Avicenna J Med Biochem. 2015 March; 3(1): e23205. DOI: 10.17795/ajmb-23205

Published online 2015 March 21. **Research Article**

Combination of Ezetimibe and Garlic Reduces Serum Lipids and Intestinal Niemann-Pick C1-like 1 Expression More Effectively in Hypercholesterolemic Mice

Abbas Mohammadi [1]; Seyyed Alireza Vafaei [2]; Mohammad Nabi Moradi [3]; Mehrdad Ahmadi [4]; Mona Pourjafar [5]; Ebrahim Abbasi Oshaghi [3,*]

[1]Department of Biochemistry, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, IR Iran
[2]Department of Biochemistry, Science and Research Branch, Islamic Azad University, Sanandaj, IR Iran
[3]Department of Biochemistry, School of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran
[4]Research Center for Molecular Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran
[5]Department of Molecular Medicine and Genetics, Hamadan University of Medical Sciences, Hamadan, IR Iran

*Corresponding author: Ebrahim Abbasi Oshaghi, Department of Biochemistry, School of Medicine, Hamadan University of Medical Sciences, Hamadan, IR Iran. Tel: +98-9198029847, E-mail: 7abbasi@gmail.com

Received: September 3, 2014; **Revised:** November 24, 2014; **Accepted:** December 27, 2014

Background: Combination therapy plays an important role in the management of cardiovascular disease (CVD).
Objectives: The aim of this experiment was to study the influence of garlic combined with ezetimibe on lipid profile as well as intestinal Niemann-Pick C1-like 1 (NPC1L1) expression in normal and hypercholesterolemic mice.
Materials and Methods: A total of 40 mice were randomly divided into five groups: Group 1: hypercholesterolemic group (received 2% w/w cholesterol + 0.5% w/w cholic acid in their diet), Group 2: garlic group (hypercholesterolemic diet + 4% w/w garlic extract), Group 3: ezetimibe group (hypercholesterolemic diet + 0.005% w/w ezetimibe), Group 4: combination group (hypercholesterolemic diet + 0.005% w/w ezetimibe + 4% w/w garlic) and Group 5: control (chow only).
Results: Serum low-density lipoprotein-cholesterol (LDL-C) and total cholesterol (TC) levels were significantly decreased in ezetimibe, garlic (both $P < 0.05$), and combination groups ($P < 0.001$). Also, triglycerides and very low-density lipoprotein-cholesterol (VLDL-C) were significantly lower in garlic and combination groups ($P < 0.05$). Liver enzymes, alanine aminotransferase (ALT) and aspartate aminotransferase (AST), were also significantly decreased in garlic, ezetimibe (both $P < 0.05$) and combination groups ($P < 0.001$) in comparison with hypercholesterolemic animals. Analysis of semi quantitative RT-PCR results showed that the levels of NPC1L1 was also significantly less ($P < 0.01$) in the garlic, ezetimibe, and combination groups ($P < 0.001$) compared with the controls. Based on the results, the combination of garlic and ezetimibe can lower serum lipids and liver enzymes more effectively in hypercholesterolemic mice.
Conclusions: This experiment revealed that a possible mechanism for the beneficial effects of garlic and ezetimibe combination in lowering plasma LDL-C and TC is inhibition of intestinal cholesterol absorption. More research might be necessary to determine the efficacy and the exact mechanism of this co-administration.

Keywords: Cholesterol; Garlic; Ezetimibe; Herbal Medicine; Hypercholesterolemia

1. Background

Heart disease is a major cause of mortality and morbidity in the world. The main risk factors, such as family history and age cannot be changed. However, other risk factors including, obesity, diabetes, smoking, diet, high blood pressure, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and low levels of high-density lipoprotein cholesterol (HDL-C) can be changed or treated (1, 2). These risk factors can be controlled and corrected by diet, exercise, hypolipidemic drugs and herbal medicine (3). Ezetimibe is a novel drug which inhibits cholesterol absorption in the intestine without affecting absorption of triglycerides (TGs) or fat-soluble vitamins (4). Bays HE, et al. showed that ezetimibe administration reduced LDL-C by 18.5%, and increased HDL-C by 3.5% in humans. They also reported that this drug was well tolerated, with side effects similar to the placebo (5). Most chemical drugs have adverse effects and several have been associated with possible carcinogenicity. For these reasons, many patients tend to use herbal medicines for their ailments (6-8). Traditional medicine is still very much relied-on all over the world, consequently, the World Health Organization (WHO) has announced that around 80% of people in developing countries chiefly choose traditional medicine for their primary health care needs, and around 85% of such traditional medicine includes the usage of herbal plant extracts (9). In this respect, it has been shown that garlic has many useful effects on cardiovascular risk factors, including hypoglycemic, hypolipidemic, anti-dia-

betic, anti-hypertensive, anti-carcinogenic, anti-bacterial, anti-thrombotic, anti-fungal and antioxidant effects (10-12). Sulfur compounds present in garlic, including S-allylcysteine (SAC), allicin, ajoene, diallyl disulfide (DADS), S-allylcysteinesulfoxide and S-methylcysteinesulfoxide, areprobably responsible for the therapeutic effects of garlic (10). Therefore, the first major aim of this study was to determine the effect of combination therapy with one chemical drug (ezetimibe) plus one potential herbal medicine (garlic). Liver and the small intestine are two important organs in regulation of cholesterol homeostasis. Niemann-Pick C1-like 1 (NPC1L1) protein plays a vital role in intestinal cholesterol absorption. This protein is mainly expressed in the small intestine and liver. Altman SW, et al. showed that NPC1L1-deficient mice display a significant decline in intestinal cholesterol absorption (13). Studies showed that ezetimibe binds to brush-border membranes of the intestine in wild-type mice yet not those of NPC1L1 knockout mice, suggesting that this drug acts on the NPC1L1 and turns off this transporter (14). Ezetimibe inhibited absorption of intestinal cholesterol, while garlic inhibited cholesterol synthesis in the liver as well as intestinal cholesterol absorption (13).

2. Objectives

In this study we investigated whether the combination of garlic and ezetimibe would result in a greater reduction of lipids than ezetimibe and garlic alone. We also measured NPC1L1 levels in the intestine.

3. Materials and Methods

3.1. Animals

Male mice were kept on a 12 hours light/dark cycle at a temperature of 22 ± 1°C. After acclimatizing for one week in a cage, animals were randomly divided into five groups (n = 8):

Group 1: hypercholesterolemic group (received chow + 2% w/w cholesterol + 0.5% w/w cholic acid) (11).

Group 2: garlic group (received hypercholesterolemic diet + 4% w/w garlic extract).

Group 3: ezetimibe group (received hypercholesterolemic diet + 0.005% w/w ezetimibe).

Group 4: combination group (received hypercholesterolemic diet + 0.005% w/w ezetimibe + 4% w/w garlic extract).

Group 5: control (chow only).

The TG and TC levels were at the baseline prior to the experiment and were similar between animals. Garlic extract was dissolved in physiological saline and mixed with animal's diet. An equivalent amount of saline was added to the diet of hypercholesterolemic and control groups. Ezetimibe was dissolved in corn oil and mixed with the hypercholesterolemic diet. An equivalent amount of corn oil was also added to the diet of hypercholesterolemic and control groups. At the end of four weeks, mice were fasted for 12 hours, and then anesthetized and sacrificed. Blood was then collected from the heart and serum was separated by centrifugation at 3000 g for 10 minutes. Duodenum of small intestine was removed instantly and washed with PBS buffer, quickly frozen with liquid nitrogen and was kept at -80 degrees (15-18).

3.2. Water Soluble Garlic Extract

The garlic extract was prepared as described previously (19).

3.3. Biochemical Factors

Lipids and liver enzymes were measured using commercial kits (Pars Azmoon, Tehran, Iran). Low-density lipoprotein-cholesterol was calculated according to the Friedewaldequation (20-22).

3.4. Semi-Quantitative RT-PCR

The intestinal RNA was extracted using the Accuzol reagent (Bioneer, Korea) according to the manufacturers' protocol. Synthesis of cDNA was completed according to the manufacturer's protocol (Fermentas, Lithuania) using 5ng of mRNA for each sample. For each RT-PCR reaction, 2 μL of cDNA, 1 μL of forward primer (10 pm), 1 μL of reverse primer (10 pm), 13 μL of PCR Master Mix (Cinnagen, Iran) and 8 μL of deionized water were added to a sterile tube on ice, and briefly centrifuged. Thirty-five cycles of amplification were performed with denaturation at 95°C for 30 seconds, annealing at 61°C for 30 seconds, and extension at 72°C for 30 seconds. All reactions were finished with a single extra cycle at 72°C for five minutes. The primers used in this experiment were: mouse β-actin; forward: 5'-*TGG AAT CCT GTG GCA TCC ATG AAA C-3*', reverse: 5'-*TAA AAC GCA GCT CAG TAA CAG TCC G-3*', npc1l1; forward: 5'-*GCT TCT TCC GCA AGA TAT ACA CTC CC-3*', and reverse: 5'-*GAG GAT GCA GCA ATA GCC ACA TAA GAC-3*' (23, 24).

3.5. Statistical Analysis

Data are shown as means ± standard error of the mean. One-way analysis of variance (ANOVA) and the SPSS 15 software were used statistical analysis. Differences were considered statistically significant if $P < 0.05$.

4. Results

4.1. Lipids

The levels of TC and LDL-C were significantly lower in ezetimibe and garlic-treated animals ($P < 0.05$) (Figures 1 - 2). In the combination group, these reductions were more significant ($P < 0.001$). Triglycerides and VLDL-C significantly declined in combination and garlic groups ($P < 0.05$). Serum ALT and AST were significantly lower in the combination ($P < 0.01$), garlic ($P < 0.05$) and ezetimibe ($P < 0.05$) groups as compared with hypercholesterolemic animals (Table 1).

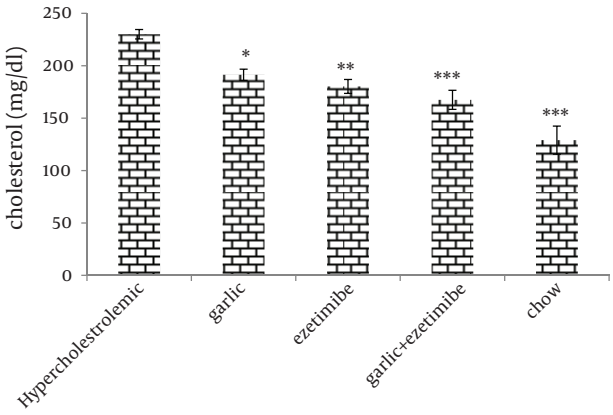


Figure 1. Effect of Different Treatments on Cholesterol Levels Compared With Hypercholesterolemic Animals. *P < 0.05, **P < 0.01 and ***P < 0.001

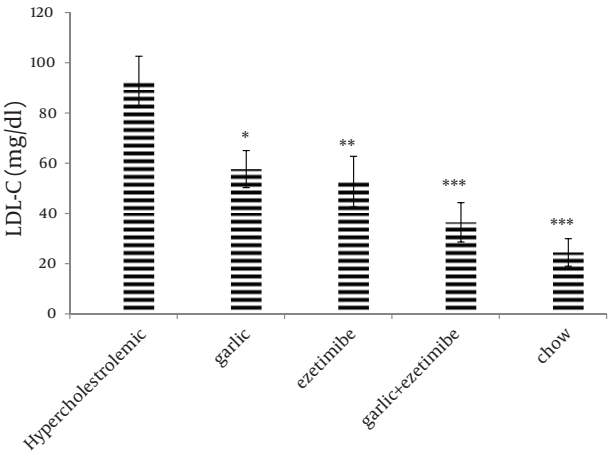


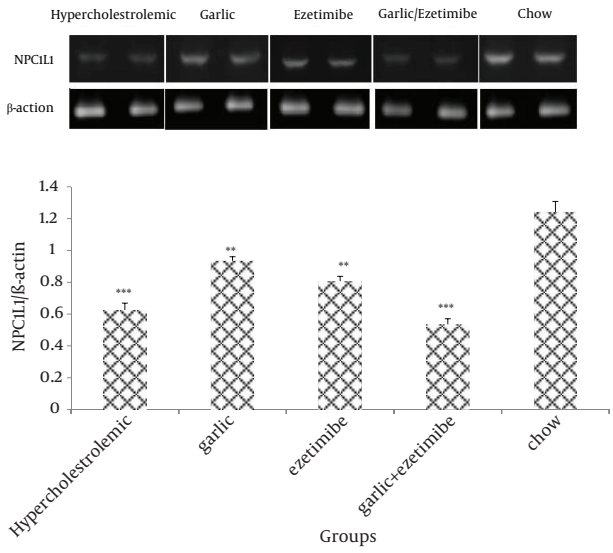
Figure 2. Effect of Different Treatments on Low-Density Lipoprotein-Cholesterol Levels Compared With Hypercholesterolemic Animals; *P < 0.05, **P < 0.01 and ***P < 0.001

Table 1. Comparison of Biochemical Factors Among Different Groups (n = 8)

Biochemical Factors	Hypercholesterolemic	Garlic	Ezetimibe	Garlic/Ezetimibe	Chow
TG, mg/dL	160.5 ± 7.5	137.4 ± 4.5 [a]	144.2 ± 4.1	135.2 ± 2.7 [a]	133.5 ± 4.0
VLDL-C, mg/dL	32.1 ± 1.5	27.5 ± 0.9 [a]	28.5 ± 0.8	27.1 ± 0. 6 [a]	26.7 ± 0.8
HDL-C, mg/dL	110.2 ± 8.1	106.2 ± 3.8	95.0± 8.4	104.0 ± 8.0	87.4 ± 9.7
AST, u/L	59.8 ± 5.1	42.1± 3.9 [a]	43.3 ± 4.0 [a]	40.3 ± 3.8 [b]	41.5± 4.7
ALT, u/L	61.4 ± 4.3	43.7 ± 2.9 [a]	45.1± 3.6 [a]	41.1± 4.2 [b]	43.1± 5.2

[a] P<0.05.
[b] P<0.001 with hypercholesterolemic. TG: triglycerides, VLDL-C: very low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol.

Figure 3. Expression of NPC1L1 mRNA in intestine of mice (n = 8)



The levels of NPC1L1 mRNA was significantly declined in the combination group (***P < 0.001 and **P < 0.01) as compared to the chow group. Data are presented as mean ± SEM.

4.2. Gene Expression

Results of semi-quantitative RT-PCR showed that the levels of intestinal NPC1L1 were markedly reduced in garlic and ezetimibe groups (P < 0.01), however the reduction in the combination group was more significant (P < 0.001) (Figure 3).

5. Discussion

Cardiovascular disease (CVD) includes conditions such as heart failure, congenital heart disease, coronary heart disease, arrhythmia, stroke, heart valve disease and hypertension. It has been estimated that CVD affects 83.6 million people and accounted for 32.3% of deaths in the United States in 1992 (25-28). Statins (or 3-hydroxy-3-methyl-glutaryl (HMG)-CoA reductase inhibitors) are the most broadly used hypolipidemic drugs and are frequently prescribed in monotherapy. However, not all patients achieve their LDL-C lowering goal by statin monotherapy or are worried about side effects (29). Consequently, combination therapy was suggested as a method to normalize lipid profile with outraising the statin dose or as an approach to decrease side effects (4, 30). In this study the combination of garlic and ezetimibe reduced total

cholesterol by 27.4%, triglycerides by 15.6%, LDL-C by 57% and VLDL-C by 16%. However, reduction in garlic and ezetimibe groups was far less than the combination group. A decrease of LDL-C by at least 30% to 50% can medically be advantageous to patients at high risk of heart disease; however, it is difficult to reach this goal with statins alone. Recent clinical experiments have suggested that statins in combination with ezetimibe significantly decrease LDL-C levels in the serum of hypercholesterolemic patients, indicating greater hypolipidemic activity compared with statins alone (4, 30-32). Van Heek M, et al. showed that co-administration of ezetimibe with simvastatin caused 44% to 57%, atorvastatin 50% to 60%, lovastatin 33% to 45% and pravastatin 34% to 41% decrease in plasma LDL-C levels (31). Co-administration of garlic and ezetimibe in hypercholesterolemic animals synergistically decreased plasma TC, TG, LDL-C and VLDL-C levels; this was a result of effective inhibition of intestinal cholesterol absorption and hepatic cholesterol biosynthesis. Salen G, et al. showed that administration of ezetimibe, 10 mg/day, for eight weeks, significantly reduced plant sterol concentrations in the plasma of sitosterolemia patients. They suggested that ezetimibe inhibited intestinal absorptions of both plant sterols and cholesterol (33). Sudhop T, et al. also reported that ezetimibe markedly reduced intestinal plant sterol absorption (34). Administration of ezetimibe in humans, at a dose of 10 mg/day, decreased absorption of cholesterol in the intestine by 54% compared with the placebo. The levels of plasma LDL-C was also reduced by 20% with an 89% compensatory rise in cholesterol biosynthesis in hepatic cells (35). On the other hand, use of garlic may play a vital role in the management of diabetes and CVD. Garlic has been shown to have several beneficial properties, including antimicrobial, antifungal, antioxidant, immunomodulatory, anti-inflammatory, anti-diabetic, hepatoprotective, anti-atherosclerosis, hypolipidemic, anthelmintic, anticoagulant and fibrinolytic, wound healing, anticancer and antihypertensive activity (10). Therefore, combination therapy with administration of other drugs which inhibit cholesterol biosynthesis is very useful. On the other hand, HMG-CoA reductase inhibitors, even at high doses, are often inadequate for reaching the LDL-C lowering goal for many hypercholesterolemic patients (36). Administration of ezetimibe plus garlic extracts offers clinicians a new opportunity to simultaneously inhibit two key pathways in the metabolism of cholesterol; biosynthesis of hepatic cholesterol by garlic and the intestinal cholesterol absorption by ezetimibe. We have previously suggested that, administration of garlic plus ezetimibe is very effective in attenuation of CVD risk factors (30). Lipid transports in the small intestine play a vital role in cholesterol homeostasis. Ezetimibe by inhibition of NPC1L1 significantly decreased plasma cholesterol and LDL-C, and consequently, inhibited atherosclerotic plaque formation and liver steatosis (13, 37). Ezetimibe markedly decreased LDL-C when administrated in mono therapy or in combination with HMG-CoA reductase inhibitors (statins) (13). Altmann SW, et al. demonstrated that cholesterol absorption in NPC1L1 knockout animals was 70% lower than wild-type mice. These animals did not respond to treatment with ezetimibe (13). Davis HR et al. reported that feeding animals with an atherogenic diet (1% cholesterol and 0.5% cholate) leads to 75% decline in NPC1L1 expression in the intestine of mice (38). We also previously reported that consumption of 2% cholesterol and 0.5% cholate or garlic significantly reduced intestinal NPC1L1 expression (19). In this experiment, garlic and ezetimibe reduced NPC1L1 expression, while this reduction was more significant in the combination group. Temel RE, et al. reported that over-expression of NPC1L1 in the liver of transgenic mice, significantly reduced biliary cholesterol and raised plasma cholesterol level, demonstrating that in addition to cholesterol absorption in the intestine, NPC1L1 mediates reabsorption of biliary cholesterol in the liver as well (39). These results show that NPC1L1 plays important roles in biliary and dietary cholesterol absorption in the liver and intestine (40). In Conclusion, this experiment indicated the useful effects of ezetimibe and garlic combination on cardiovascular risk factors. This treatment disclosed a possible novel mechanism for the lipid lowering properties of ezetimibe and garlic combinations. More research is needed to assay NPC1L1 protein levels and other transporters.

Acknowledgements

We are thankful to Kerman University of Medical Sciences for funding this study.

References

1. Anderson KM, Odell PM, Wilson PW, Kannel WB. Cardiovascular disease risk profiles. *Am Heart J*. 1991;**121**(1 Pt 2):293–8.
2. Malik S, Wong ND, Franklin SS, Kamath TV, L'Italien GJ, Pio JR, et al. Impact of the metabolic syndrome on mortality from coronary heart disease, cardiovascular disease, and all causes in United States adults. *Circulation*. 2004;**110**(10):1245–50.
3. Malloy MJ, Kane JP. A risk factor for atherosclerosis: triglyceride-rich lipoproteins. *Adv Intern Med*. 2001;**47**:111–36.
4. Zynolebadi N, Moradi MN, Ghasemi H, Totonchi A, Goodarzi M, Oshaghi EA, et al. New insight in ezetimibe/garlic combination in hypercholesterolemic mice. *Res Pharm Sci*. 2012;**7**(5):S757.
5. Bays HE, Moore PB, Drehobl MA, Rosenblatt S, Toth PD, Dujovne CA, et al. Effectiveness and tolerability of ezetimibe in patients with primary hypercholesterolemia: pooled analysis of two phase II studies. *Clin Ther*. 2001;**23**(8):1209–30.
6. Goodarzi MT, Tootoonchi AS, Karimi J, Abbasi Oshaghi E. Antidiabetic effects of aqueous extracts of three Iranian medicinal plants in type 2 diabetic rats induced by high fructose diet. *Avicenna J Med Biochem*. 2013;**1**:7–13.
7. Dubey NK, Kumar R, Tripathi P. Global promotion of herbal medicine: India's opportunity. *Curr Sci Bangalore*. 2004;**86**(1):37–41.
8. Firenzuoli F, Gori L. Herbal medicine today: clinical and research issues. *Evid Based Complement Alternat Med*. 2007;**4**(Suppl 1):37–40.
9. Ajose FO. Some Nigerian plants of dermatologic importance. *Int J Dermatol*. 2007;**46 Suppl 1**:48–55.
10. Londhe VP. Role of garlic (allium sativum) in various diseases-an overview. *J Pharm Res Opin*. 2014;**1**(4).
11. Mohammadi A, Norouzian P, Jamshidi M, Najafi N, Oshaghi EA.

Effect of garlic (Allium sativum) on lipid profiles, antioxidant activity and expression of scavenger receptor class B type I (SR-BI) in liver and intestine of hypercholesterolemic mice. *J Adv Chem.* 2013;**5**(3):784–91.
12. Mohammadi A, Oshaghi EA. Effect of garlic on lipid profile and expression of LXR alpha in intestine and liver of hypercholesterolemic mice. *J Diabetes Metab Disord.* 2014;**13**(1):20.
13. Altmann SW, Davis HR,Jr., Zhu LJ, Yao X, Hoos LM, Tetzloff G, et al. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science.* 2004;**303**(5661):1201–4.
14. Garcia-Calvo M, Lisnock J, Bull HG, Hawes BE, Burnett DA, Braun MP, et al. The target of ezetimibe is Niemann-Pick C1-Like 1 (NPC1L1). *Proc Natl Acad Sci U S A.* 2005;**102**(23):8132–7.
15. Shahryari J, Poormorteza M, Noori-Sorkhani A, Divsalar K, Abbasi-Oshaghi E. The Effect of Concomitant Ethanol and Opium Consumption on Lipid Profiles and Atherosclerosis in Golden Syrian Hamster's Aorta. *Addict Health.* 2013;**5**(3-4):83–9.
16. Moradi MN, Tootoonchi A, Rezaei A, Abbasi Oshaghi E, Zynolebadi N. Effect of flaxseed on biochemical markers. *Res Pharm Sci.* 2012;**7**(5):S771.
17. Mohammadi A, Mirzaei F, Moradi MN, Jamshidi M, Ghiasvand T, Yari R, et al. Effect of flaxseed on serum lipid profile and expression of NPC1L1, ABCG5 and ABCG8 genes in the intestine of diabetic rat. *Avicenna J Med Biochem.* 2013;**1**(1):1–6.
18. Abbasi Oshaghi E, Noori Sorkhani A, Rezaei A. Effects of walnut on lipid profile as well as the expression of sterol-regulatory element binding protein-1c (SREBP-1c) and peroxisome proliferator activated receptors β (PPARβ) in diabetic rat. *Food Nutr Sci.* 2012;**2012**(3):255–9.
19. Mohammadi A, Bazrafshani MR, Oshaghi EA. Effect of garlic extract on some serum biochemical parameters and expression of npc1l1, abca1, abcg5 and abcg8 genes in the intestine of hypercholesterolemic mice. *Indian J Biochem Biophys.* 2013;**50**(6):500–4.
20. Mohammadi A, Mirzaei F, Jamshidi M, Yari R, Pak S, Norouzian P, et al. Influence of Flaxseed on Lipid Profiles and Expression of LXRa, in Intestine of Diabetic Rat. *Int J Biol.* 2013;**5**(4).
21. Mohammadi A, Mirzaei F, Jamshidi M, Yari R, Pak S, Noori Sorkhani A, et al. The In vivo Biochemical and Oxidative Changes by Ethanol and Opium Consumption in Syrian Hamsters. *Int J Biol.* 2013;**5**(4).
22. Zynolebadi N, Moradi M, Heydarian E, Rezaei A, Abbasi Oshag E. Influence of trientine and flaxseed oil combination on the lipid profiles, antioxidant capacity, malon dialdehyde levels and regeneration of cardiovascular injury in diabetic rats. *Res Pharm Sci.* 2012;**7**(5):S838.
23. Abbas M, Ebrahim Abbasi O, Arash Noori S, Farhad O, Roghaye Hosseini K, Rezaei A. Effect of opium on lipid profile and expression of liver X receptor alpha (LXRβ) in normolipidemic mouse. *Food Nutr Sci.* 2012;**3**(2):249–54.
24. Mohammadi A, Yari R, Farnoosh G, Oshaghi EA. Effect of Ezetimibe on some biochemical factors and expression of Intestinal Scavenger receptor class B type I (SR-BI) in obsess mouse. *Int Res J Biological Sci.* 2013;**3**(3):10–3.
25. Barbi G, Corder CN, Koren E, McConathy W, Ye SQ , Wilson P. Effect of pravastatin and cholestyramine on triglycerideβrich lipoprotein particles and Lp (a) in patients with type II hypercholesterolemia. *Drug Dev Res.* 1992;**27**(3):297–306.
26. Cooper R, Cutler J, Desvigne-Nickens P, Fortmann SP, Friedman L, Havlik R, et al. Trends and disparities in coronary heart disease, stroke, and other cardiovascular diseases in the United States: findings of the national conference on cardiovascular disease prevention. *Circulation.* 2000;**102**(25):3137–47.
27. Vatannejad A, Khodadadi I, Amiri I, Vaisi-Raygani A, Ghorbani M, Tavilani H. Genetic variation of hormone sensitive lipase and male infertility. *Syst Biol Reprod Med.* 2011;**57**(6):288–91.
28. Sacks FM, Tonkin AM, Craven T, Pfeffer MA, Shepherd J, Keech A, et al. Coronary heart disease in patients with low LDL-cholesterol: benefit of pravastatin in diabetics and enhanced role for HDL-cholesterol and triglycerides as risk factors. *Circulation.* 2002;**105**(12):1424–8.
29. Cannon CP, Steinberg BA, Murphy SA, Mega JL, Braunwald E. Meta-analysis of cardiovascular outcomes trials comparing intensive versus moderate statin therapy. *J Am Coll Cardiol.* 2006;**48**(3):438–45.
30. Ballantyne CM, Weiss R, Moccetti T, Vogt A, Eber B, Sosef F, et al. Efficacy and safety of rosuvastatin 40 mg alone or in combination with ezetimibe in patients at high risk of cardiovascular disease (results from the EXPLORER study). *Am J Cardiol.* 2007;**99**(5):673–80.
31. Van Heek M, Farley C, Compton DS, Hoos L, Davis HR. Ezetimibe selectively inhibits intestinal cholesterol absorption in rodents in the presence and absence of exocrine pancreatic function. *Br J Pharmacol.* 2001;**134**(2):409–17.
32. Schrott HG, Stein EA, Dujovne CA, Davidson MH, Goris GB, Oliphant TH, et al. Enhanced low-density lipoprotein cholesterol reduction and cost-effectiveness by low-dose colestipol plus lovastatin combination therapy. *Am J Cardiol.* 1995;**75**(1):34–9.
33. Salen G, von Bergmann K, Lutjohann D, Kwiterovich P, Kane J, Patel SB, et al. Ezetimibe effectively reduces plasma plant sterols in patients with sitosterolemia. *Circulation.* 2004;**109**(8):966–71.
34. Sudhop T, von Bergmann K. Cholesterol absorption inhibitors for the treatment of hypercholesterolaemia. *Drugs.* 2002;**62**(16):2333–47.
35. Labonte ED, Camarota LM, Rojas JC, Jandacek RJ, Gilham DE, Davies JP, et al. Reduced absorption of saturated fatty acids and resistance to diet-induced obesity and diabetes by ezetimibe-treated and Npc1l1-/- mice. *Am J Physiol Gastrointest Liver Physiol.* 2008;**295**(4):G776–83.
36. de Bari O, Neuschwander-Tetri BA, Liu M, Portincasa P, Wang DQ. Ezetimibe: its novel effects on the prevention and the treatment of cholesterol gallstones and nonalcoholic Fatty liver disease. *J Lipids.* 2012;**2012**:302847.
37. Yeh YY, Lin RI, Yeh SM, Evans S. Garlic reduces plasma cholesterol in hypercholesterolemic men maintaining habitual diets. *Food Factors for Cancer Prevention.*: Springer; 1997. pp. 226–30.
38. Davis HJ, Zhu LJ, Hoos LM, Tetzloff G, Maguire M, Liu J, et al. Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. *J Biol Chem.* 2004;**279**(32):33586–92.
39. Temel RE, Tang W, Ma Y, Rudel LL, Willingham MC, Ioannou YA, et al. Hepatic Niemann-Pick C1-like 1 regulates biliary cholesterol concentration and is a target of ezetimibe. *J Clin Invest.* 2007;**117**(7):1968–78.
40. Ge L, Wang J, Qi W, Miao HH, Cao J, Qu YX, et al. The cholesterol absorption inhibitor ezetimibe acts by blocking the sterol-induced internalization of NPC1L1. *Cell Metab.* 2008;**7**(6):508–19.