and determination of cholesterol and phospholipids bound to these fractions. Lipid Res., 11:583.

Campbell, J. A. (1961). Methodology of protein Evaluation, Nutrition. Doc. R 10/add 37. WHO/FAO. UNICEF.

Carr, A. and F. Balz (1999). Toward a new recommended dietary allowance for vitamin C based on antioxidant and health effects in humans. Am. J. Clin. Nutr., 69, 1086-1107.

Das, S.; L. Snehlata and I. Srivastava (1997). Effect of ascorbic acid on lipid profile and lipid peroxidation in hypercholesterolemic rabbits. Nutr. Res., 17 (2): 231-241.

Drabkin, D. I. (1949). The standardization of Hemoglobin measurements.Am. J. Med. Sci., 21 (7): 710.

Diplock, A. T. (1995). Safety of antioxidant vitamins and β. Carotene. Am. J. Clin. Nutr.: 92 (suppl.): 1510S- 6S.

Eichholzer, M.; J. Luthy; F. Gutzwiller and H. B. Stahelin (2001) The role of folate, antioxidant vitamins and other constituents in fruit and vegetables in the prevention of cardiovascular disease. Int. J. vitam. Nutr. Res., 71 (1): 5-17.

Finely, P. (1978). Enzymatic colorimetric determination of serum total cholesterol. Clin. Chem. 24: 391.

Fossati, P. and L. Principe (1982). Enzymatic colorimetric determination of serum Triglycerides. Clin. Chem.; 28: 2077.

Friedewald, W. T.; R. I. Levy and D. S. Fredriekson (1972). Estimation of concentration of low density lipoproteins separated by three differents. Clin. Chem.; 28: 2077.

Hamilton, I. M. J.; Gilmore, w. S.; Benzie, I., F. F.; Mulholland, C. W. and Strain, J. J. (2000). Interaction between vitamins C and E in human subjects. British J. of Nutr. 84, 261-267.

Hathcock, J. (1997): Vitamins and minerals: efficiency and safety. Am. J.Clin. Nutr. (66): 427- 437.

Hegsted, D. M.; Mills, R. C.; Elvehjen, C. A. and Hart, E. B. (1941). Salt mixture. J. Biol. Chem., 138: 459.

Jeng, K. G.; Yang, C.; Siu, W.; Tsai, Y. Liao, W. and Kuo, J. (1996).Supplementation with vitamins C and E enhances cytockine production by peripheral blood mononuclear cells in healthy adults. Am. J. Clin. Nutr., 64 (6): 960- 965.

Jewell, C. and O'Brien, N. M. (1999). Effect of dietary supplementation with carotenoids on xenobiotic metabolizing enzymes in the liver, lung, kidney and small intestine of the rat.

Kahlon, T. S.; Chow, F. I.; Irving, D. W. and Sayre, R. N. (1997). Cholesterol response and fatty streak formation in hamsters fed two levels of saturated fat and various levels of cholesterol. Nutr. Res., 17 (11/12): 1693-1707.

Kaikkonen, J.; Porkkala-Saratahe, E.; Morrow, J. D.; Roberts, L. J.;Nyyssonen, K.; Salonen, R.; Tuomainen, T.P.; Ristonmaa, U.; Poulsen, H. E. and Salonen, J. T. (2001). Supplementation with vitamin E but not vitamin C lowers lipid peroxidation in vivo in mildly hypercholesterolemic men. Free- Radic- Res. Dec.; 35 (6): 967-78.

Kappus, H. and Diplock, A. T. (1992). Tolerance and safety of vitamin E:a toxicological position report. Free Radic Biol. Med. : 13: 55-74.

Khachik, F.; Bemstein, P. and Garland, D. L. (1997). Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. Investig. Ophthamol. Vis. Sci. 38: 1802-1811.

Lichtenstein, A. H.; Kennedy, E.; Barrier, P.; Danford, D.; Ernst, N. D.; Grundy, S. M.; Leveille, G. A.; Van Horn, L.; Williams, C.L. and Both, S. L. (1998): Dietary fat consumption and health. Nutr. Rev., 56 (5): 3S-19S.

Liu, J. F. and Lee, Y. W. (1998): Vitamin C supplementation restores the impaired vitamin E status of guinea pigs fed oxidized Frying oil. J. Nutr., 128: 116-122.

Mahfouz, M. M.; Kawano, H. and Kummerow, F. (1997): Effect of cholesterol rich diets with and without added vitamin E and C on the severity of atherosclerosis in rabbits. Am. J. Clin. Nutr., 66: 1240-1249.

Mares- Perlman, J. A. and Erdman, J. W. (2002). Can Lutein protect against chronic disease? A multidisciplinary approach involving basic science and epidemiology to weight evidence and design analytic strategies. J. Nutr. 132: 517S.

Mares- Perlman. J. A.; Millen, A. E.; Ficek, T. L. and Hankinson, S. E. (2002). The body of evidence to supporta protective role for lutein and zeaxanthin in delaying chronic disease. J. Nutr. 132. 518S-520S.

Mc-Inory (1954). A mirco Hematocrit for determining the packed cell and Hemoglobin concentration on capillary blood. J. Clin. Path., (7), 32.

Meydani, M. (1995): Vitamin E. Lancet, 345: 170-175.

Meydani, S. N.; Meydani, M.; Blumberg, J. B.; Leka, L. S.; Perdrosa, M.and Diamond, R. (1998). Assessment of the safety of supplementation with different amounts of vitamin E in healthy older adults. Am. J. Clin. Nutr., 68 (2): 311-318.

Mietus- Snyder, M. and Malloy, M. J. (1998). Endothelial dysfunction occurs in children with two genetic hyperlipidemia improvement with antioxidant vitamin therapy. J. Pedietrics, 133 (1): 35-40.

Mosinger, B. J. (1999). Higher cholesterol in human LDL is associated with the increase of oxidation susceptibility and the decrease of antioxidant defence: experimental and simulation data. Biochem-Biophys- Acta. Jan. 6, 1543 (1): 180-4.

Ness, A.; Khaw, K. and Bingham, D. (1996). Vitamin C status and serum lipids. Am. J. Clin. Nutr. (50): 724-729.

Niki, E.; Noguchi, N.; Tsuchihashi, H., and Gotoh, N. (1995). Interactionamong vitamin C, vitamin E and β- carotene. Am. J. Clin. Nutr., 62 (5): 1322 S- 1326 S.

Oldham, K. M. and Bowen, P. E. (1998). Oxidative stress in critical care: is antioxidant supplementation beneficial?. J. Am. Diet. Assoc., 98: 1001-1008.

Paul, A.; Calleja, L.; Joven, J.; Vilella, E.; Ferre, N.; Camps, J.; Girona, J. and Osada, J. (2001). Supplementation with vitamin E and/or Zinc does not Attenuate Atherosclerosis in Apolipoprotein E-deficient Mice fed a High-Fat, High-Cholesterol Diet. Int. vitam. Nutr. Res. 71(1): 45-52.Rein, D.; Yokoyama, W. H..; Xu, R.; Walzem, R.L. and German, J.B. (1998). Dietary vitamin E in an atherogenic hamster model. Nutr. Res., 18 (3): 567-579.

Reitman, S. and Frankel, S. (1957). Acolorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Path., 28, 56-63.

Robins, S. J.; Fasulo, J. M.; Pritzker, C. R.; Patton, G. M.; Morey, K. M.; Smith, D.; Ordovas, J. M. and Schaefer, E. J. (1994). Response of Hamsters to an Atherogenic Diet: Late Compensation by Enhanced Biliary Secretion. Gastroenterology, 106: A 970.

Rowley, K.G.; Su, Q.; Cincotta, M.; Skinner, M.; Skinner, K.; Pindan, B.; White, G. A. and O'Dea, K. (2001). Improvements in circulating cholesterol, antioxidants, and homocysteine after dietary intervention in an Australian Aboriginal Community. Am. J. Clin. Nutr. Oct.; 74 (4): 442-8.

Sahin, K.; Kucuk, O.; Sahin, N. and Sari, M. (2002). Effects of vitamin C and vitamin E on lipid peroxidation status, serum hormone, metabolite and mineral concentrations of Japanese quails reared under heat stress (34 °C). Int. J. vitam. Nutr. Res., 72 (2), 91-100.

Salem, I. S. (1999). Comparative evaluation of two different forms of dietary fiber on atherosclerosis in rats. MS. c. Thesis, Faculty of Home Economics, Helwan University.

Sardar, S.; Chakraborty, A. and Chatterjee, M. (1996).Comparative effectiveness of vitamin D, and dietary vitamin E on peroxidation of lipids and enzymes of the hepatic antioxidant system in Sprague-dawley rats. Internat. J. Vit. Nutr. Res. 66: 39-45.

SAS (1996). Statistical Analysis System. SAS User's Guide: Statistics.SAS Institute Inc. Editors, Cary, NC.

Schouten, J. A.; Beyenen, A. C; Popp-Snijders, C. and Mulder, C. (1985). effect of a hypercholesterolemic dieton the fatty acid composition of rat erythrocyte phospholipids. Nutr. Res. Inter.; 31: 229-236.

Schwenke, D. C.; Rudel, L.L; Sorci- Thomas, M. G. and Thomas, M. J. (2002). α-Tocopherol protects against diet induced atherosclerosis in New Zealand white rabbits. J. of Lipid Res. (43), 1927-1938.

Sharma, N.; Desigan, B.; Ghosh, S.; Sanyal, S. N.; Ganguly, N. K. and Majumdar, S. (1999). Effect of antioxidant vitamin E as a protective factor in experimental atherosclerosis in rhesus monkeys. Ann., Nutr. Metab. 43 (3): 181-90.

Shinnick, F. L.; Ink., S. L. and Marlett, J. A. (1990). Dose response to a dietary oat bran fraction in cholesterol fed rats. J. Nutr., 120: 561-568.

Sies, H. and Stahl, W. (1995). Vitamins E, C, β-carotene and other carotenoids as antioxidants. Am. J. Clin. Nutr., 62 (5): 1315 S- 1321 S.

Simon, J. A.; Schreiber, G. B.; Crawford, P. B.; Frederick, M.M. and Sabry, Z. I. (1993). Dietary vitamin C and serum lipids in black and white girls. Epidemiology. Nov., 4 (6): 537-42.

Sulli, K. C.; Sun, J.; Giraud, D. W.; Moxley, R. A. and Driskell, J. A. (1998). Effects of β-carotene and α-tocopherol on the levels of tissue cholesterol and triglyceride in hypercholesterolemic rabbits. J. Nutr. Biochem. 9: 344-350.

Sun, J.; Girand, D.; Moxley, R.; Driskell, J. and Sun, J. (1997). Beta-carotene and α-tocopherol inhibit the development of atherosclerotic lesions in hypercholesterolemic rabbits. Int. J. for Vit. and Nutr. Res. 67 (3): 155-163.

Turley, E.; Armstrong, N. C.; Wallace, J. M. W.; Gilmore, W. S.; Mckelvey-Martin, V. J.; Allen, J. M. and Strain, J. J. (1999). Effect of cholesterol feeding on DNA damage in male and female Syrian hamsters. Ann. Nutr. Metab.; 43: 47-51.

Tyssandier, V.: Cardinault, N. Caris-Veyrat, C.; Amiot, M.; Groleir, P.; Bouteloup, C.; Azais- Braesco, V. and Borel, P. (2002). Vegetable-borne lutein, lycopene and β-carotene compete for incorporation into chylomicrons, with no adverse effect on the medium-term (3-wk) plasma status of carotenoids in humans. Am. J. Clin. Nutr.; 75: 526-34.

Van den Berg, H.; Faulks, R.; Granado, H.; Hirschberg, J.; Olmedilla, B.; Sandmann, G.; Southon, S. and Stanhl, W. (2000). The potential for the improvement of carotenoid levels in foods and the likely systemic effects. J. Sci. Food Agri. 80, 880-912.

Weldon, G. H.; Bhatt, A.; Keller, P. and Hurmmler, H. (1983). α-Tocopherol Acetate (vitamin E): a long term toxicity and carcinogenicity study in rats. Internat. J. Vit. Nutr. Res., 53: 287-296.

Xiao, Q.; Buchanan, W.; Matthews, A. J.; Chung, B.H. and Bagdade, J. D. (1997). Lack of effects of vitamin E on cholesteryl ester transfer composition in cholesterol fed rabbits. Biochemistry and Molecular Biology, 117-B (4): 553-559.

Yamamoto, K.; Fukuda, N.; Shiroi, S.; Shiotsuki, Y.; Nagata, Y.; Tani, T. and Sakai, T. (1995). Effect of dietary antioxidants on the susceptibility to hepatic microsomal lipid peroxidation in the rat. Ann. Nutr. Metab., 39: 99-106.

تأثير بعض مضادات الأكسدة الطبيعيةعلى الفئران المصابة بارتفاع في مستوى الكوليسترول

عبير صلاح عبد الحافظ فياض* – عايده رشاد موافي **

*** كلية الاقتصاد المنزلي – جامعة حلوان**

**** معهد التغذية**

يهدف هذا البحث إلى دراسة تأثير كفاءة مضادات الأكسدة (الليوتين – فيتامين هـ – فيتامين ج) كل على حدة أو كل اثنين معا أو الثلاثة معا على الفئران المصابة بارتفاع في مستوى الكوليسترول . وقد تم استخدام مجموعة من فئران الألبينو في وزن ١٢٠-١٣٠ جم وتغذيتها بغذاء يحدث ارتفاعا في دهون الدم وذلك لمدة ٣ أسابيع كذلك تم تغذية مجموعة أخرى من الفئران على الغذاء الأساسي طول مدة التجربة ثم بعد ذلك قسمت فئران المجموعة الأولى (ذات المستوى المرتفع في دهون الدم) إلى (٨) مجموعات تركت مجموعة منها بدون اخذ مضادات الأكسدة وتم إعطاء باقي المجاميع جرعات من مضادات الأكسدة لمدة (٥) أسابيع تبعا لما يلي (الليونين) – (فيتامين هـ) – (فيتامين جـ) – (فيتامين جـ +الليوتين) – (فيتامين هـ + الليوتين) – (فيتامين جـ+هـ) – (فيتامين جـ + هـ + الليوتين) وقد كانت كمية الجرعات كما يلي:

١٠٠٠ مجم/ كجم من الطعام بالنسبة لفيتامين جـ، ٨٠٠ مجم/كجم من الطعام بالنسبة لفيتامين هـ، ٣٠٠ مجم/كجم من الطعام بالنسبة للليونين.

- خلال فترة التجربة تم تسجيل وزن الفئران لتقدير الزيادة الأسبوعية في الوزن و حساب المتناول من الطعام و كذلك معدل كفاءة الغذاء.
- كما تم جمع السيرم لقياس كلا من الكوليسترول الكلى – كوليسترول الليبوبروتينات عالية الكثافة – كوليسترول الليبوبروتينات منخفضة الكثافة – الجليسريدات الثلاثية – إنزيمات وظائف الكبد – الهيموجلوبين– الهيماتوكريت

وقد أظهرت الدراسة أن الغذاء العالي في محتواة من الدهون و الكوليسترول أدى إلى ارتفاع ملحوظ في كل من الكوليسترول و الليبوبروتينات منخفضة الكثافة و الجليسريدات الثلاثية وكذلك أدى إلى انخفاض ملحوظ في مستوى كوليسترول الليبوبروتينات عالية الكثافة وكذلك في مستوى الهيموجلوبين و الهيماتوكريت .

كما أوضحت الدراسة أن كل المعاملات فيما عدا فيتامين هـ أدت إلى نقص معنوي في الكوليسترول . بينما أدى إعطاء كلا من فيتامين جـ ، (فيتامين جـ + الليوتين) وكذلك أعضاء كلا من (فيتامين جـ + هـ + الليوتين) إلى زيادة معنوية في مستوى كوليسترول الليبوبروتينات عالية الكثافة . كما انه لم يلاحظ أي تأثير لجميع المعاملات على مستوى الجليسريدات الثلاثية للفئران . بينما أدى إعطاء فيتامين جـ و الليونين و (فيتامين جـ + الليوتين) إلى نقص معنوي في نشاط GOT (الاسبرتيت أمينو ترانس فريز) . نتائج الهيموجلوبين و الهيماتوكريت أظهرت أن كلا من فيتامين هـ و فيتامين جـ أدت إلى رفع معنوي في كلا المعاملتين.

أوضحت النتائج وجود ارتباط واضح بين تناول مضادات الأكسدة وتقليل الإصابة بارتفاع الكوليسترول في الدم (السبب الرئيسي لأمراض القلب وتصلب الشرايين) كذلك أوضحت أن استخدام خليط من مضادات الأكسدة الطبيعية التي تم دراستها (اثنين أو ثلاثة) يساعد في أظهار كفاءتها كعوامل خافضة لمعدل كلسترول الدم.

ويوصي بإجراء مزيد من الدراسات في هذا المجال للتعرف علي مدي إمكانية الاستفادة من إضافة مضادات الأكسدة الطبيعية في صورة خليط من المنتجات الغذائية خاصة المقدمة إلى الأفراد الذين يعانون من ارتفاع معدل كلسترول الدم كوسيلة علاجية.