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Activity of serum paraoxonase 1, lipid profile and atherogenic indexes in diabetic induced rats treated with alpha lipoic acid

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ABSTRACT

Background: Paraoxonase 1 (PON1) is an enzyme attached to high density lipoprotein (HDL-C) which has antioxidant and anti-atherogenic activities.
Objectives: In this study, we investigated the effects of alpha lipoic acid on PON1 activity, lipid profile and atherogenic index as well as correlation between PON1 activities and HDL-C in diabetic rats.
Materials and Methods: Thirty adult male rats were distributed in three experimental groups in this study. Control (group I), diabetic (group II) and diabetic animals treated with alpha lipoic acid (group III) group. Diabetes mellitus was induced in rats in groups II and III by a single dose of alloxan monohydrate (100 mg/kg; subcutaneous) and then treatment was performed with administration of alpha lipoic acid (100 mg/kg intraperitoneally) in group III for 6 weeks. Blood samples were collected from animals to measure the levels of triglyceride (TG), total cholesterol (TC), low density lipoprotein (LDL-C), HDL-C, PON1 activity and correlation the between HDL-C and atherogenic index by PON1.
Results: Statistical analysis showed that alpha lipoic acid significantly ($P<0.05$) inhibited the increase of TG, TC, LDL-C, VLDL, atherogenic index, atherogenic coefficient (AC), and cardiac risk ratio (CRR) when compared to diabetic rats in group II. HDL-C level was increased by alpha lipoic acid. The activity of PON1 was significantly ($P<0.05$) decreased in diabetic rats and treatment with alpha lipoic acid increased the PON1 activity. Moreover, the activity of PON1 correlated positively with HDL-C and negatively with AC, CRP 1 and CRP2.
Conclusions: This study demonstrated that the administration of alpha lipoic acid can improve PON1 activity, lipid metabolism, atherogenic index and is able to reduce the risk of coronary artery diseases and atherosclerosis in diabetic rats.

Original Article

Implication for health policy/practice/research/medical education:
Alpha-lipoic acid is a natural antioxidant that effective on improving of paraoxonase 1 activity, lipid profile and atherogenic index in diabetic rats.

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1. Background

Hyperlipidemia is a key risk factor in cardiovascular diseases caused by diabetes. Diabetes increases the levels of total cholesterol (TC), triglyceride (TG), and low density lipoprotein (LDL-C) and decreases the high density lipoprotein (HDL-C) level which can cause the formation of small dense LDL-C particles with high potential for oxidation (1). Therefore, it increases the ability of HDL-C to protect against lipid oxidation (2). Since the PON1 is correlated to HDL-C, under the condition of diabetes it/HDL-C can reduce the level of the antioxidant enzyme. The process that is postulated to

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play a central role in endothelial cell damage (3). Hence diabetes is involved in the elevation of microvascular and macrovascular abnormalities in coronary heart diseases (1,3).

Human serum paraoxonase 1 (aryl dialkyl phosphatase EC 3.1.8.1) has serum esterase activity in the liver, kidney and small intestine. High amount of this enzyme in the serum is contained in HDL-C particles. Paraoxonase 1 (PON1) is firmly connected with N-terminal domain of apolipoprotein A1 in HDL-C and very low in other lipoprotein such as chylomicrons and VLDL (4). PON1 does the enzymatic hydrolysis of organophosphorus compounds such as oxidized cholesteryl esters and phosphatidylcholine core aldehydes (5). It is postulated that PON1 has two active sites: one for hydrolysis of oxygen analog and anti-atherogenic enzyme that is known to attenuate the formation of plaques. The antiatherogenic of ability PON1 causes protection of lipoprotein particle against oxidation- free radicals because of the hydrolysis of the cholesteryl ester and steroid nucleus phosphatidylcholine (6,7).

Different studies have shown that PON1 and HDL-C prevent oxidative LDL-C *in vitro* and *in vivo*. Oxidized forms of LDL aggregate in the subendothelial space of arteria starting the initial steps of atherosclerosis (8). Thus, there is inverse relationship between oxidized LDL-C and PON 1 activity. Hence, decreasing levels of PON 1 activity in people with high risk of developing atherosclerosis have been detectable (8). PON1 activity, inhibits the lipid oxidation on macrophage and erythrocyte and increase the expression of monocyte chemotactic protein 1 (MCP-1) due to the induction of inflammatory responses in endothelial cells preventing the complication of atherosclerosis (9). PON 1, Hydrolyze hydrogen peroxide as a major active oxygen radical and thereby is effective in reduction of oxidative stress (10). Alpha lipoic acid is a supplement with high antioxidant activity that contracts in mitochondria membrane as a main co-factor. Previous studies have proven alpha lipoic acid antioxidant effects on improving diabetic nephropathy and neurologic diseases (11). Thus, alpha lipoic acid is an antioxidant compound with antidiabetic properties that improves diabetes complications such as hyperlipidemia and atherosclerosis (12).

2. Objectives

The aim of current study is to investigate the effects of alpha lipoic acid on PON1 activity, lipid profile, atherogenic index and correlation between HDL-C and atherogenic index in alloxan monohydrate-induced diabetic rats.

3. Materials and Methods

3.1. Experimental design

The study was carried out on thirty adult male Sprague Dawley rats (180±20 g). The rats were purchased from the animal house of the Shahid Beheshti Medical University, Tehran, Iran. The animals were kept for 1 week in animals laboratory to adapt themselves with conditions. established standard laboratory conditions (temperature of 22°C and humidity of 50±10%), and 12-hour light/dark cycles. The animals were randomly divided into three groups of tens. control (group I), non-treated diabetics (group II) and diabetic rats treated with alpha lipoic acid (group III) groups. Diabetes was inducted with injection of 100 mg/kg alloxan monohydrate (SC) for animals in II and III groups (Sigma, St. Louis, MO, USA) (13). FBS was measured after three days. The animals with glucose level of ≥250 mg/dL were recognized diabetics and separated for the study (9). Alpha lipoic acid powder (Sigma, St. Louis, MO) 100 mg/kg dissolved in 0.9% NaCl and ethanol (96%) was injected to animals in group III for 6 weeks daily (14). then the animals were anesthetized with ketamine (87 mg/kg intraperitoneally) and xylazine (13 mg/kg intraperitoneally) and blood samples were obtained from animals in all groups. The blood samples were placed in laboratory temperature for 20 minute so as to clot and then centrifuged at 3000 rpm for 15 minutes for serum separation.

3.2. Determination of PON1 activity

PON1 activity in serum was determined with spectrophotometry at 412 nm according to our previous study (7) with some modifications. Determination of PON1 activity was made using paraoxon. The activity was measured by adding 10 µL of serum to 1 mL Tris– HCl buffer (100 mM at pH 8) containing 2 mM CaCl2 and 5 mM of paraoxon. The production of 4-nitrophenol and detected with spectrophotometer at 412 nm (Caution XS-D5520M, France) after 3 minutes. PON1 activity was expressed with unit/mg-pr.

3.3. Determination of lipid profile and atherogenic index

The serum levels of fasting blood sugar (FBS), TG, TC, low-density lipoprotein (LDL-C), very low-density lipoprotein (VLDL), HDL-C, and atherogenic index were measured. Concentrations of cholesterol and TG were measured by biochemical analyzer (Olympus AU-600, Tokyo, Japan). using commercial kits ((Pars Azmoon Co., Tehran, Iran). HDL-C was analyzed by a Pars Azmoon kit (Pars Azmoon Co., Tehran, Iran). LDL and VLDL were calculated by using Friedewald et al equation (15) with some modifications. The atherogenic

index ((unite) [log TG/HDL-C]), atherogenic coefficient (AC) (TC-HDL-C/HDL-C), cardiac risk ratio 1 (CRR 1): (TC/HDL-C) and cardiac risk ratio 2 (CRR 2): (LDL/HDL-C) were calculated by using the Ikewuchi and Ikewuchi equation (16) with some modifications.

3.4. Ethical approval

All of the experimental protocols were conducted in accordance with the manuals of the Animal Ethics Committee of the Lorestan University of Medical Sciences. Additionally, all experimental protocols and steps of the tests were conducted in compliance with the regulations of the Research Ethics Committee of our university and Iranian Ethical Guidelines for the use of animals in research. Additionally All animal experiments were in accordance with protocols approved by the United States National Institutes of Health (NIH, 1978). This research was supported by Lorestan University of Medical Sciences (Grant# 21/91).

3.5. Statistical analysis

All values are expressed as mean± SD. The data were compared between groups by Mann–Whitney U test. Statistical analysis was conducted using the SPSS 22 (Chicago, IL) for windows software. A *P* value of ≤0.05 was considered statistically significant.

4. Results

4.1. The effects of alpha lipoic acid on FBG and lipid profile

The levels of FBS and lipid profile are shown in Table 1. The level of serum FBS was significantly ($P<0.05$) increased in non-treated diabetic group compared to control group. Treatment with alpha lipoic acid significantly ($P<0.05$) inhibited the increase of FBS (39.31%) in treated diabetic group compared to untreated diabetic group. The levels of TC, TG, VLDL and LDL-C were significantly ($P<0.05$) increased in non-treated diabetic group compared to control group. Treatment with alpha lipoic acid significantly ($P<0.05$) inhibited the increase of TC, TG, VLDL and LDL-C in treated diabetic group with alpha lipoic acid compared to diabetic group. The level of HDL-C was significantly ($P<0.05$) decreased in non-treated diabetic group compared to control group. Treatment with alpha lipoic acid significantly ($P<0.05$) increased HDL-C in treated diabetic group compared to diabetic group.

4.2. The effects of alpha lipoic acid on atherogenic index, AC and CRR

The levels of atherogenic index, ACand CRR are shown in Table 1. The level of atherogenic index ([unite] [log TG/HDL-C]) and AC (TC-HDL-C/HDL-C) were significantly ($P<0.05$) increased in non-treated diabetic group compared to control group. Treatment with alpha lipoic acid significantly ($P<0.05$) inhibited the increase of atherogenic index and AC in diabetic group treated with alpha lipoic acid compared to diabetic group. The levels of CRR1 (TC/HDL-C) and CRR2 (LDL/HDL-C) were significantly ($P<0.05$) increased in non-treated diabetic group compared to control group. Treatment with alpha lipoic acid significantly ($P<0.05$) inhibited the increase of CRR1 and CRR2 levels in diabetic group treated with

Table 1. Effect of alpha lipoic acid on TC, TG, LDL, HDL-C, VLDL atherogenic index, AC, CRR1, CRR2 and PON1 activity in diabetic rats

Parameter	Control	Non-treated diabetic	Lipoic acid-treated diabetic	P value
FBS (mg/dL)	179.16±15.95	402±43.1*	244±19.07#	0.02
TG (mg/dL)	61±2	81±6*	71±2#	0.001
TC (mg/dL)	104.4±5	149.6±8*	124.4±4#	0.001
HDL-C (mg/dL)	48.6±2.07	39.02±1*	44.72±1#	0.005
LDL (mg/dL)	43.5±6	94.2±10.2*	65.3±4#	0.003
VLDL (mg/dL)	12.28±0.00	16.36±1*	14.45±0.00#	0.009
Atherogenic index ([unite][log TG/HDL-C])	0.101±0.02	0.0318±0.02*	0.0203±0.02#	0.002
CRR1 (TC/HDL-C)	2.12±0.06	3.84±0.00*	2.78±0.36#	0.02
CRR2 (LDL/HDL-C)	0.933±0.00	2.42±0.00*	1.43±0.08#	0.003
AC(TC-HDL-C/HDL-C)	1.14±0.6	2.84±0.00*	1.77±0.3#	0.007
PON1 activity (unit/mg-protein)	91.94±11.25	48.97±8.88*	85.89±7.12#	0.03

Values are represented as mean ± SD; * Significant change in comparison with Control at $P < 0.050$. # Significant change in comparison with Lipoid acid-treated diabetic at $P < 0.050$; FBG: Fasting blood glucose; TG: Triglyceride; TC: Total cholesterol; HDL-C: High-density lipoprotein cholesterol; LDL: Low-density lipoprotein; VLDL: Very low density lipoprotein; PON1: Paraoxonase 1; AC: Atherogenic coefficient; CRR: Cardiac risk ratio.

alpha lipoic acid compared to untreated diabetic group.

4.3. The effects of alpha lipoic acid on PON 1 activity and correlation with HDL-C, AC and CRR

The activity of PON1 and its correlation with HDL-C, AC and CRR are shown in Table 1 and Figures 1, 2, 3 and 4. activity of PON1 was significantly ($P < 0.05$) decreased in non-treated diabetic group compared to control group. Treatment with alpha lipoic acid significantly ($P<0.05$) increased the activity of PON1 in diabetic group treated with alpha lipoic acid compared to untreated diabetic group. The activity of PON1 was positively associated with HDL-C (r=0.829, $P=0.03$; Figure 1). The activity of PON1 was inversely associated with AC (r= -0.847, $P=0.002$; Figure 2), CRR 1 (r= -0.815, $P=0.02$; Figure 3) and CRR 2 (r= -0.802, $P=0.003$; Figure 4).

5. Discussion

5.1. Effect of alpha lipoic acid on serum level of PON1 activity and correlation between PON 1 activity with HDL-C and atherogenic index

All findings of the present study support a role of PON1 in cardiovascular diseases causes by diabetes. Hyperglycemia, hyperinsulinemia and dyslipidemia are associated with oxidative stress and dysfunctional endothelium (17). Diabetes significantly decreased the serum PON1 activity and HDL-C concentration in comparison to control group. Treatment with alpha lipoic acid inhibited the reduction in PON1 activity and HDL-C concentration compared to non-treated diabetic group. There is strong evidences that show PON1 has beneficial effect against atherosclerosis by protection of HDL-C from peroxidation and plasma membrane from injury thereof. In clinical studies on patients with type 1 diabetes have demonstrated the reduced level of PON1 activity compared to healthy group (15). Different studies have shown that antioxidant therapy is one of the most important treatment strategies for diabetic patients (18). All results of the current study obviously demonstrated that the activity of PON1 correlated positively with HDL-C and negatively with AC, CRR1 and CRR2 in treated diabetic rats. PON1 has an antioxidant enzyme that decreased LDL-oxidation (19). HDL-C can inhibit LDL-C oxidation via the transition of metals and the

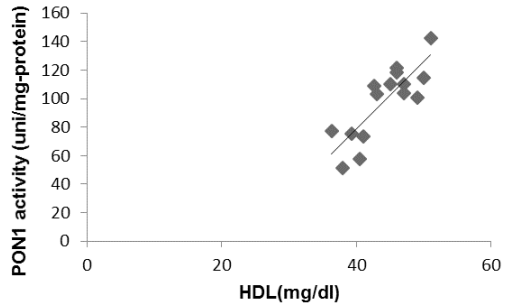

Figure 1. Correlation between serum PON 1 activity and levels of HDL in diabetic rats treated with alpha lipoic acid (r= 0.829, $P= 0.03$)

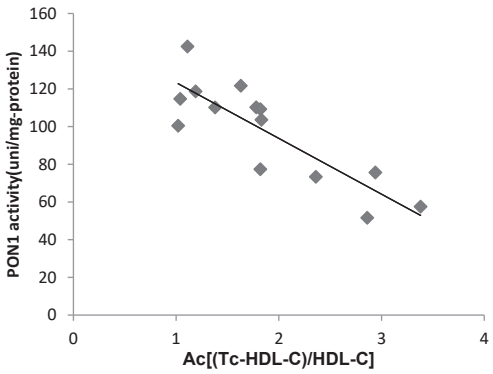

Figure 2. Correlation between serum PON 1 activity and levels of AC [atherogenic coefficient (TC-HDLC/HDLC)] in diabetic rats treated with Alpha lipoic acid (r= - 0.847, $P= 0.002$)

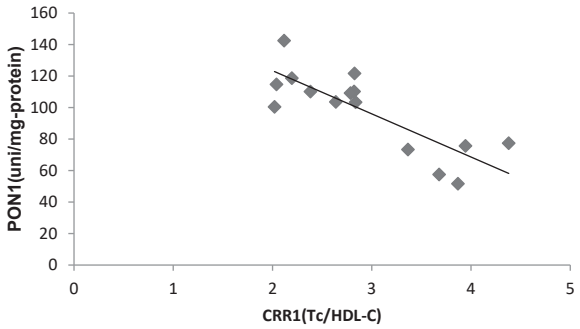

Figure 3. Correlation between maternal serum PON 1 activity and levels of CRR1 (cardiac risk ratio (TC/HDL-C)) in diabetic rats treated with Alpha lipoic acid (r= - 0.815, $P= 0.02$)

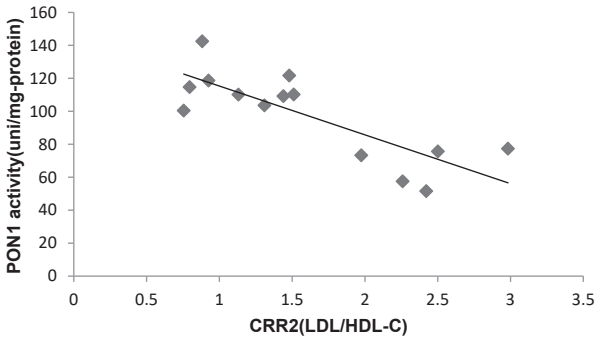

Figure 4. Correlation between maternal serum PON 1 activity and levels of CRR2 (cardiac risk ratio (LDL/HDL-C) in diabetic rats treated with alpha lipoic acid (r= - 0.802, $P = 0.003$)

prevention of lipid hydro peroxides and ester hydro peroxides formation (20,21).

Several study showed HDL-C has anti-atherogenic and anti-inflammatory activities by inhibiting the expression of adhesion molecules on the endothelial cells and the transmigration of monocytes (22). PON1 activity was positivity correlated with HDL-C level. In return, PON1 activity was inversely correlated with atherogenic index. Therefore, it may be suggested that reduced PON 1 activity is probably due to the expenditure of PON1 for the protection of oxidation (10,11). All the results of the present study were in accordance with the previous reports (10,11). Therefore, alpha lipoic acid has an antioxidant properties that may cause an increase in PON1 activity and has a positive correlation with HDL-C and negative correlation with the atherogenic index. Numerous studies have indicated that oxidative stress has an important role in coronary artery diseases, diabetes and complications such as dyslipidemia and hyperinsulinemia (3, 22). Therefore the previous studies and the results of our study demonstrated the efficacy of oxidative supplements in improving such complications. Administration of antioxidants has improving impact on the each stage of diabetes and its complications.

5.2. Effect of alpha lipoic acid on serum lipid profile and atherogenic index

Hyperlipidemia occurs when the amount of lipoproteins in the serum is abnormal. It has been detected that the concentration of cholesterol in HDL-C has inverse relationship while serum TC, TG and LDL-C levels are positively related to atherosclerosis (23). In the current study, there was significantly increased serum FBS, TG, TC, VLDL-C and LDL-C concentrations in diabetic group comparison with the control group. Treatment of diabetic rats with alpha lipoic acid significantly inhibited the increase of serum FBS, TG, TC, VLDL and LDL-C concentrations in comparison with diabetic group. Additionally, atherogenic index, AC, CRP1 and CRP2 as risk factors in cardiovascular diseases, were decreased on diabetic rats when treated with alpha lipoic acid. In addition, various studies on rabbits, indicated that atherogenic index and the levels of LDL-C were increased in diabetes condition when treated with alpha lipoic acid (24, 25). Findings of the recent researches on patients with chronic kidney diseases shown that decreased levels of PON1 activity, were accompanied by the increased levels of lipid profile and atherogenic index that was in accordance with our results (24,25). The HDL-C serum concentration were significantly decreased in diabetic rats in comparison to control. Treatment with alpha lipoic acid increased HDL-C concentration in diabetic rats. Several studies demonstrated increased concentrations of lipid profile in diabetics (24,25). Therefore, antioxidant therapy may be one of the modalities for improvement of lipid and glucose levels. Natural antioxidants such as vitamin E, vitamin C and Q10 show hypoglycemic and hypolipidemic effects in diabetes conditions (7). Other studies have demonstrated beneficial effects of alpha lipoic acid on lipid profile in insulin resistance (24,25). Findings of our study are consistent with other studies. The mechanism by which alpha lipoic acid can decrease TC and LDL-C concentration is unknown. However, it may be conducted through lipoprotein lipase (LPL) activity or through cholesterol metabolism by the liver. Antioxidant effects of alpha lipoic acid to reduce lipid profile in diabetes condition may be due to the ability of alpha lipoic acid to initiate LDL-C receptor synthesis in liver. Thus, alpha lipoic acid in return augments the uptake of cholesterol back to the hepatocyte and raise the synthesis of apolipoprotein that is present in HDL-C particles to inverse cholesterol transport (12,26). Therefore, natural antioxidants such as olive and *Satureja khozestanica* essential oil improve LDL-C oxidation in-vitro and hyperlipidemia, glycation and peroxidation in diabetes condition. In addition, Alpha lipoic acid as a natural antioxidant can have beneficial effects to decrease oxidative stress (27,28).

6. Conclusions

The results of our study showed that alpha lipoic acid has modulatory effects on lipid profile and atherogenic index. Alpha lipoic acid increased PON1 activity and serum HDL-C levels in diabetic rats. Alpha lipoic acid therapy is correlated positively with HDL-C and negatively with AC, CRR1, and CRR2 in rats.

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Authors' contribution

HA designed the project. PJ collected the data. PJ and MB analyzed the data. HA and PJ wrote the manuscript. HA and PJ revised English version. HA edited the final draft. All author signed the manuscript.

Conflicts of interest

The authors declare no conflict of interest.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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References

1. Yatsuya H, Li Y, Hilawe EH, Ota A, Wang C, Chiang C ,et al. Global trend in overweight and obesity and its association with cardiovascular disease incidence. Circ J. 2014;78(12):2807-18.
2. Elsøe S, Ahnström J, Christoffersen C, Hoofnagle AN, Plomgaard P, Heinecke JW, et al. Apolipoprotein M binds oxidized phospholipids and increases the antioxidant effect of HDL. Atherosclerosis. 2012; 221(1): 91-7. doi: 10.1016/j.atherosclerosis.2011.11.031.
3. Patil VP, Patil AB, Patil VS, Ingleshwar DG. Paraoxonase Activity and Lipid Profile in Paediatric Nephrotic Syndrome: A Cross-sectional Study. J Clin Diagn Res. 2016;10(3):BC17-20. doi: 10.7860/JCDR/2016/18524.7440.
4. Zachurzok A, Deja G, Gawlik A, Drosdzol-Cop A, Klimek K, Malecka-Tendera E. Lipid Profile in Adolescent Girls with Type 1 Diabetes Mellitus and Hyperandrogenemia. Int J Endocrinol. 2016: 9473158. doi: 10.1155/2016/9473158.
5. Androutsopoulos VP, Kanavouras K, Tsatsakis AM. Role of paraoxonase 1 (PON1) in organophosphate metabolism: implications in neurodegenerative diseases. Toxicol Appl Pharmacol. 2011; 256(3): 418-24. doi: 10.1016/j.taap.2011.08.009.
6. Orekhov AN, Bobryshev YV, Sobenin IA, Melnichenko AA, Chistiakov DA. Modified low density lipoprotein and lipoprotein-containing circulating immune complexes as diagnostic and prognostic biomarkers of atherosclerosis and type 1 diabetes macrovascular disease. Int J Mol Sci. 2014; 15(7): 12807-41. doi: 10.3390/ijms150712807.
7. Ahmadvand H, Ghasemi Dehnoo M, Dehghani A, Bagheri S, Cheraghi RA.
8. Serum paraoxonase 1 status and its association with atherogenic indexes in gentamicin-induced nephrotoxicity in rats treated with coenzyme Q10. Ren Fail. 2014 Apr;36(3):413-8. doi: 10.3109/0886022X.2013.865154.
9. James RW. A long and winding road: defining the biological role and clinical importance of paraoxonases. Clin Chem Lab Med, 2006. 44(9): 1052-9.
10. Kulka M. A review of paraoxonase 1 properties and diagnostic applications. Pol J Vet Sci. 2016;19(1):225-32. doi: 10.1515/pjvs-2016-0028.
11. García-Heredia A, Marsillach J, Rull A, Triguero I, Fort I, Mackness B, et al. Paraoxonase-1 inhibits oxidized low-density lipoprotein-induced metabolic alterations and apoptosis in endothelial cells: a nondirected metabolomic study. Mediators Inflamm. 2013:156053. doi: 10.1155/2013/156053. Epub 2013 May 22.
12. Patil VP, Patil AB, Patil VS, Ingleshwar DG. Paraoxonase Activity and Lipid Profile in Paediatric Nephrotic Syndrome: A Cross-sectional Study. J Clin Diagn Res. 2016 Mar;10(3):BC17-20. doi: 10.7860/JCDR/2016/18524.7440.
13. Ahmadvand H, Jamor P. Effects of alpha lipoic acid on level of NO and MPO activity in diabetic rats. Ann Res Antioxid. 2017;2(2):e04.
14. Ahmadvand H, Ghasemi-Dehnoo M. Antiatherogenic, hepatoprotective, and hypolipidemic effects of coenzyme Q10 in alloxan-induced type 1 diabetic rats. ARYA Atheroscler. 2014 Jul;10(4):192-8.
15. Gurcay AG, Gurcan O, Kazanci A, Bozkurt I, Senturk S, Bodur E, et al. Comparative Biochemical and Motor Function Analysis of Alpha Lipoic Acid and N-Acetyl Cysteine Treatment on Rats with Experimental Spinal Cord Injury. Turk Neurosurg. 2016; 26(1):119-26. doi: 10.5137/1019-5149.JTN.14594-15.0.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem, 1972. 18(6):499-502.
17. Ikewuchi, C. Alteration of plasma lipid profiles and atherogenic indices by Stachytarpheta jamaicensis L.(Vahl). Biokemistri, 2009. 21(2).
18. Hoffman RP. Hyperglycemic endothelial dysfunction: does it happen and does it matter? J Thorac Dis. 2015; 7(10): 1693-5. doi:10.3978/j.issn.2072-1439.2015.10.24.
19. Vergès B. Pathophysiology of diabetic dyslipidaemia: where are we? Diabetologia. 2015; 58(5): 886-99. doi: 10.1007/s00125-015-3525-8.
20. Boemi M, Leviev I, Sirolla C, Pieri C, Marra M, James RW. Serum paraoxonase is reduced in type 1 diabetic patients compared to non-diabetic, first degree relatives; influence on the ability of HDL to protect LDL from oxidation. Atherosclerosis. 2001; 155(1): 229-35.
21. Clifton, PM. Effect of Grape Seed Extract and Quercetin on Cardiovascular and Endothelial Parameters in High-Risk Subjects. J Biomed Biotechnol. 2004; 5: 272-8.
22. Rye KA, Bursill CA, Lambert G, Tabet F, Barter PJ. The metabolism and anti-atherogenic properties of HDL. J Lipid Res. 2009; 50 Suppl:S195-200
23. Nobécourt E, Jacqueminet S, Hansel B, Chantepie S, Grimaldi A, Chapman MJ, et al. Defective antioxidative activity of small dense HDL3 particles in type 2 diabetes: relationship to elevated oxidative stress and hyperglycaemia. Diabetologia. 2005; 48(3): 529-38.
24. Cheraghi, M, Shahsavari GH, Maleki A, Ahmadvand H. Paraoxonase 1 Activity, Lipid Profile, and Atherogenic Indexes Status in Coronary Heart Disease. Reports of Rep Biochem Mol Biol. 2017; 6(1): 1-7.
25. Budin SB, Kee KP, Eng MY, Osman K, Bakar MA, Mohamed J. Alpha lipoic acid prevents pancreatic islet cells damage and dyslipidemia in streptozotocin-induced diabetic rats. Malays J Med Sci. 2007;14(2):47-53.
26. Ahmadvand H, Noori A, Ghasemi Dehnoo M, Bagheri S , Rooh Angiz Cheraghi RA. Hypoglycemic, hypolipidemic

and antiatherogenic effects of oleuropein in alloxan-induced Type 1 diabetic rats Asian Pac J Trop Dis 2014; 4(Suppl 1): S421-S425

27. Amom Z, Zakaria Z, Mohamed J, Azlan A, Bahari H, Taufik Hidayat Baharuldin M, et al. Lipid Lowering Effect of Antioxidant Alpha-Lipoic Acid in Experimental Atherosclerosis. J Clin Biochem Nutr. 2008; 43(2): 88-94. doi: 10.3164/jcbn.2008051.
28. Bagheri S, Ahmadvand H, Khosrowbeygi A, Ghazanfari F, Jafari N, Nazem H, Hosseini RH. Antioxidant properties and inhibitory effects of Satureja khozestanica essential oil on LDL oxidation induced-CuSO(4) in vitro. Asian Pac J Trop Biomed. 2013 Jan;3(1):22-7. doi: 10.1016/S2221-1691(13)60018-0.
29. Ahmadvand H, Bagheri S, Khosrobeigi A, Boshtam M, Abdolahpour F. Effects of olive leaves extract on LDL oxidation induced-CuSO(4) in vitro. Pak J Pharm Sci. 2012 Jul;25(3):571-5.