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Large intake of polyunsaturated fatty acid alters antioxidant/ oxidant balance in hypercholesterolemic rats

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ABSTRACT

This study aimed to investigate the effect of flaxseed oil (FO) alone or with lipoic acid (LA) on oxidative stress status in rats fed on high cholesterol diet (HCD). Thirty six male Wistar albino rats were used in this study divided into 6 groups. The rats were received either normal diet or HCD with or without FO, LA or combination of FO and LA. These results revealed that HCD feeding of rats caused significant decrease of liver glutathione and protein thiols content by 58% and 47% respectively, while glutathione peroxidase activity was increased by 122%. Supplementation of rats with FO (1 g/kg body weight) keeps these parameters at values similar that of control rats. On another hand FO at dose (2 g/kg body weight) not ameliorates these parameters as compared with the control. HCD supplementation elicit significantly increase of serum protein carbonyl and malondialdehyde by 287% and 127%. While values of these parameters in rats received LA and FO beside HCD near that of control ones. It concluded that large dose of oil rich with polyunsaturated fatty acids (PUFA) may be provoking oxidative stress. Administration of antioxidants like LA is necessary during the lipids lowering therapy particularly with PUFAs.

KEYWORDS

Hypercholesterolemia;
Flaxseed oil;
Lipoic acid;
Oxidative stress;
Rats.

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are known to be exert protective effects against many diseases; in particularly when they are supplemented in quantities[1]. Administration of such lipids in excess of normal dietary levels can resulted in an increase of cells susceptibility to the free radical attack[2]. Reactive oxygen species (ROS) react with the double bond of these unsaturated lipids resulting in lipid peroxidation, and increased the risk of oxidative damage[3].

Many of vegetable oils contain high percent of PUFAs; therefore they are used in the treatment of hypercholesterolemia (HC)[4]. On the other side it has been reported that ingestion of large amount of these oils may be overwhelming the biological antioxidant /oxidant balance[5]. Flax-seed oil (FO) is nature richest source of essential fatty acids, it contains high ratio of PUFAs

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particularly alpha linolenic acid, so that it used to decrease the risk of atherosclerosis. Each one gram of FO contains alpha linolenic acid (0.55 g). Alpha linolenic acid is converted in the body to eicosapentanoic acid and docosahexanoic acid that have beneficial effects in several diseases[6].

High cholesterol diet (HCD) feeding is associated with increase of cholesterol accumulation in the serum and tissues leading to oxidative stress[7]. The activation of both xanthine oxidase and NADPH oxidase are the main causes for increasing of free radical production in HC[8]. In response to oxidative stress, xanthine dehydrogenase is converted into oxidase form that uses oxygen molecules as the electron acceptor to produces uric and superoxide radicals[9]. Under the affect of superoxide dismutase, the superoxide is converted to hydrogen peroxide which provokes oxidative damage to the cellular structure[10].

Lipids in mostly those rich with PUFAs as well as proteins are liable to attach by ROS. Oxidative products of lipids and proteins can react with the structural and regulatory elements of the cells leading to disturbance of many cellular functions[11]. The measurement of the oxidative products, malondialdehyde (MDA) as marker for lipids peroxidation and protein carbonyl (PCO) as marker for proteins oxidation in the blood and the tissues are a good indicator for oxidative damage of these molecules[12]. Enzymatic defense against ROS include superoxide dismutase, catalase, and glutathione peroxidase (GPx). GPx is able breakdown hydrogen peroxide formed after the dismutation of superoxide, as well as it detoxifies the lipid hydroperoxides[13].

Supplementations of antioxidants preserve the endogenous antioxidant systems either by maintaining their normal levels or by minimizing ROS production[14]. Lipoic acid (LA) is one of the antioxidants that inhibit oxidative stress and it has cholesterol lowering effect. Moreover, LA maintains the cellular glutathione (GSH) which is the major cellular antioxidant[15]. Therefore, administration of LA has beneficial effect in treatment of many diseases in which ROS have been implicated[16].

Many studies reported that PUFAs supplementation have contradictory effects. Within this scope, the FO was chosen as example of oils richer with PUFAs to investigate the large intakes of PUFAs on oxidative stress status during the induction of HC in rats. This has been accomplished through studying the effect of low dose of FO (LDFO), high dose of FO (HDFO) as well as LA administration alone or in combination on the lipids profile as well as oxidative stress markers (GPx, GSH, PCO, MDA and uric acid) in rats received HCD and compared with those received normal diet.

MATERIAL AND METHODS

Chemicals

The chemicals of cholic acid, trichloroacetic acid, thiouracil and thiobarbituric acid were purchased from (Fluka Buchs, Switzerland). Tetramethoxy propane, glutathione (GSH), Alpha lipoic acid (LA), Tris HCl, Ellman's reagent, and guanidine–HCl were supplied from (Sigma Chemical Co., St. Louis, MO, USA). FO was obtained from ISIS Company, Cairo, Egypt. The remaining chemicals of the analytical grade were commercially available.

Instruments and apparatus

Beckman XL-70 ultracentrifuge, 100,000 *rpm* (USA), JENWAY spectrophotometer model 6105 UV/VIS. Centrifuge Sigma 3K 20, up to 10,000g (Germany), Homogenizer: Janke and Kunkel IKA 8,000 - 20.500 RPM (Germany).

Animals and diet

Male Wistar albino rats were obtained from our animal facility. The animals were housed in animal house unit, in the Pharmacology Department, College of Pharmacy, Al–Azhar University. The animals were received standard diet and water under standardized conditions away from stress condition. The standard diet was composed of 72.2 % carbohydrate, 3.4 % fats, 19.8 % proteins, 3.6% cellulose, 0.5 % vitamins and minerals and 0.5 % salts. The animals were housed in metabolic cages under standard laboratory conditions (12 h light/dark cycles at 25 ± 2°C) with free access to standard rat pellet food and tap water. The rats were left for 2 weeks for acclimatization.

Experimental design

This study was carried out on 36 male Wister albino rats weighing 180 ± 210 g and rats were divided into 6 groups, 6 rats in each group.

Group 1, normal control in which the rats were fed on standard diet pellets.

In group 2, the rats were fed HCD whereas the dietary chow was supplemented with 4% cholesterol, 1% cholic acid and 0.5% thiouracil[17].

In group 3 the rats were received low dose of FO (LDFO) (1 g/kg body weight) orally in addition to HCD[18].

In group 4 the rats were received high dose of FO (HDFO) (2mg/kg body weight) orally with HCD. In the fifth group, the rats were treated with LA at dose (20 mg/kg body weight) by oral gavages concurrent with HCD[7].

The final group received the combination of HDFO and LA in addition to HCD.The experimental protocol was carried out for 2 months. Experiments were conducted according to the guidelines of institutional animal ethical committee of College of Pharmacy, Al–Azhar University, Nasr City, Cairo, Egypt.

Samples collection

At the end of experiment, all rats were allowed to fast for 12 hours, and sacrificed under ether anesthesia. Blood samples were drown by cardiac puncture and collected into centrifuge tubes and left to stand at room temperature for 10 minutes, then centrifuged at 3000 rpm for 10 minutes. The isolated serum samples were stored at–20°C until analysis.

The homogenate of liver tissue was prepared on ice in a ratio of 1 gm wet tissue for 9 ml phosphate buffer (50 mM, pH 7). The first part was used for the assessment of MDA while the second part was mixed with an equal volume of ice–cold meta–phosphoric acid (12% w/v), incubated for 30 min on ice, and centrifuged for 10 min at 5000 rpm at 4°C and supernatants were used for determination of GSH. The third part was centrifuged at 5000 rpm for 10 min at 4°C, and supernatant was collected and stored at –80°C until analysis of GPx activities and protein oxidation[19].

Biochemical analysis

Serum level of Serum total cholesterol (TC), high density lipoprotein cholesterol (HDL–C), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL–C) and serum uric acid were determined using a commercially available kit (Biocon DiagnostiK, Germany).

Liver lipids were extracted by a modified Folch method Folch *et al.*,[20]. One gram of liver tissue was homogenized for 5 min in 6ml of Folch solution [chloroform (2): methanol (1)] and 2ml H2O. After centrifugation for 10 min, the lower phase that contains liver lipids was separated. Lower phase of lipid fractions was assayed after treating with triton X-100: chloroform (25 µl: 475 µl) for total cholesterol or with methanol for triglyceride, using enzymatic kits.

Serum PCO levels was measured by using previously described method[21] while MDA measured by the method of Draper and Hadley[22]. Liver homogenate was use for determination of PCO[23], protein thiols (PSH)[24], GSH[25], MDA[26], GPx[27], and total proteins[28].

Statistical analysis

Results were expressed as means ± standard deviation (SD) and were analyzed for statistically significant differences using one–way analysis of variance (ANOVA) followed by the Tukey–Kramer post analysis test to compare all groups. *P* values less than 0.05 were considered significant. GraphPad Prism® was used for statistical calculations (Version 5.00 for Windows, GraphPad Software, San Diego California USA).

RESULTS

"Body weight and liver weight gain" of the rats fed with HCD ($P < 0.001$) as well as rats received LDFO ($P < 0.05$), HDFO ($P < 0.01$) or LA ($P < 0.05$) with HCD was significantly increased as compared with the rats that fed with normal diet. No statistically significant changes were noted in group received HDFO plus LA in comparison with those fed with normal diet (Figure 1a and b).

TABLE 1 displays the results of TC, HDL-C, TAG, LDL-C and uric in the serum of the investigated groups. Feeding of the rats with HCD was associated with a significant increase of serum TC level by about 131% in comparison with the rats received normal dietary chow. On another side the administration of either FO or LA alone or in combination causes marked reduction of serum TC by about 39.6%, 42% and 51% respectively regarding to HCD fed rats. The results also, revealed that HCD induce a marked reduction of serum HDL–C level in comparison with the rats that supple-

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mented with normal diet by 47%. The treatments of rats with HDFO plus LA preserve HDL-C level at values similar to that of control ones.

HCD administration revealed 47% elevation of se-

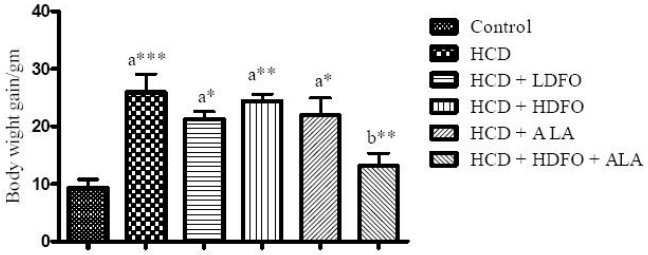

Figure1a : Effect of high cholesterol diet, low dose of flaxseed oil, high dose of flaxseed oil and alpha lipoic acid on body weight gain by (gm)

Values were expressed as mean ± SD, (N= 6). a: Significantly increased from control group. b: Significantly decreased from HCD group. *: P < 0.05, **: P < 0.01, ***: P < 0.001. Abbreviations: HCD, high cholesterol diet; LDFO, low dose of flaxseed oil; HDFO, high dose of flaxseed oil; ALA, alpha lipoic acid.

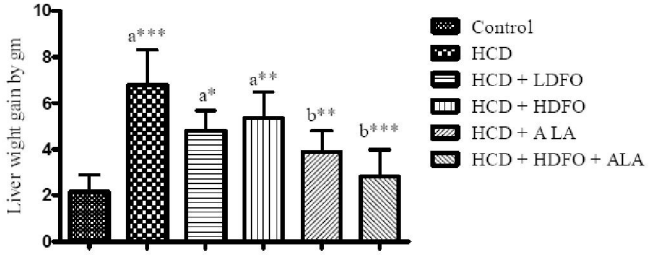

Figure1b : Effect of high cholesterol diet, low dose of flaxseed oil, high dose of flaxseed oil and alpha lipoic acid on liver weight gain by (gm)

Values were expressed as mean ± SD, (N= 6). a: Significantly increased from control group, b: Significantly decreased from HCD group *: P < 0.05, **: P < 0.01, ***: P < 0.001. Abbreviations: HCD, high cholesterol diet; LDFO, low dose of flaxseed oil; HDFO, high dose of flaxseed oil; ALA, alpha lipoic acid.

rum TAG as compared with control rats. On the contrary, administration of FO and LA alone or in combination were significantly decreased the serum level of TAG by about 31% than HCD. In respect to LDL-C level, rats received HCD have high-level of LDL-C than that of control by 86%. Conversely supplementations of our tested substances alone or in blend to rats received HCD causes reduction of LDL–C level in comparison with cholesterol rich diet feeding by 58%, 56 % and 76 % respectively. Additionally, feeding of rats with HCD produces 175% increase of serum uric acid in comparison with normal diet feeding. In contrast, treatment with FO was significantly reducing uric acid level by 44%, LA 47% and 62% for blend of FO and LA as compared with HCD. Our results revealed that there is no significant different between supplementation of FO either at LDFO or HDFO on the lipids profile.

Concerning to liver PSH, the present results re-

TABLE 1 : Serum levels of total cholesterol, high density lipoprotein cholesterol, triacylglycerol, low density lipoprotein cholesterol, uric acid, and malondialdehyde and protein carbonyl in rats received normal diet, high cholesterol diet or high cholesterol diet plus low dose of flaxseed oil, high dose of flaxseed oil and alpha lipoic acid

Markers	Control	HCD	Treated groups			
			LDFO	HDFO	LA	HDFO + LA
TC	55.6±4.16	127±13.8^{a}***	77.9±7.29^{a,b}***	82.3±7.27^{a,b}***	80.1±7.25^{a,b}***	67.9±5.82^{b}***
HDL-C	36.8 ± 4.70	19.4± 3.98^{a}**	36.1 ±10.7 ^{b}*	33.6 ± 12.3^{b}*	29.2 ± 8.63	37.6 ± 7.02^{b}**
TAG	61.4 ± 11.7	117± 10.1^{a}***	76.2 ± 29.8 ^{b}*	81.8 ± 30.5^{b}*	82.0 ± 16.9^{b}*	67.5 ± 15.51^{b}**
LDL-C	6.55 ± 2.37	32.5± 12.2^{a}***	13.9 ±5.41^{b} ***	20.6 ± 5.81^{b}***	18.1 ± 6.01$^{a, b}$***	11.85 ± 6.95^{b}***
UA	2.65 ± 0.94	7.32 ± 1.41^{a}***	3.42 ± 0.59^{b}***	4.13± 0.82^{b}***	3.85 ± 1.06^{b}***	2.79 ± 1.03^{b}***
MDA	0.61 ± 0.09	1.88± 0.62^{a}***	1..02 ±0.35 ^{b}*	1.83 ±0 .81^{b}***	0.97 ± 0.20^{b}*	0.77 ± 0.13^{b}**
PCO	0.56 ± 0.22	2.12± 0.91^{a}***	1.05 ±0.37 ^{b}*	1.59 ± 0.63^{a}*	0.93 ± 0.37^{b}**	0.51 ± 0.26^{b}***

Values were expressed as mean ± S.D, N = 6. Units: TC, HDL–C, TAG, LDL–C and uric acid were expressed as mg/dl, while MDA µ mol/L and PCO nmol/mg protein. a: Significantly different from control group, b: Significantly different from HC group. *: P < 0.05, **: P < 0.01, ***: P < 0.001. Abbreviations: TC; total cholesterol, HDL–C; high density lipoprotein cholesterol, TAG; triacylglycerol, LDL–C; low density lipoprotein cholesterol, uric acid, MDA; malondialdehyde and PCO; protein carbonyl, HCD; high cholesterol diet, LDFO; low dose of flaxseed oil, HDFO; high dose of flaxseed oil, ALA; alpha lipoic acid.

vealed that this parameter was significantly decreased by cholesterol feeding versus control animal ($P < 0.001$); furthermore rats received HDFO have low level of PSH. Also, there is significant difference of PSH between rats received HDFO and LDFO ($P < 0.05$). Treatment of animals with LA alone or in combination with HDFO maintains PSH when compared with HCD rats. Moreover, our results showing that HCD as well

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as consumption of HDFO beside HCD causes marked reduction of GSH level in liver tissues ($P < 0.001$, $P < 0.05$) compared with normal rats. Administration of FO at high dose cause greater reduction of GSH level than consumption of FO at low dose ($P < 0.05$). Supplementation of rats with LDFO, LA or LA with HDFO treatment maintains the GSH level at value similar that of control rats. Moreover, our finding revealed that there is significant elevation of GPx activity by HCD feeding ($P < 0.001$) as well as in group treated with HDFO compared with the normal group ($P < 0.05$). GPx activity is maintained either, by utilization of LDFO, LA alone or LA plus HDFO when compared with HCD ($P < 0.001$). TABLE 2 shows the statistical data of these parameters.

Figure 3 depicts the statistical data of hepatic MDA content which showed that liver MDA markedly elevated by feeding of HCD alone or in combination with HDFO compared with normal diet feeding ($P < 0.001$). The rats treated with LA or HDFO plus LA have MDA level similar to that of the control. Our observation indicated that there is significant difference between liver MDA level by administration of FO at low dose and at high dose ($P < 0.001$). Additionally, in rats received HCD as well as rats treated with HDFO; the serum level of this parameter was significantly increased comparison with normal control rats. HDFO plus LA exert noticeable reduction of MDA level by 142% in comparison to HCD (TABLE 1).

In comparison with rats feeding of normal dietary chow, administration of HCD caused significant elevation of PCO level in both hepatic tissues and serum (P

TABLE 2 : Liver tissue levels of total lipids, total cholesterol, triacylglycerol, protein thiols, glutathione, and glutathione peroxidase in rats received in rats received normal diet, high cholesterol diet or high cholesterol diet plus low dose of flaxseed oil, high dose of flaxseed oil and alpha lipoic acid

Markers	Control	HCD	Groups of rats received HCD with			
			LDFO	HDFO	LA	HDFO +LA
TL	42.5 ± 11.9	94.5±12.2^{a***}	53.0 ± 9.57^{b***}	56.6±13.5^{b***}	51.7±14.0^{b***}	44.9±14.3^{b***}
TC	6.55±2.15	32.2±6.49^{a***}	14.3±2.68^{a*,b***}	18.0±4.33^{a***,b***}	14.8±2.97^{a*,b***}	10.1±3.17^{b***}
TAG	4.47±1.33	17.8±3.47^{a***}	8.15±2.33^{b***}	9.11±2.73^{a*,b***}	8.02±1.57^{b***}	5.97±1.6^{b***}
PSH	105 ± 9.95	56.3 ± 13.5***	87.0±12.4^{b***}	63.4 ± 11.8^{a**,b*}	91.3 ± 13.3^{b***}	104 ± 9.97^{b***}
GSH	12.1 ± 2.61	5.12 ± 1.81^{a**}	10.9±2.76	6.16± 2.77^{a**}	10.7 ± 3.06^{b**}	12.7 ± 2.25^{b***}
GPx	2.34 ± 0.39	5.20 ±1.59^{a***}	3.44±0.95^{b*}	4.14± 1.01^{a*}	2.54 ± 0.73^{b***}	2.57± 0.60^{b***}

Values were expressed as mean ± S.D, N = 6. Units: TL, TC, TAG were express as mg/g of tissues, where PSH nmol/mg protein, GSH µ mol/gm of liver homogenate and GPx U/mg protein. a: Significantly different from control group, b: Significantly different from HCD group .*: P < 0.05,: P < 0.01, ***: P < 0.001. Abbreviations: TL; total lipids, TC; total cholesterol, TAG; triacylglycerol, HCD; high cholesterol diet, LDFO; low dose of flaxseed oil, HDFO, high dose of flaxseed oil, ALA; alpha lipoic acid.**

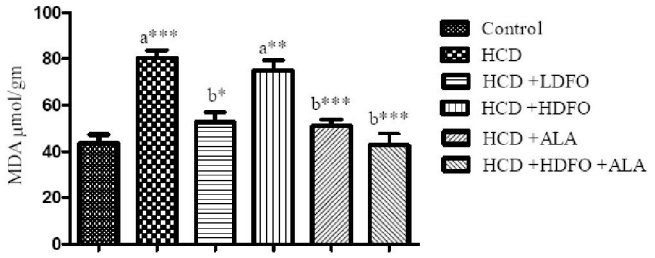

Figure 3 : Effect of high cholesterol diet, low dose of flaxseed oil, high dose of flaxseed oil and alpha lipoic acid on hepatic malondialdehyde level µ mol/gm of liver tissues

Values were expressed as mean ± SD, (N= 6). a: Significantly increased from control group, b: Significantly decreased from HCD group .*: P < 0.05, **: P < 0.01, ***: P < 0.001. Abbreviations: HCD, high cholesterol diet; LDFO, low dose of flaxseed oil; HDFO, high dose of flaxseed oil; ALA, alpha lipoic acid, MDA; malondialdehyde.

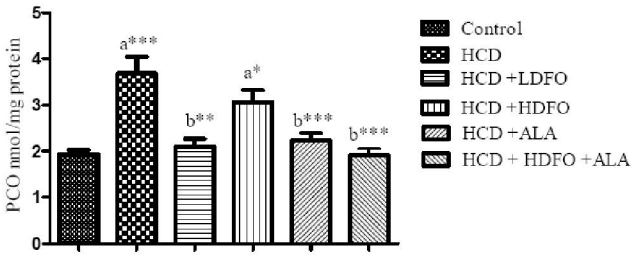

Figure 4 : Effect of effect of high cholesterol diet, low dose of flaxseed oil, high dose of flaxseed oil and alpha lipoic acid on hepatic protein carbonyl content nmol/mg protein

Values were expressed as mean ± SD, (N= 6). a: Significantly increase from control group, b: Significantly decreased from HCD group .*: P < 0.05, ***: P < 0.001. Abbreviations: HCD, high cholesterol diet; LDFO, low dose of flaxseed oil; HDFO, high dose of flaxseed oil; ALA, alpha lipoic acid, PCO; protein carbonyl.

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< 0.001); likewise rats supplemented with HDFO in addition to HCD still have high PCO level ($P < 0.05$). In contrary LA administration with HCD significantly attenuated PCO level compared with HCD feeding alone ($P < 0.01$). Group received HDFO has significantly high level of liver PCO when compared with group of animals received LDFO ($P < 0.05$). Moreover the treatment of rats with HDFO plus LA preserves PCO level at values similar to that of controls one. Serum PCO is represented in (TABLE 1) while Figure 4 shows the hepatic PCO content.

DISCUSSION

Previous study reported that consumption of large amounts of PUFAs can reduce antioxidant protection, leading to increased peroxidation of lipoproteins which may be result in undesirable effects[29]. Therefore, this study was conducted to assess the effect of large intake of FO on lipids profile in addition to some biomarkers of oxidative stress in rats fed on HCD in presence or absence of LA as lipophilic and hydrophilic antioxidants.

Dyslipidemia induced in our rat's model received HCD due to the presence of cholic acid as one component of HCD which inhibits the 7 α-hydroxylase activity, the key enzyme required for the conversion of cholesterol into bile acids. Moreover HC itself is associated with oxidative stress which increases the activity of hydroxyl-methyl-glutaryl-CoA reductase, key enzyme, in cholesterol biosynthesis[30]. The hypolipidemic effect of FO is due to the presence of high content of PUFAs particularly alpha linolenic acid which plays an important role in metabolism of TAG as well as reverse transport of cholesterol to the liver to be metabolized into bile acids[18]. Lipids lowering effect of LA is attributed to preservation the activity lipids metabolizing enzymes through antioxidant effect[31].

HC is associated with increase of xanthine oxidase activity; thereby provoke superoxide production[32]. In the present study activity of xanthine oxidase was indicated by estimation of serum uric acid level. Activation of xanthine oxidase as a result of increased ROS production by HCD feeding is the possible cause for increasing the uric acid in the serum of rats receiving this diet. In contrast the LA has uric acid lowering effect due to its beneficial role in the restoration of normal activity of xanthine oxidase by ROS scavenging effect[7]. The hypolipidemic effect of FO may be responsible for restoration of the normal xanthine oxidase activity, therefore normalize the serum uric acid. In addition, the supplementation with FO plus LA gives greater reduction of lipids profile as well as uric acid when compared with HCD supplementation.

GPx is an oxidative stress inducible enzyme; the increase in the activity of this enzyme to counteract the increased oxidative stress. GPx provides an effective protection by utilizing GSH in the degradation of peroxides[33]. In our findings, the increase of GPx activity by HCD feeding may be defense mechanism against increased free radicals production. However GSH is the substrate of GPx, the GSH level was decreased due to the increased of GPx activity. These findings, are similar to the observation of previous studies suggested that increase of GPx activity in different forms of oxidative stress[34]. It has been reported that, antioxidants supplementation inhibits superoxide production along with retaining the normal antioxidant status[35]. The restoration of GPx activity and GSH level by LA treatment is due to either free radicals scavenging ability or by maintaining the level of endogenous antioxidants.

Conversely, the GPx activity is still high and GSH status is low in rats received high dose FO beside HCD. This may be due to the consumption of GSH in protection against ROS produced by HCD or utilization of GSH to minimize the peroxidation of PUFAs present in FO. Furthermore, another study reported that ingestion of PUFAs rich oils without sufficient antioxidants is associated with a decrease in the level of endogenous antioxidants[36]. Additionally alpha linolenic acid present in FO may be act as substrate for lipooxygenase which consume the GSH in biosynthesis of leukotrienes.

The decrease of GSH level by HCD makes the lipids more target for ROS attack; moreover, superoxide can be reacted with nitric oxide to form peroxynitrite which propagate the lipids peroxidation[37]. HCD feeding is associated with significant increase of both serum and liver MDA level in comparison with rats received normal diet. However, LA is water and fat soluble antioxidant makes it highly effective in reducing MDA level in both aqueous and fatty phases[38]. On the other hand rats treated with high dose of FO still have high level of

MDA. These finding are in agreement with several study reported that administration of PUFAs diet rich is associated with increased of MDA level[39,40]. Moreover MDA is formed as by product during the production of eicosanoids from PUFAs.

The protein carbonyl content in serum and liver of HCD fed animals was found to increase while, PSH content in liver tissues were markedly decreased compared with normal rats. These findings are in agreement with the previous study demonstrated that there is an increase of protein oxidation in response to oxidative stress[41]. GSH is restoring the damage molecules through hydrogen donation, reduction of peroxide formation in addition to preservation of protein thiols[42]. The decline of GSH level is the possible cause for increased protein oxidation as a result of HCD feeding. Treatment with LA restores PSH levels and attenuates PCO formation in rats compared with HC animals. As we mentioned before, LA increases GSH level either by maintaining of level or by increasing the cellular uptake of the substrates for GSH biosynthesis[15]. This clearly indicates that the treatment with LA attenuates the oxidative stress, by the prevention of oxidative damage of protein which involved in cellular damages under the effect of HC.

CONCLUSION

Although FO administration improves lipids abnormality that caused by feeding of HCD, the large intake of FO has deleterious effects on GSH and protein thiols levels thus increase the propensity of lipids and protein oxidation. LA normalizes the elevated oxidative stress biomarker (GPx, MDA, PCO and uric acid) in addition to lipids lowering effects. It is suggested that supplementation of antioxidants like LA is necessary during the lipids lowering therapy especially with oils rich in PUFAs.

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REFERENCES

[1] D.B.Jump; J.Biol.Chem., **277**, 8755 **(2002)**.
[2] K.Arab, A.Rossary, L.Souleere, J.P.Steghens; Br.J.Nutr., **96(5)**, 811 **(2006)**.
[3] P.C.Calder, G.L.Jensen, B.V.Koletzko, P.Singer, G.J.Wanten; Intensive Care Med., **36(5)**, 735 **(2010)**.
[4] H.D.Ramachandran, R.Kusum, P.L.Raina; I.J.E.B, **44**, 32 **(2006)**.
[5] T.Holm, R.K.Berge, T.Uel; J.Coll.Cardiol., **37**, 279A **(2001)**.
[6] K.Prasad; Drug News Perspect., **13(2)**, 99 **(2000)**.
[7] A.K.Sekar, S.Varatharajan, V.Palaninathan; Prostaglandins Leukot.Essent.Fatty Acids, **75**, 25 **(2006)**.
[8] R.M.Nageswara, V.Aleksandr, S.R.Marschall; Arterioscler.Thromb.Vasc.Biol., **25**, 29 **(2005)**.
[9] R.Asai, N.Tomoko, M.Tomohira, O.Ken, I.Kiyohiko, F.Emil, N.Takeshi; J.Biochem., **141(4)**, 525 **(2007)**.
[10] M.Shouji, K.Shintaro, I.Tomomi, M.Hidenori, I.Naoki, O.Yukihiro, Y.Takashi, S.Kenji, T.Hiroyuki; Am.J.Physiol.Heart Circ.Physiol., **291**, H2237 **(2006)**.
[11] R.T.Dean, S.Fu, R.Stocker; Biochemical J., **324**, 1 **(1997)**.
[12] U.Cakatay, R.Kayali; Biogerontology, **6**, 87 **(2005)**.
[13] T.Shiomi, H.Tsutsui, H.Matsusaka, K.Murakami, S.Hayashidani, M.Ikeuchi, J.Wen, T.Kubota, H.Utsumi, A.Takeshita; Circulation, **109**, 544 **(2004)**.
[14] M.C.Toufektsian, M.de Lorgeril, N.Nagy, P.Salen, M.B.Donati, L.Giordano; J.Nutr., **138**, 747 **(2008)**.
[15] H.S.Jung, T.S.Eric, D.M.Jason, D.M.C.Brian, E.R.Alma, E.R.F.Balz, M.H.Tory; FASEB J., **15**, 700 **(2001)**.
[16] A.E.Midaoui, A.Elimadi, L.Wu, P.S.Haddad, J.de Champlain; Am.J.Hypert., **16(3)**, 173-179 **(2003)**.
[17] P.R.Deepa, P.Varalakshmi; Intern.J.Cardiol., **106**, 338 **(2006)**.
[18] K.Vijaimohan, J.Mallika, K.E.Sabitha, S.Subramaniyam, C.Anandhan, C.S.Shyamala Devi; Life Sciences, **79**, 448 **(2006)**.
[19] C.Feillet-Coudray, T.Sutra, G.Fouret, J.Ramos, C.Wrutniak-Cabello, G.Cabello, G.Cristol, C.Coudray; Free Rad.Biol.Med., **46**, 624 **(2009)**.
[20] J.Folch, M.Lee, G.H.Sloane Stanley; J.Biol.Chem.,

Regular Paper

226, 497 **(1957)**.
[21] A.Z.Reznick, L.Packer; Methods Enzymol., **233**, 357 **(1994)**.
[22] H.H.Draper, M.Hadley; Methods Enzymol., **186**, 421 **(1990)**.
[23] L.Levine, J.Williams, R.Stadtman, E.Shacter; Methods Enzymol., **233**, 346 **(1994)**.
[24] J.Koster, P.Biemond, A.Swaak; Ann.Rheum.Dis., **45(1)**, 44 **(1986)**.
[25] G.Ellman; Arch.Biochem.Biophys., **82**, 70 **(1959)**.
[26] C.Fraga, B.Leibovitz, Tappel; Free Rad.Biol.Med., **4**, 155 **(1988)**.
[27] D.E.Paglia, W.N.Valantine; J.Lab.Clin.Med., **70**, 158 **(1967)**.
[28] O.Lowery, N.Rosenbrough, R.Randall; J.Biol. Chem., **193(1)**, 265 **(1951)**.
[29] L.Meilin, W.Agneta, W.Rolf, S.Tom; Nutr.Res., **23**, 1027 **(2003)**.
[30] L.Gesquiere, N.Loreau, A.Minnich, J.Davignon, D.Blache; Free Rad.Biol.Med., **27**, 134 **(1999)**.
[31] K.Malarkodi, P.Varalakshmi; Nutr.Res., **23**, 539 **(2003)**.
[32] C.White, D.Parks, D.R.Patel, J.Shelton, M.Tarpy, B.Freeman, V.Darley-Usmar; FEBS Lett., **561**, 94 **(2004)**.
[33] K.M.Surapaneni, V.Vishnu Priya; J.Clinic Diagnostic Research, **3**, 827 **(2008)**.
[34] S.Dahingra, U.Singh, M.P.Bansal; Indian J.Biochem. Biophys., **40**, 260 **(2003)**.
[35] X.Chen, R.Touyz, J.Park, E.Schiffrin; Hypert., **38(2)**, 606 **(2001)**.
[36] O.Johansen, I.Seljeflot, A.T.Hostmark, H.Arnesen; Arterioscler.Thromb.Vasc.Biol., **19**, 1681 **(1999)**.
[37] Y.Ohara, T.E.Peterson, D.G.Harrison; J.Clin.Invest., **91**, 2546 **(1993)**.
[38] G.P.Biewenga, G.R.Haenen, A.Bast; Gen.Pharmacol., **29**, 315 **(1997)**.
[39] P.Loris, R.Enrico, Z.Carola, S.Lucia, I.Elena, B.Andrea, M.Alessandra, P.Sandro, T.Maurizio, P.Arturo, M.Mario; Am.J.Clini.Nutr., **68**, 888 **(1998)**.
[40] J.K.Min, S.S.Myoung, N.P.Jung, S.L.Sang; British J.Nutr., **94**, 526 **(2005)**.
[41] O.Gul, K.Selda, O.Yusuf, A.Gulcin, U.Mujdat; Clin.Biochem., **34**, 335 **(2001)**.
[42] L.N.Grinberg, E.A.Rachmilewitz, H.Newmark; Biochem.Pharmacol., **48**, 643 **(1994)**.