Cholesterol lowering Effect of Subchronic Inhalation Particulate Matter 10 Coal Dust on Rats

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Abstract

Aim of this study was have to known an effect of inhaled particulate matter 10 (PM_{10}) of coal dust on lipid profile, hematopoetic stem cells (HSC) and circulating endothelial cells (CECs) in rats. A total of 32 Wistar male rats, were randomly divided into four groups of 8 rats each, including one control group and three groups for inhaled coal dust (concentration 6.25 mg/m^3; 12.5 mg/m^3; and 25 mg/m^3). The exposure to coal dust exposure was conducted using equipment that was designed by and available from Pharmacology Laboratory, Medical Faculty, Brawijaya University of Malang. The level of total cholesterol was lower significantly in rats inhaled to coal dust at compared with the control rats ($p<0.05$). The level of triglycerydes was higher significantly in rats inhaled to coal dust at concentration 25 mg/m^3 compared with the control rats ($p<0.05$). The level of LDL-c was lower significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m^3 compared with the control rats ($p<0.05$), but the level of HDL-c, HSCs and CECs were not different significantly ($p>0.05$). The level of HDL-c/LDL-c ratio was higher significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m^3 compared with the control rats ($p<0.05$). The level of cholesterol/HDL-c ratio and atherogenic index were lower significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m^3 compared with the control rats ($p<0.05$). The level of hematopoetic stem cells and circulating endothelial cells were not different significantly in rats inhaled to coal dust compared with the control rats ($p>0.05$). Sub-chronic inhalation particulate matter 10 (PM_{10}) coal dust have lowering effect on atherogenic index due to decreasing cholesterol and LDL levels. Beside that, sub-chronic inhalation particulate matter 10 (PM_{10}) coal dust also have dyslipidemia which marked by increasing of triglyceride levels.

Keywords: dyslipidemia; inhaled pollution; endothelial damage; hematopoetic stem cells.

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Introduction

Unhealthy lifestyle and polluted environment were risk factors for increasing incidence of coronary heart disease (CHD) and peripheral vascular disease due to dyslipidemia. Deposition of cholesterol, triglyceryde, calcium and other substances within vascular are accelerated factor of atherosclerosis progression. To date, inhalation of occupational and atmospheric coal dust has not only contributed significantly to the development of several respiratory disorders, also cardiovascular disease [1, 2]. Epidemiologic study demonstrated that the population residing near coal mine facilities suffer from a higher rate of cardiovascular disease [3], but the association of coal dust exposure and lipid profile or dyslipidemia is still unknown.

Mesoderm-derived adult stem cells, such as cardiac-derived stem cells, mesenchymal stem cells, skeletal myoblasts, hematopoietic stem cells (HSCs), and endothelial progenitor cells, represent a more suitable cell source for cell therapy intervention [4]. HSCs are multipotents stem cells that give rise to all cells of the blood lineage. In recent years, it has been proposed that these cells can circulate to the site of injury, where they contribute to myocardial repair and regeneration. As we know, there is no study to evaluate the effect of coal dust on hematopoietic stem cells.

Circulating endothelial cells (CECs) are mature cells that are not associated with vessel walls, but are detached from the endothelium and circulate within peripheral blood. The number of CECs present in the blood has been found to increase in response to cardiovascular disease, vasculitis, infectious disease, and various cancers [5]. Indeed, the level of CECs has been recognized as a useful biomarker for vascular damage. The endothelial cell damages due to dyslipidemia and proinflammatory cytokines have been demonstrated previous studies [6, 7]. Increased levels of triglycerides and lipoproteins correlate with impairment of endothelial function [8, 9]. Endothelial cell damage due to dyslipidemia plays a critical role in the development and progression of atherosclerosis [10, 11]. Several studies showed that coal dust produces proinflammatory cytokines and free radicals in vitro [12, 13] in rats [1, 14] and humans [15, 16] but for dyslipidemia and endothelial damage is still unknown.

To the best of our knowledge, no report has evaluated the effect of coal dust exposure on endothelial damage and its regeneration. Accordingly, we performed the subchronic inhalation of particulate matter 10 (PM_{10}) of coal dust on hematopoetic stem cells and

circulating endothelial cells. In addition, we also measured the change of lipid profile in subchronic inhalation of particulate matter 10 (PM_{10}) of coal dust.

Material and Methods

Animals and diets

Thirty two male Wistar rats, weighing 100–125 g, purchased from Central Animal House of Bandung were housed in an air-conditioned room at 25 ± 1◦C and 65–70% relative humidity with a 12 h light-dark cycle. The protocol used in this study was approved by the Ethic Committee for Animal Experimentation of the University of Lambung Mangkurat. Diets were made following American Institute of Nutrition (AIN) recommendations. The animals were given food and water *ad libitum* during the experimental period.

Coal dust preparation

Coal dust was made from gross coal by pulverizing using a Ball Mill, Ring Mill and Raymond Mill in Carsurin Coal Laboratories of Banjarmasin, resulting coal dust with a diameter of <75 μm. This particle coal dust was then filtered by Mesh MicroSieve (BioDesign, USA) with size of pores less than 10 μm of diameter. This specimen of coal dust then saving in Tissue and Specimen Banking of Banjarmasin referenced as voucher code 2012-2-CD. The characteristics of coal dust were then analyzed by Scanning Electron Microscope and X-Ray Fluorescence at the Physic and Central Laboratory Faculty of Mathematic and Natural Science University of Malang.

Scanning Electron Microscope has show the aerodinamic diameter of coal dust particle is less than 10 μm in one dimension, so its call PM_{10}. In addition, the morphology of particle consisting singlet or aggregate particle. X-Ray Fluorescence has show inorganic composition (%) of coal dust, which were iron (29.3 ± 0.1), silicon (29.0 ± 0.2), calcium (12.00 ± 0.07), aluminium (10 ± 0.2), titanium (6.31 ± 0.19), phosphorus (5.90 ± 0.04), potassium (4.5 ± 0.06), and barium (1.00 ± 0.09) and several inorganic minerals is less than <1% including europium (0.70 ± 0.00), chromium (0.48 ± 0.04), nickel (0.41 ± 0.00), copper (0.34 ± 0.02), zinc (0.22 ± 0.03), vanadium (0.20 ± 0.02), and manganese (0.15 ± 0.09).

Coal dust exposure

The groups for coal dust exposure receiving coal dust inhalation at concentration 6.25 mg/m^3, 12.5 mg/m^3, 25 mg/m^3 one hour per day for 28 days which modified from previous study [17]. Coal dust exposure was done by coal dust inhalation equipment that was designed and available in Pharmacology Laboratorium, Medical Faculty, Brawijaya University of Malang. The principal of this equipment is to provide an ambient environment which contains coal dust which inhaled by rat. The airstream of thus equipment is setting at 1.5-2 liter/minutes whic similar to environmental airstream.

Plasma lipid profile and atherogenic index analysis

At the end of the treatment, rats in all groups were anesthetized by ether; blood samples were collected by cardiac puncture and allowed to clot for 2 hours at room temperature, followed by centrifugation at 3500 g for 10 minutes to obtain the serum. The serum was stored at −80◦C until analysis. The levels of total cholesterol triglycerides (TG), high-density lipoprotein cholesterol (HDL-c), and low-density lipoprotein cholesterol (LDL-c) were measured in the serum of rat using an automated enzymatic technique [18]. The atherogenic index (AI) was defined as (TC−HDL-c)/HDL-c) and was calculated for the experimental groups.

Measurement of hematopoetic stem cells

Hematopoetic stem cells were isolated from peripheral blood as described elsewhere [19]. Briefly, 10 mL of venous ethylenediaminetetraacetic acid (EDTA) blood was obtained by peripheral venepuncture, stored at 4^{o}C to 10^{o}C, and processed within 6 hours after collection. Peripheral blood mononuclear cells were isolated by density-gradient centrifugation using Ficoll-Paque Plus (Amersham Pharmacia Biotech, Uppsala, Sweden). Isolated cells were washed twice with PBS and resuspended in 20 0mL of PBS supplemented with 0.5% of bovine serum albumin and 2 mM of EDTA. CD34$^+$ cells in peripheral blood were evaluated by immunostaining with PE-conjugated CD34 monoclonal antibody (Biolegend) and detected by flow cytometry (BD FACSCalibur Flow Cytometer).

Measurement of circulating endothelial cells

Circulating endothelial cells were isolated from peripheral blood as described elsewhere [18], with minor modification. Briefly, 10 mL of venous ethylenediaminetetraacetic acid (EDTA) blood was obtained by peripheral venepuncture, stored at 4^{o}C to 10^{o}C, and processed within 6 hours after collection. Peripheral blood mononuclear cells were isolated by density-gradient centrifugation using Ficoll-Paque Plus (Amersham Pharmacia Biotech, Uppsala, Sweden). Isolated cells were washed twice with PBS and resuspended in 20 0mL of PBS supplemented with 0.5% of bovine serum albumin and 2 mM of EDTA. CD146^{+} cells in peripheral blood were evaluated by immunostaining with FITC-conjugated CD146^{+} monoclonal antibody (Biolegend) and detected by flow cytometry (BD FACSCalibur Flow Cytometer).

Ethics

This research has been approved by research ethics committee Faculty of Medicine University of Lambung Mangkurat, Banjarmasin, Indonesia.

Statistical analysis

Data are presented as mean ± SD and differences between groups were analyzed using 1-way ANOVA with SPSS 15.0 statistical package. Post Hoc test was used if the ANOVA was significant. $p < 0.05$ was considered statistically significant.

Results

Effect PM10 coal dust on lipid profile

Table 1 showed the levels of total cholesterol, triglycerydes, HDL-c, LDL-c, and atherosclerosis index, HDL-c/LDL-c ratio, and cholesterol/HDL-c ratio in serum of control and experimental groups of rats. The level of total cholesterol was lower significantly in rats inhaled to coal dust compared with the control rats ($p<0.05$). The level of triglycerydes was higher significantly in rats inhaled to coal dust at concentration 25 mg/m^3 compared with the control rats ($p<0.05$). The level of HDL-c was not different significantly in all groups ($p>0.05$). The level of LDL-c was lower significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m^3 compared with the control rats ($p<0.05$). The level of HDL-

c/LDL-c ratio was higher significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m^3 compared with the control rats ($p<0.05$). The level of cholesterol/HDL-c ratio was lower significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m^3 compared with the control rats ($p<0.05$). The level of atherogenic index was lower significantly in rats inhaled to coal dust at concentration 12.5 and 25 mg/m^3 compared with the control rats ($p<0.05$).

Table 1. Lipid profile in rats inhaled to subchronic PM_{10} of coal dust

(mg/dL)	Concentration of coal dust			
	0 mg/m^3	6.25 mg/m^3	12.5 mg/m^3	25 mg/m^3
Cholesterol	98.400 ± 9.044	80.400 ± 16.134[a]	66.600 ± 11.610[a]	69.000 ± 9.083[a]
Triglyceride	73.400 ± 4.037	60.600 ± 22.300	123.600 ± 36.487[b]	103.400 ± 22.434[ab]
HDL-c	38.080 ± 4.683	33.560 ± 2.901	31.980 ± 7.982	33.140 ± 5.081
LDL-c	45.640 ± 6.975	34.720 ± 19.080	9.900 ± 8.679[ab]	15.180 ± 3.697[a]
HDL-c/LDL-c	0.851 ± 0.179	1.342 ± 0.975	5.631 ± 5.037[ab]	2.270 ± 0.530[a]
Chol/HDL-c	2.602 ± 0.257	2.396 ± 0.422	2.110 ± 0.194[a]	2.090 ± 0.117[a]
AI	1.602 ± 0.257	1.396 ± 0.422	1.110 ± 0.194[a]	1.090 ± 0.117[a]

Note: AI: atherogenic index; values are presented as mean ± SD; [a]$p<0.05$; in comparison with control group; [b]$p<0.05$; in comparison with rats inhaled to 6.25 mg/m^3; [c]$p<0.05$; in comparison with rats inhaled to 12.5 mg/m^3

There is negative correlation significantly between total cholesterol and concentration of coal dust ($p=0.001$; $r=-0.694$). There is positive correlation significantly between triglycerides and concentration of coal dust ($p=0.019$; $r=0.520$). There is negative correlation significantly between LDL-c and concentration of coal dust ($p=0.000$; $r=-0.738$). There is negative correlation significantly between atherogenic index and concentration of coal dust ($p=0.003$; $r=-0.631$).

Effect PM10 coal dust on HSC and CECs

Table 2 showed the levels of hematopoetic stem cells and circulating endothelial cells of control and experimental groups of rats. The level of hematopoetic stem cells and circulating endothelial cells were not different significantly in rats inhaled to coal dust compared with the control rats ($p>0.05$).

Table 2. HSCs and CECs in rats inhaled to subchronic PM_{10} of coal dust

(% cells)	Concentration of coal dust			
	0 mg/m^3	6.25 mg/m^3	12.5 mg/m^3	25 mg/m^3
HSCs	0.118 ± 0.043	0.158 ± 0.093	0.086 ± 0.046	0.072 ± 0.051
CECs	0.060 ± 0.050	0.026 ± 0.017	0.022 ± 0.008	0.044 ± 0.025

Note: values are presented as mean ± SD; [a]$p<0.05$; in comparison with control group; [b]$p<0.05$; in comparison with rats inhaled to 6.25 mg/m^3; [c]$p<0.05$; in comparison with rats inhaled to 12.5 mg/m^3

Discussion

Lipids have been observed to play important roles in pathological changes observed in disease conditions and pre-condition diseases. Serum lipids bounding to lipoproteins can be elevated by an increase in biosynthesis and/or by a decrease in their elimination process. As we know, this is the first time a study has been designed to evaluate an effect of inhaled particulate matter 10 of coal dust on a lipid profile, hematopoetic stem cells, and circulating endothelial cells in rats.

Reverse cholesterol transport (RCT) is the mechanism by which cholesterol is transported from the peripheral tissues and cells to the liver, where it is transformed into bile acids, and then finally eliminated from the body. The main function of RCT is to prevent the formation and development of atherosclerosis, by decreasing the plasma cholesterol level [19]. The process of RCT can be divided into three stages: 1) the efflux of cellular cholesterol from peripheral cells as high-density lipoprotein, 2) conversion of cholesterol in HDL to cholesteryl esters (CE) by the enzyme lecithin-cholesterol acyltransferase (LCAT), then cholesterol is carried as CE within the core of HDL particles to the liver, and 3) delivery of CE from HDL to hepatocytes [20]. In the present study, inhalation of coal dust over four weeks resulted in 18.29% to 32.31% lower total plasma cholesterol levels, compared to the control group. Besida that, there is negative correlation significantly between total cholesterol and concentration of coal dust ($p=0.001$; $r=-0.694$). We suggest that the mechanism by which coal dust inhalation lowers cholesterol may be related to increased RCT; however, the detailed mechanisms need to be clarified in a future study. The inorganic components of coal dust that can decrease cholesterol levels are silicon, chromium and vanadium. Elevated silicon ingestion impedes cholesterol-induced atherogenesis in rabbits [21]. Chromium induced a reduction in cholesterol *in vitro* [22] and *in vivo* [23, 24]. In addition, vanadium effectively lowered hyperlipidemia in diabetic rats [25].

Increased levels of triglycerides are not as strongly associated with increased cardiovascular risk as increased plasma cholesterol levels. Nevertheless, elevated plasma triglyceride levels are clinically significant, as they are an indicator of dyslipidemia [26]. The level of triglycerides was significantly higher in rats inhaling coal dust at a concentration of 25 mg/m^3 compared with the control rats ($p<0.05$). There is positive correlation significantly between triglycerides and concentration of coal dust ($p=0.019$; $r=0.520$). This finding indicates that coal dust inhalation at a concentration of 25 mg/m^3 only lead to dyslipidemia; however, dyslipidemia is not strongly associated with increased cardiovascular risk which confirmed by negative correlation significantly between atherogenic index and concentration of coal dust ($p=0.003$; $r=-0.631$).

High-density lipoprotein has antiatherosclerotic effects, including augmentation of ability of reverse cholesterol transport [29, 30]. Cholesteryl ester transfer protein (CETP) promotes the transfer of CE from HDL to the apoB-containing lipoprotein particles, which subsequently are cleared from the circulation by the liver. Thus, CETP plays a critical role in the intravascular remodeling and recycling of HDL particles [31]. The level of HDL-c is not different significantly in the coal dust exposure group compared to the control group ($p > 0.05$), but the level of LDL-c was lower significantly in rats inhaling to coal dust at concentrations of 12.5 and 25 mg/m^3 compared with the control rats ($p<0.05$). in addition, There is negative correlation significantly between LDL-c and concentration of coal dust ($p=0.000$; $r=-0.738$). The level of HDL-c/LDL-c ratio was higher significantly in rats that inhaled coal dust at concentrations of 12.5 and 25 mg/m^3 compared with the control rats ($p<0.05$). This result indicated that coal dust maintained HDL-c at basal level and in ideal proportion to LDL-c which is important for CETP function. In addition, atheroprotective properties of HDL-c have been associated to its role in preserving endothelial function [31]. We also find that coal dust does not increase endothelial damage. This means that the basal level of HDL-c, which is found in control and coal dust inhalation groups has a beneficial effect in preventing endothelial damage.

Decrease in the level of total-cholesterol/HDL-cholesterol ratio and HDL-cholesterol/LDL-cholesterol ratio is linked to a reduction in the risk of morbidity and mortality in cardiovascular diseases [32]. The level of total-cholesterol/HDL ratio is lower significantly in coal dust exposure at concentrations of 12.5 mg/m^3 and 25 mg/m^3 compared to the control group. This finding indicated that subchronic coal dust exposure at concentrations of 12.5

mg/m^3 and 25 mg/m^3 decrease the risk of cardiovascular disease. We also find that the atherogenic index is decreased in the coal dust inhaled groups, reach different significances at coal dust concentrations of 12.5 and 25 mg/m^3.

Endothelial cells appear to regulate the trafficking and release of HSCs from bone marrow [33]. Endothelial cell damage due to dyslipidemia plays a critical role in the development and progression of atherosclerosis [34]. The level of hematopoetic stem cells and circulating endothelial cells is not different in coal dust exposure groups compared to control group. This finding indicated that subchronic coal dust particle exposure does not induce cell death of hematopoetic stem cells and endothelial damage. The viability of endothelial cells determine the trafficking and release hematopoetic stem cells from bone marrow.

In conclusion, sub-chronic inhalation particulate matter 10 (PM_{10}) coal dust have dual effect on lipid profile. First effect is lowering effect on atherogenic index due to decreasing cholesterol and LDL levels. Second effect is dyslipidemia which marked by increasing of triglyceride levels.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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References

1. Pinho RA, Silveira PCL, Piazza M, Tuon T, Silva GA, Dal-Pizzol F, Moreira JCF. Regular physical exercises decrease the oxidant pulmonary stress in rats after acute exposure to mineral coal. Rev Bras Med Esporte. 2004:12(2);71e-74e.
2. Stachowska E, Baskiewicz M, Marchlewicz M, Czuprynska K, Kaczmarczyk M, Wiszniewska B, Machalinski B, Chlubek D. Conjugated linoleic acids regulate triacylglycerol and cholesterol concentrations in macrophages/foam cells by the modulation of CD36 expression. Acta Biochim Pol. 2010:57(3);379-84.
3. Hendryx M, Zullig KJ. Higher coronary artery disease and heart attack morbidity in Appalachian coal mining regions. Preventive Medicine. 2009:49(5);355-9.
4. Avitabile D, Crespi A, Brioschi C, Parente V, Toietta G, Devanna P, Baruscotti M, Truffa S, Scavone A, Rusconi F, Biondi A, D'Alessandra Y, Vigna E, Difrancesco D, Pesce M, Capogrossi MC, Barbuti A. Human cord blood CD34+ progenitor cells acquire functional cardiac properties through a cell fusion process. Am J Physiol Heart Circ Physiol. 2011;300(5):H1875-84.
5. Kondo S, Ueno H, Hashimoto J, Morizane C, Koizumi F, Okusaka T, Tamura K. Circulating endothelial cells and other angiogenesis factors in pancreatic carcinoma patients receiving gemcitabine chemotherapy. BMC Cancer. 2012;12:268.
6. Brown CD, Higgins M, Donato KA, Rohde FC, Garrison R, Obarzanek E, Ernst ND, Horan M. Body mass index and the prevalence of hypertension and dyslipidemia. Obes Res. 2000;8(9):605-19.
7. Caballero EA. Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease. Obes Res. 2003;11(11):1278-89.
8. Lundman P, Eriksson MJ, Silveira A, Hansson LO, Pernow J, Ericsson CG, Hamsten A, Tornvall P. Relation of hypertriglyceridemia to plasma concentrations of biochemical markers of inflammation and endothelial activation (C-reactive protein, interleukin-6, soluble adhesion molecules, von Willebrand factor, and endothelin-1). Am J Cardiol. 2003;91(9):1128-31.
9. Ferreira AC, Peter AA, Mendez AJ, Jimenez JJ, Mauro LM, Chirinos JA, Ghany R, Virani S, Garcia S, Horstman LL, Purow J, Jy W, Ahn YS, de Marchena E. Postprandial hypertriglyceridemia increases circulating levels of endothelial cell micro-particles. Circulation. 2004;110(23):3599-603.
10. Libby P. Vascular biology of athrosclerosis: overview and state of art. Am J Cardiol. 2003;91(3A):3A-6A.
11. Ross R. Cell biology of atherosclerosis. Annu Rev Physiol. 1995;57:791-804.
12. Zhang Q, Dai J, Ali A, Chen L, Huang X. Roles of bioavailable iron and calcium in coal dust-induced oxidative stress: Possible implications in coal workers' lung disease. Free Radic Res. 2002;36(3):285-94.
13. Zhang Q, Huang X. Induction of ferritin and lipid peroxidation by coal samples with different prevalence of coal workers' pneumoconiosis: role of iron in the coals. Am J Ind Med. 2002;42(3):171-9.

14. Armutcu F, Gun BD, Altin R, Gurel A. Examination of lung toxicity, oxidant/antioxidant status and effect of erdosteine in rats kept in coal mine ambience. Environ Toxicol Pharmacol. 24(2);106-13.

15. Avila Júnior S, Possamai FP, Budni P, Backes P, Parisotto EB, Rizelio VM, Torres MA, Colepicolo P, Wilhelm Filho D. Ecotoxicology. 2009;18(8):1150-7.

16. Ulker O, Yucesoy B, Demir O, Tekin I, Karakaya A. Serum and BAL cytokine and antioxidant enzyme level at different stages of pneumoconiosis in coal workers. Hum Exp Toxicol. 2008;27(12):871-7.

17. Donaldson K, Brown GM, Brown DM, Robertson MD, Slight J, Cowie H, Jones AD, Bolton RE, Davis JM. Contrasting bronchoalveolar leukocyte responses in rats inhaling coal mine dust, quartz, or titanium dioxide: effects of coal rank, airborne mass concentration, and cessation of exposure. Environ Res. 1990;52(1):62-76.

18. Lu Y, He Z, Shen X, Xu X, Fan J, Wu S, Zhang D. Cholesterol-lowering effect of allicin on hypercholesterolemic ICR mice. Oxid Med Cell Longev. 2012;2012:489690.

19. Guo X, Liu L, Zhang M, Bergeron A, Cui Z, Dong JF, Zhang J. Correlation of $CD34^{+}$ cells with tissue angiogenesis after traumatic brain injury in a rat model. J Neurotrauma. 2009;26(8):1337-44.

20. Yazdanyar A, Yeang C, Jiang XC. Role of phospholipid transfer protein in high-density lipoprotein-mediated reverse cholesterol transport. Curr Atheroscler Rep. 2011;13(3):242-8.

21. Song G, Liu J, Zhao Z, Yu Y, Tian H, Yao S, Li G, Qin S. Simvastatin reduces atherogenesis and promotes the expression of hepatic genes associated with reverse cholesterol transport in apoE-knockout mice fed high-fat diet. Lipids Health Dis. 2011;10:8.

22. McCarty MF. Reported anti atherosclerotic activity of silicon may reflect increased endothelial synthesis of heparan sulfate proteoglycans. Med Hypotheses. 1997;49(2):175-6.

23. Pattar GR, Tackett L, Liu P, Elmendorf JS. Chromium picolinate positively influences the glucose transporter system via affecting cholesterol homeostasis in adipocytes cultured under hyperglycemic diabetic conditions. Mutat Res. 2006;610(1-2):93-100.

24. Boyd SG, Boone BE, Smith AR, Conners J., Dohm GL. Combined dietary chromium picolinate supplementation and an exercise program leads to a reduction of serum cholesterol and insulin in college-aged subjects. J. Nutr. Biochem. 1998;9(8): 471-5.

25. Wang MM, Fox EA, Stoecker BJ, Menendez CE, Chan SB. Serum cholesterol of adults supplemented with brewer's yeast or chromium chloride. Nutr Res. 1989;9(9):989-98.

26. Crans DC, Mahroof-Tahrir M, Johnsons MD, Wilkins PC, Yang L, Robbins K, Johnson A, Alfano JA, Godzala ME, Austin LT, Willsky GR. Vanadium(IV) and vanadium(V) complexes of dipicolinic acid and derivatives. Synthesis, X-ray structure, solution state properties: and effects in rats with STZ-induced diabetes. Inorganic Chimica Acta, 2003;356:365-78.

27. Watts GF, Karpe F. Triglycerides and atherogenic dyslipidaemia: extending treatment beyond statins in the high-risk cardiovascular patient. Heart. 2011;97(5):350-6.

28. Silva M, Silve ME, de Paula H, Carneiro MC, Pedrosa ML. Iron overload alters glucose homeostasis, cause liver steatosis, and increases serum triacylglycerols in rats. Nutr Res. 2008;28(6),391-8.
29. Ansell BJ, Watson KE, Fogelman AM, Navab M, Fonarow GC. High-density lipoprotein function: recent advances. J Am Coll Cardiol. 2005;46(10):1792-8.
30. Davignon J. Beneficial cardiovascular pleiotropic effects of statins. Circulation. 2004;109(23),39–43.
31. Bighetti EJB, Patricio PR, Casquero AC, Berti J, Oliveira HCF. Ciprofibrate increases cholesteryl ester transfer proteoin gene expression and the indirect reverse cholesterol transport to the liver. Lipids Health Dis. 2009;8:50.
32. Bleda S, de Haro J, Varela C, Esparza L, Rodriguez J, Acin F. (2012). Improving total-cholesterol/HDL-cholesterol ratio results in an endothelial dysfunction recovery in peripheral artery disease patients. Cholesterol. 2012;2012:895326.
33. Kiernan TJ, Boilson BA, Witt TA, Dietz AB, Lerman A, Simari RD. Vasoprotective effects of human CD34+ cells: towards clinical applications. J Transl Med. 2009;7:66.
34. Mikirova NA, Casciari JJ, Hunninghake RE, Beezley MM. Effect of weight reduction on cardiovacular risk factors and CD34-positive cells in circulation. Int J Med Sci. 2011;8(6):445-52.