Iranian Journal of Health and Physical Activity (2012) 3 (1), 1-7

ABCG1 Gene Responses to Treadmill Running with or without *Pistachio-Atlantica* in Female Rats

Navabeh Zare Kookandeh[1], Hamid Deldar [2]*, Abbas Ghanbari Niaki [1], Zarbakht Ansari Pirsaraei [2], Saleh Rahmati Ahmadabad [1], Zahra Raoof [1]

[1] *Department of Physical Education and Sport Science, University of Mazandaran, Baboulsar, Iran.*
[2] *Department of Animal Science, Sari Agricultural Sciences and Natural Resources University, Sari, Iran.*

Received 26 December 2011 Accepted 15 March 2012

Abstract

Intrudaction: ABCG1 is the first member of the ABCG subfamily. The protein is expressed in many cell types and in numerous tissues including the kidney, lung, liver, and intestine. The aim of this study was to determine the small intestine and kidney ABCG1 relative gene expression in response to treadmill-running training in female rats.

Material and Methods: Twenty Wistar rats (6-8 weeks old and 125-135 g weight) were used. Animals were randomly assigned into saline-control (SC), saline-training (ST), and Baneh-control (BC), and Baneh-training (BT). Training groups were given exercise on a motor-driven treadmill at 25 m/min (0% grade) for 60 min/dayand5 days/week for eight weeks. Subjects were fed orally, with Baneh extraction and saline for four weeks. ABCG1 relative gene expression was detected by Real-time PCR method.

Results: results demonstrated that Baneh extraction significantly reduces ABCG1relative gene expression in small intestine ($P < 0.02$).

Discussion and Conclusion: exercise training increases ABCG1relativegeneexpressionin small intestine; therefore exercise training may adjust the reduction in Baneh exercise group.

Key Words: ABCG1, female rats, Treadmill exercise, ABC transporters, Pistachio atlantica

Introduction

Cholesterol and phospholipids are essential to the body, but toomuchof cholesterol or lipids are toxic and a risk factor for arteriosclerosis. Cholesterol transport through the body is an important process in maintaining cholesterol homeostasis. One specific component is theretune of excessive cholesterol from the peripheral tissues to the live r by the high density lipoprotein (HDL). This process is called reverse cholesterol transport [1,2]. In RCT process, the formation and remodeling of HDL-C in plasma requires several factors such as ABC family, lecithin cholesterol acyltransferase (LCAT), lipoprotein lipase (LPL), hepatic lipase (HL), cholesterylester transport protein (CETP), and phospholipid transport protein (PLTP) [3-5]. ATP-binding cassette (ABC) transporters mediate the translocation of a wide variety of substrates such as ions, sugars, amino acids, vitamins, lipids, antibiotics and drugs to larger molecules [6].ABCG1 is the first member of the ABCG subfamily. The protein is expressed in many cell types (including macrophages, endothelial and epithelial cells, T and B cells, type II cells, astrocytes and neurons) and in numerous tissues including the brain, eye, kidney, spleen, lung, liver, and intestine [7-9].In contrast to ABCA1, which transports phospholipids and other lipophilic compounds as well as cholesterol, ABCG1 is largely a cholesterol transporter. [10, 11]. The expression of ABCG1 but not ABCA1 correlates with cholesterol release from cerebellar astroglia [12]. These findings suggest a significant role for ABCG1 and possibly for ABCG4 in the release of cholesterol from cells into the reverse cholesterol transport pathway.

In recent years,many researches have been done on the ABC family. One of these researches was carried out by Ghanbari-Niaki*et al*andinvestigated the effect of 6 weeks' endurance exercise on ABCA1 gene expression in rats.They reported that ABCA1 gene expression increases in rats' liver and that the plasma levels of high density lipoprotein-cholesterol (HDL-C), Pre-β HDL and lecithin cholesterol acyltransferase (LCAT), significantly

* Coresponding author E-mail:
h.deldar@sanru.ac.ir or hamiddeldar@gmail.com

increased [13]. Khabazian*et al* showed that 12 weeks of aerobic exercise, increased mRNA expressions of ABCA1 gene expression in rat's small intestine[14]. The role of various medicinal plants in reducing blood cholesterol and heart disease, including coronary artery disease is well known. More than two thousand years ago, doctors began to use herbs to treat obesity;some of these herbs werewalnut, *pistachio* and *silymarin*. Vecera*et al*reported that *silymarin* positively affects the plasma lipoprotein profile via up-regulation of ABC transporters involved in lipid metabolism [15]. Many researches showed that *pistachio* and Baneh contain high levels of polyunsaturated fatty acids [16]. High levels of polyunsaturated fatty acids found in nuts maylead to appetiteloss,body weight loss and reduction of plasma lipids.

So far, no research has been done on the effect of exercise on ABCG1 relative gene expression; also there is no report about the effect of *Pistachiaatlantica* on ABCG1 relative gene expression. In this research, the effect of exercising and *Pistachiaatlantica*(Baneh) on relative gene expressionof ABCG1 was studied.

Material and Methods

Plant material

The ripped fruit samples of *Pistachiaatlantica* (Baneh) were collected from the fields of Maibod in the Yazd province of Iran, and were stored at –18 ° C until use. Plant Material was identified by herbarium collection in department of physical education and sport science, university of Mazandaran, Baboulsar , Iran.

Preparation of the extracts

The extracts were prepared by maceration (72h) of the coarsely powdered hull and kernel of *Pistachiaatlantica,* with 150 ml tap water for 45 min at room temperature and werefiltered twice through filter paper. The volume of the filtered solution was increased to 100 ml with tap water so that one ml was equivalent to 100 mg of the preparatory material [17].The freshly prepared extracts were cooled and immediately used in the experiments. To the herbalists' recommendation, distilled water was not usedfor the extraction. After training, 100 mg/kg liquid extraction of Baneh was orally assigned to the Baneh groups and the same amount of saline was fed to saline groups.

Animals

All experiments involving the animals were conducted according to the policy of the Iranian convention for the protection of vertebrate animals used for experimental and other scientific purposes; and the protocol was approved by the Ethics Committee of the Sciences, University of Mazandaran (UMZ) and Babol University of Medical Sciences (BUMS, Mazandaran, Iran). Twenty Wistar female rats (6-8 weeks old125-135 gweight) were acquired from Pasteur's Institute (Amol, Mazandaran) and maintained in the Central Animal House of Faculty ofPhysical Education and Sports Science of UMZ. Five rats were housedper cage (46-L volume) with a 12-hour: 12-hour light-dark cycle.Temperature was maintained at 22°C ± 1.4°C. Diets (a pellet form) and water were provided *adlibitum*. Animals were randomly assigned into control (n = 10) and training (n = 10) groups. Rats were divided further into saline-control (SC), saline-training (ST), and Baneh-control (BC), and Baneh-training (BT). The control group remained sedentary, whereas the training group underwent a moderate running exercise program.

Exercise training protocol

At first, the animals were familiarized with the rat treadmill apparatus,everyday and for 4 days [(the 14-lane motorized-driven treadmill was designed by the primary author (UMZ, Babolsar, Mazandaran, Iran)]. The exercise group was trained for 8 weeks using the same training methods previously described [13,14]. The rats run at 25 m/min for 60 minutes, 5 d/wk. The animals were killed 72 hours after the last exercise session. Food but not water was removed from the rat cages 4 hours before the sacrifices.

Tissue biopsies

Seventy-two hours after the last training session, rats were anesthetized with intra peritoneal administration of a mixture of ketamine (30– 50 mg / kg body weight) and xylazine (3– 5 mg / kg body weight). The kidney andsmall intestinewere excised, cleaned, divided into two pieces, washed in ice-cold saline, and immediately frozen in liquid nitrogen and stored at − 80 ° C until RNA extraction.

RNA isolation, cDNA synthesis and Real-time PCR

Total RNA was extracted from 80 to 100 mg of

tissue using RNA purification kits (AccuZol, Bioneer, Cat.No: k3090). Complementary DNA (cDNA) was extended from 1loligo-$(dt)_{18}$primers (0.25 µgper reaction) using cDNA synthesis kit (AccuPower RT PreMix, Bioneer, Cat.No: k2041-B) according to the manufacturer's instructions. Real-time quantitative PCR was performed using Quanti Fast SYBR Green PCR Kit (Cat. No. 204052; Qiagen, GmbH, Germany) in using 15 µl reaction containing 0.5µl single-strand cDNA,7.5µl Master Mix, 1µl of the each forward and reverse primers (5 pmol/µl) and 5µl dH_2O. The primers for ABCG1 and β-actin (as normalizer) were taken from Sporstøl et al, 2007 and Gao, &Yuan, 2010, respectively [18,19]. Expected fragment size and gene bank accession numbers are listed in Table 1. The PCR was carried out on the RotorGene 3000 Real time PCRsystem from Corbett is listed in Table 2.

Product specificity was confirmed in the initial experiments by 3% agarose gel electrophoresis and routinely by melting curve analysis.

Table1: Oligonucleotide primer sequences and real-time PCR amplification parameters

Gene	Forward and reverse primer sequences	Annealing temperature	Amplicon size (bp)	Gene accession no.
ABCG1	F:5′-GAAGGTTGCCACAGCTTCTC-3′ R:5-′CATGGTCTTGGCCAGGTAGT-3′	55 **°C**	339	NM_053502
β-actin	F:5′-TATCGGCAATGAGCGGTTCC-3′ R:5′-AGCACTGTGTTGGCATAGAGG-3′	55 **°C**	145	NM_031144

Table 2: Real-time Cycler conditions

Steps		Time	Temperature
PCR initial activation step		5 min	95 **°C**
Two-step cycling			
Denaturation		10s	95 **°C**
35-45 Cycle	Combined annealing/extension	30s	55 **°C**
Melting Curve		5 min	62 to 95**°C**
Cooling		End	40**°C**

Statistical analysis

The relative levels of mRNA were analyzed by the $2^{-\Delta\Delta Ct}$method [20]. The Kolmogorov-Smirnov test was used to determine the normality of distribution, and variables were found to be normally distributed. All results are expressed as means ± SEM. Statistical analysis were performed using a one way analysis of variance. Least significant difference post hoc test was used in the event of a significant ($P < .05$) F ratio. All statistical analysis was performed with SPSS (Version 13; SPSS, Chicago, IL).

Results

ABCG1 relative gene expression in small

intestine and kidney weredetermined in female rats.Data analysis revealed a significant difference in small intestine ABCG1mRNA relative abundance between groups (F=4.423, P<0.02) (Fig.1). Using a suitable following post hoc test, data were showed that small intestine relative expression of ABCG1 was highest in ST group (2.078±0.52) compared to other groups at the end of program (Fig.1). Also relative expression of ABCG1 was significantly lower in Baneh treated (0.857±0.363) animals when compared with saline-treated groups (P<0.021) (Fig.2). In this regard exercise increases the ABCG1 relative mRNA expression (P<0.049) (Fig. 2). In addition ABCG1 relative mRNA expression in saline group was significantly higher than Baneh groups (P<0.021) (Fig. 2). There was no significant difference between groups, in ABCG1mRAN expression fold change in kidney tissue (F=1.63, P<0.23) (Fig. 3).

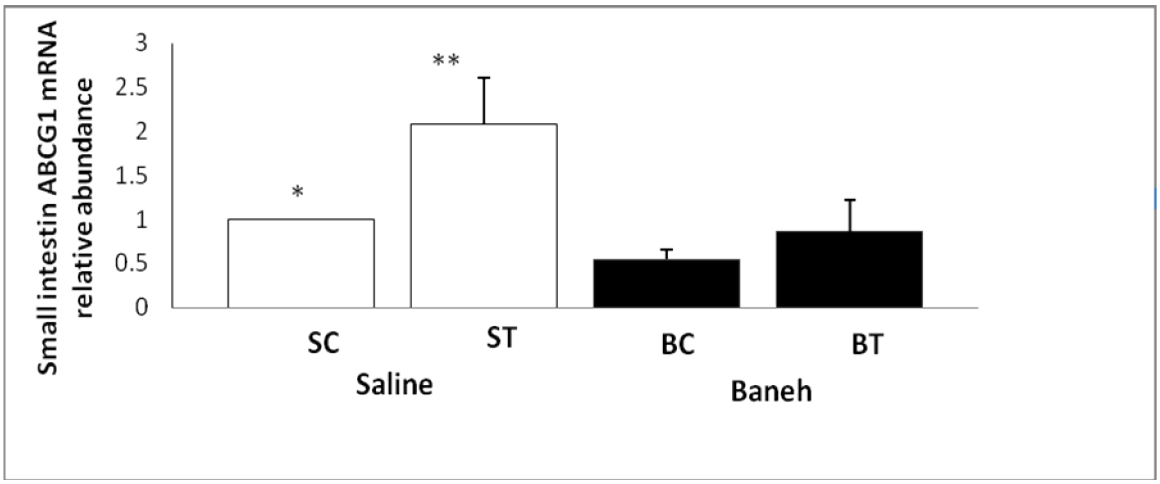

Figure1: Real-time PCR of small intestine ABCG1relative mRNA expression in saline- control (SC), saline-training (ST), Baneh-control (BC), and Baneh-training (BT) wild-type female rats. Data expressed as mean ± SEM. Each column is assigned to one group and 5 rats per each group.*, SC vs ST, (P < 0.029) **,STvs BT, (P < 0.021).

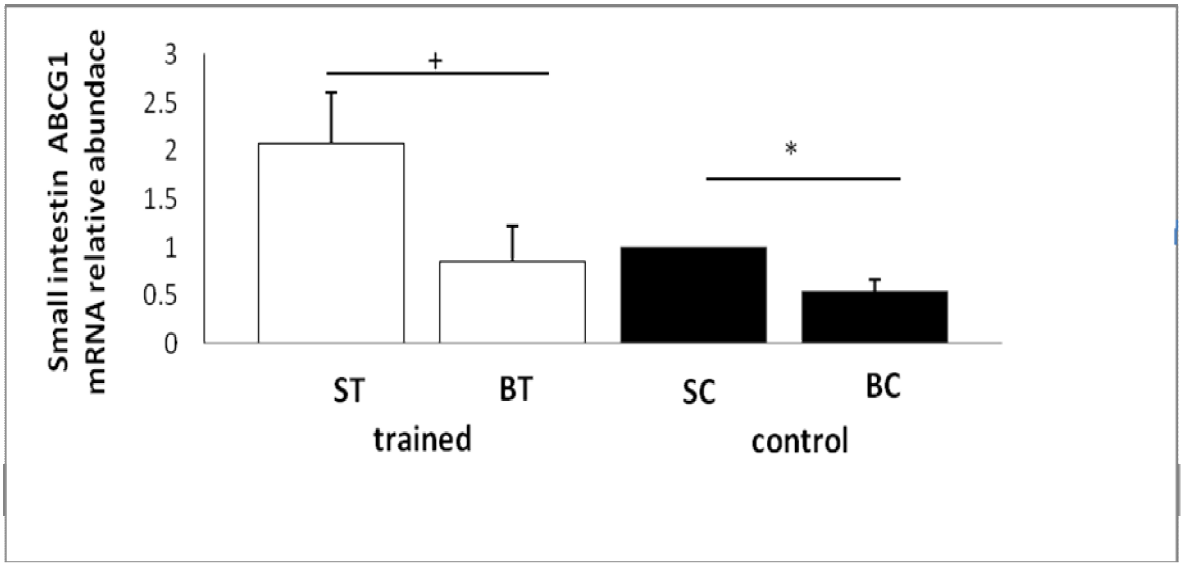

Figure 2: Real-time PCR of small intestine ABCG1relative mRNA expression. Increase of ABCG1 expression in the exercise and saline groups compared to control and Baneh group.*, trained vs Control, (P < 0.049) +, saline vsBaneh, (P < 0.021)

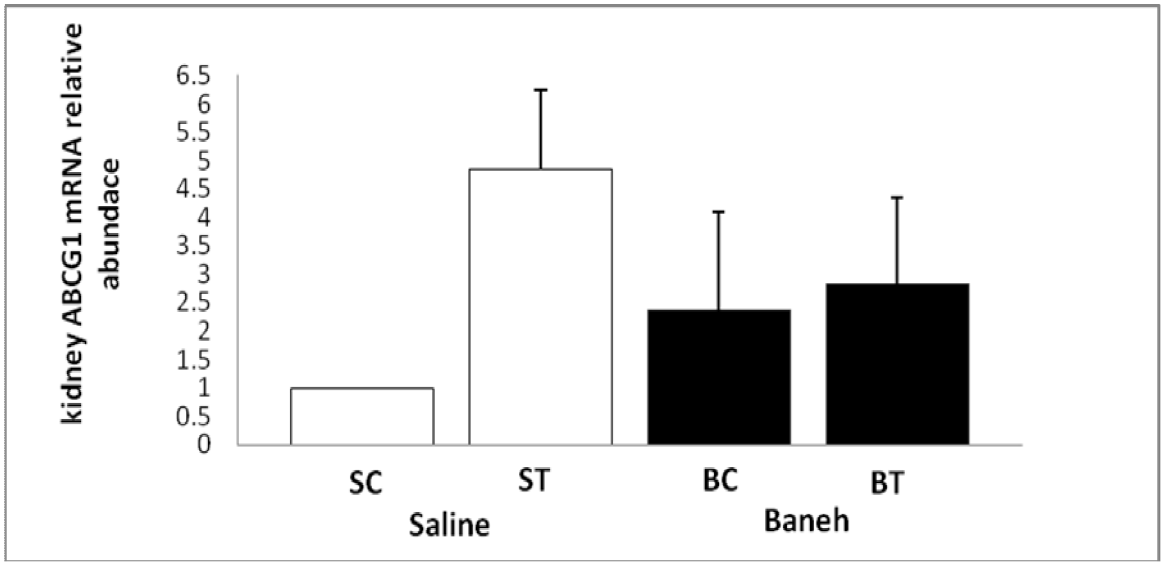

Figure 3: Real-time PCR of kidney ABCG1 relative mRNA expression. No significant differenceswere seen in ABCG1 expression between exercise and saline groups compared to control and Baneh group.

Discussion and Conclusion

To our knowledge, this is the first report to demonstrate alterations of female rat small intestine and kidney ABCG1relative gene expression in response to a treadmill running and Baneh crud extraction regime. The major finding of the present study was a higher ABCG1relative gene expression in trained small intestine and kidney than control groups. Anotherfinding was a lower relative gene expression ofABCG1 in Baneh-treated small intestine and kidney of rats when compared with saline-treated animals. mRNA expression of ABCG1 was observed in several tissues including the brain, eye, kidney, spleen, lung, liver, and intestine [8,9]. In this study we detected ABCG1relative gene expression by Real- time PCR method that is concurrent with previous reports in tissues ABCG1 gene expression. In the present study, exercise increased ABCG1mRNA relative abundance in small intestine tissue (Figures 1). ABCG1 facilitates the efflux of cholesterol from cells to HDL, rather than to free apoA-I [8, 10&11]. In recent years, several studies weredone on ABCA1 transporter. Ghanbari-Niaki et al investigated the effect of 6 weeks' endurance exercise (intensity: 25 m/min, duration: 90 min/session and five days a week), that resulted ABCA1 gene expression increase in rat'sliver. Also the plasma levels of high density lipoprotein-cholesterol (HDL-C), Pre-β HDL and lecithin cholesterol acyltransferase (LCAT), significantly increased [13]. Khabazian et al showed that 12 weeks of aerobic exercise (intensity: 25 m/min, duration: 90 min/session and five days a week, 60 minutes a day, five days a week), increase mRNA expressions of rat's small intestinal ABCA1 gene expression [14]. Also, it has been shown that eight weeks of low-intensity exercise (walking), significantly up-regulated ATP-binding cassette transporters A1 and G1 (ABCA1 and ABCG1, respectively) [21]. It has been shown that a high-fat diet suppresses ABCA1, ABCG4 and ABCG8 gene expression [22]. Previous research showed that *pistachios* are rich in essential oils [23]. Analysis of the *Pistachiaatlantica*varMutica essential oil by GC-MS method, showedthat it is composed of α-pinene (70%), β-pinene (1.94%), 3-carene (0.2%), carveol (2.18%), epoxypinene (2.15%), limonene oxide (9%), myrtenol (5.31%), limonene (0.62%), citral (5.72%), α-phellandrene (0.2%), and β-myrcene (0.3%). The total amount of essential oil obtained was 22% v/w which is higher than any other species of the genus [24]. The important pointof this research wasthe significant reduction of ABCG1 gene expression by Baneh in small intestine and lack of asignificant reduction of ABCG1 gene expression by Banehin kidney that wasprobably due to highlevels of fatty acid in the Baneh (Figures 1,2).

In conclusion, the present study showed a significantlyhigher relative expression of ABCG1 mRNA in small intestine following treadmill

running program in female rats. Also, it revealeda significantdifference in ABCG1 relative gene expression in animals fed with Baneh compared to saline groups. Our results showed that *Pistachiaatlantica* (Baneh) mayreduce ABCG1 relative gene expression in small intestine, and kidney tissuedue to its essential fatty acids.

Acknowledgements

We wish to thank the students of Molecular Genetics lab (Department of Animal Science, Sari Agricultural Sciences and Natural Resources University, Sari, Iran) for their helpful comments and guides.

References

1- Schmitz G, Kaminski WE, Orso E (2000). ABC transporters in cellular lipid trafficking. Current Opinion in Lipidology 11:493–501.

2- Van Eck M, Pennings M, Hoekstra M, Out R, Van Berkel TJ (2005). Scavenger receptor BI and ATP-binding cassette transporter A1 in reverse cholesterol transport and atherosclerosis. Current Opinion in Lipidology 16:307–315.

3- Barter PJ (2002). Hugh Sinclair lecture: the regulation and remodeling of HDL by plasma factors. Atherosclerosis Supplements 3:39–47.

4-Cavelier C, Lorenzi I, Rohrer L, von Eckardstein A (2006). Lipid efflux by the ATP-binding cassette transporters ABCA1 and ABCG1. Biochimica et Biophysica Acta 1761:655–666.

5- Zannis VI, Chroni A, Krieger M (2006). Role of ApoA-I, ABCA1, LCAT, and SR-BI in the biogenesis of HDL. Journal of Molecular Medicine 84:276–294.

6- Higgins CF (1992). ABC transporters: from microorganisms to man. Annual Review of Cell Biology 8:67–113.

7- Kennedy MA, Barrera GC, Nakamura K, Baldan A, Tarr P, Fishbein MC, Frank J, Francone OL, and Edwards P A (2005). ABCG1 has a critical role in mediating cholesterol efflux to HDL and preventing cellular lipid accumulation. Cell Metabolism 1:121-131.

8- Nakamura K, Kennedy MA, BaldanA, Bojanic DD, Lyons K, and Edwards PA (2004). Expression and regulation of multiple murine ATP-binding cassette transporter G1 mRNAs/Isoforms that stimulate cellular cholesterol efflux to high density lipoprotein. Journal of Biological Chemistry 279:45980-45989.

9- Wang N, Yvan-Charvet L, Lutjohann D, Mulder M, Vanmierlo T, Kim TW, Tall AR (2008). ATP-binding cassette transporters G1 and G4 mediate cholesterol and desmosterol efflux to HDL and regulate sterol accumulation in the brain. FASEB Journal 22:1073-1082.

10-Vaughan AM, Oram JF (2005). ABCG1 redistributes cell cholesterol to domains removable by high density lipoprotein but not by lipid-depleted apolipoproteins. Journal of Biological Chemistry 280:30150 –30157.

11- Wang N, Lan D, Chen W, Matsuura F, Tall AR (2004). ATP-binding cassette transporters G1and G4 mediate cellular cholesterol efflux to high density lipoproteins. Proceeding of the National Academy of Sciences of the U S A 101:9774–9779.

12- Karten B, Campenot RB, Vance DE, Vance JE (2006). Expression of ABCG1, but not ABCA1, correlates with cholesterol release by cerebellar astroglia. Journal of Biological Chemistry 281:4049–4057.

13- Ghanbari-Niaki A, Khabazian BM, Hossaini-Kakhak SA, Rahbarizadeh F, Hedayati M (2007). Treadmill exercise enhances ABCA1 expression in rat liver. Biochemical and Biophysical Research Communications 361:841-846.

14- Khabazian BM , Ghanbari-Niaki A, Safarzadeh-Golpordesari AR, EbrahimiM, Rahbarizadeh F, Abednazari H (2009). Endurance training enhances ABCA1 expression in rat small intestine. European Jornal of Applied Physiology 107:351-358.

15-Vecera R, Zacharova A, Orolin J, Skottova N, Anzenbacher P (2011). The effect of silymarin on expression of selected ABC transporters in the rat. Veterinarni Medicina 56:59–62.

16- Saeb M, Nazifi S, Beyzai A, Ghaysari R, Jalali J (2007). Effect of Pistacia atlantica oil consumption on serum Leptin and TSH hormone in male rat. Iran Endocrin and Metabolism 4:429- 437

17-Hamdan II, Afifi FU (2004). Studies on the in vitro and in vivo hypoglycemic activities of some medicinal plants used in treatment of diabetes in Jordanian traditional medicine. Journal of Ethnopharmacology 93:117-121.

18- Sporstøl M, Mousavi SA, Eskild W, Roos N, Berg T (2007). ABCA1, ABCG1 and SR-BI: hormonal regulation in primary rathepatocytes and human cell lines. BMC Molecular Biology 8:1-8.

19- Gao X, Yuan S (2010). High density lipoporteins-based therapies for cardiovascular disease. Journal of Cardiovascular Disease Research 1:99-103.

20-Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods 4:402-408.

21- Butcher LR, Thomas A, Backx K, Roberts A, Webb R, Morris K (2008). Low-intensity exercise exerts beneficial effects on plasma lipids via PPARgamma. Medicine & Science inSports & Exercise 40:1263-1270.

22- De Vogel-Van Den Bosch HM, De Wit NJW,

Hooiveld GJEJ, Vermeulen H, Van Der Veen JN, et al (2008). A cholesterol-free, high-fat diet suppresses gene expression of cholesteroltransporters in murine small intestine. American Journal of Physiology Gastrointestinal and Liver Physiology 294:1171-1180.

23- Gourine N, Bombarda I, Yousfi M, Gaydou EM (2010). Chemotypes of Pistacia atlantica leaf essential oils from Algeria. Natural Product Communications 5:115-120.

24- Delazar A, Reid RG, Sarker SD (2004). GC-MS Analysis of the Essential Oil from the Oleoresin of Pistacia atlantica var. Mutica Chemistry of Natural Compounds 40:24-27.