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Research Article

MYRICETIN, QUERCETIN, KAEMPFEROL AND GALANGIN ALL INCREASE SERUM PARAOXONASE ACTIVITY

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ABSTRACT
Paraoxonase is a calcium dependent enzyme which is related to HDL. Paraoxonase plays an important role in development of atherosclerotic lesions by preventing LDL from oxidation. Different antioxidants such as flavonoids affect serum paraoxonase activity. In the current study, we investigated the effect of some flavonoids on serum paraoxonase activity in rats by gavage feeding of flavonoids in dose 7.5 and 15 mg/kg body weight. Group A received only water and ethanol 25%, group B received myricetin and ethanol 25%, group C received quercetin and ethanol 25%, group D received kaempferol and ethanol 25% and group E received galangin and ethanol 25%. At the end of experimental period, serum paraoxonase activity was evaluated. All of the flavonoids increased serum paraoxonase activity.

Key Words: *Paraoxonase, Myricetin, Quercetin, Kaempferol, Galangin, Atherosclerotic Lesion*

INTRODUCTION
Paraoxonase-1 (PON-1, EC 3.1.8.1) is a calcium dependent serum esterase that bounds to HDL (Arslan *et al.,* 2011; Beltowski *et al.,* 2004b and Yeung *et al.,* 2004). PON-1 belongs to a multigene family in mammals that includes two other members: PON-2 and PON-3 (Primo-Parmo *et al.,* 1996). These three enzymes show similarity at the amino acid levels between mammalian species (Billecke *et al.,* 2000). PON-1 and PON-3 are expressed in the liver while PON-2 is expressed in some tissues such as kidney, liver, brain and testis (La Du *et al.,* 1999). Although PON-1, PON 2 and PON3 lack paraoxonase or arylesterase activities but all of them are able to hydrolyze aromatic and long-chain aliphatic lactones (Draganov *et al.,* 2000). Previous researches have established that PON-1 could hydrolyze organophosphorous compounds such as diazoxon and chlorpyrifos oxon as well as nerve agents such as sarin and soman (Van *et al.,* 2006). The most important action of PON-1 is to prevent LDL from oxidation (Lusis *et al.,* 2004) and to hydrolyze homocysteine thiolactone, so it has a main role in development of atherosclerosis (Jakubowski, 2000 and Kosaka *et al.,* 2005). There are several polymorphisms in the coding region of the human PON1 gene by which the transcriptional levels and catalytic activity of PON1 are determined (Costa *et al.,* 2003a). Two polymorphisms were observed in the coding region of PON1 gene: Gln (Q) Arg substitution at position 192 and Leu (L) Met (M) substitution at position 55 (Costa *et al.,* 2011b).

Various factors alter paraoxonase activity. One of them is flavonoids. Flavonoids are a polyphenols (Tapas *et al.,* 2008) present in human diet (Vaya *et al.,* 2003). They are natural antioxidants that inhibit lipid oxidation. They usually include one or more aromatic hydroxyl groups. This moiety is responsible for the antioxidant activity of flavonoids (Lee *et al.,* 2002). Flavonols are the most widely distributed subclass of flavonoids in nature. Flavonols are found in vegetables and fruits such as onions, apples and tea (Perez-Vizcaino and Duarte, 2010).

Because of the high incidence of cardiovascular disease and the role of paraoxonase in these illnesses, in the present study we investigated the*in vivo* effects of flavonols on serum paraoxonase activity in rats.

MATERIALS AND METHODS

Reagents

Myricetin, quercetin, Kaempferol, galangin, paraoxon and Tris-HCL were purchased from Sigma and calcium chloride was provided from Merck.

Experimental animals

Fifty male Wistar rats weighing 150±20 g were purchased from Pasteur Institute (Tehran, Iran). Rats were housed five per cage. They were fed a rat chow diet and water ad libitum. They were kept in a temperature-controlled environment ($22-25°C$) with an alternative cycle of 12 h light/dark for one week. Before starting the treatment, blood was collected from each rat by retroorbital bleeding of the eye under mild anesthesia using dry tubes. The blood was centrifuged at 3000 rpm and serum was isolated. Then, serum paraoxonase activity was evaluated by the method described by Beltowski *et al.,* (2013).

Experimental protocol

The study protocol was approved by the Ethics Committee of Isfahan University of Medical Sciences. According to the Beltowski's method (Beltowski *et al.*, 2003a), serum paraoxonase activity was measured based on the spectrophotometric measurement of p-nitrophenol levels (Gil *et al.*, 1994 and Juretic *et al.*, 2001). By using this method, Tris-HCL buffer (containing Tris-HCL, 100 mM and CaCl2, 2 mM, PH 8) and paraoxon stock 4 mM were provided. Each set of assays included 40 µL serum, 460 µL Tris-HCL buffers and 500 µL paraoxon. The absorbances were measured at 412 nm and 25°C and the paraoxonase activity was calculated according to the following formula:

$$Activity\ PoN-1(U/L)=\Delta A/T\times f$$

$$f=(TV/sV)\varepsilon 412$$

$$TV=Total\ volume(\mu l)$$

$$SV=sample\ \ volume(\mu L)$$

$$\Delta A=change\ in\ absorbance$$

$$T=Time(\text{min}\,ute)$$

$$\varepsilon_{412}=extinction\ \ \ coefficien\,t\ of\ \ paraoxon\ (\mu M^{-1}cm^{-1})=0/0182$$

(Formula 1)

Fifty rats were assigned randomly to five groups, 10 rats in each group. Experiments were performed for either four or six weeks. All of the groups received the treatments through gavage as follows:

i) Group A: 1 ml 25% ethanol in water.

ii) Group B: 7.5 mg myricetin/kg body weight + in 1 ml 25% ethanol for five rats and 15 mg myricetin/kg body weight + in 1 ml 25% ethanol for another five rats.

iii) Group C: 7.5 mg quercetin/kg body weight + in 1 ml 25% ethanol for five rats and 15 mg quercetin/kg body weight + in 1 ml 25% ethanol for another five rats.

iv) Group D: 7.5 mg kaempferol/kg body weight + in 1 ml 25% ethanol for five rats and 15 mg kaempferol/kg body weight + in 1 ml 25% ethanol for another five rats.

v) Group E: 7.5 mg galangin/kg body weight + in 1 ml 25% ethanol for five rats and 15 mg galangin/kg body weight + in 1 ml 25% ethanol for another five rats.

Gavage feeding was done once a day. The animals were given food and distilled water freely during the experiment.

At the end of the experimental period, blood was collected from heart under anesthesia by ketamine and xylazine. The blood was centrifuged at 3000 rpm. Serum paraoxonase activity was determined by Beltowski method as mentioned above.

One unit of paraoxonase activity is equal to 1 µmole of paraoxon hydrolyzed/min/l.

Statistical analysis

Statistical analysisof serum paraoxonase activities were done by one-way ANOVA using SPSS version 15. A P value<0.05 was considered significant.

RESULTS AND DISCUSSION

Serum paraoxonase activity was increased by all of the flavonoids as shown in figures 1 and 2. In both dosages of 7.5 and 15 mg/kg body weight, there was a significant difference between group B, C, D and E when compared with the control group ($p<0.009$). Our results are in accordance with other studies which stated that antioxidants such as flavonoids could preserve serum paraoxonase activity (Aviram *et al.*, 2000). Investigations *in vivo and in vitro* showed that antioxidants can increase serum paraoxonase activity by preserving the enzyme against oxidative **s**tress (Hayek *et al.*, 1997)

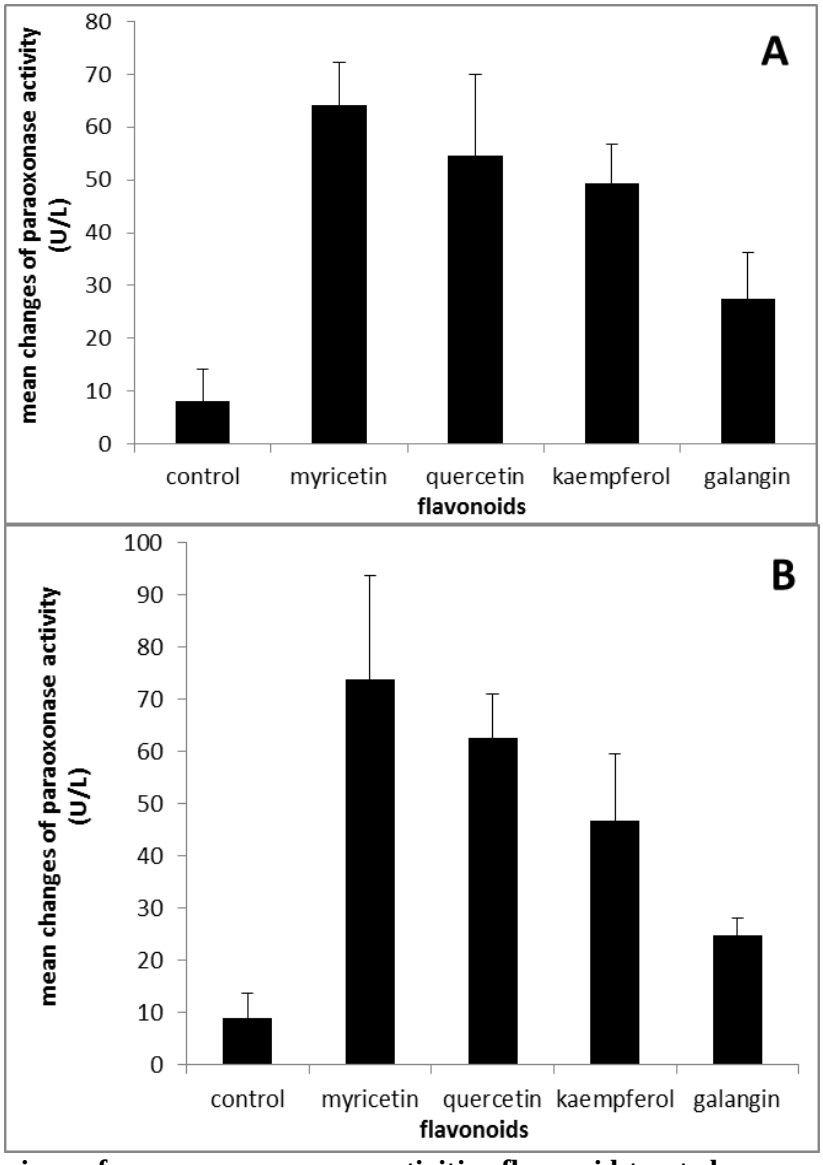

Figure 1: Comparison of serum paraoxonase activities flavonoid-treated groups and the control. Mean changes (Δ mean): of PON-1 activity after treatment –the activity before treatment. A) 7.5 mg flavonoid/kg body weight **B)** 15 mg/kg body weight

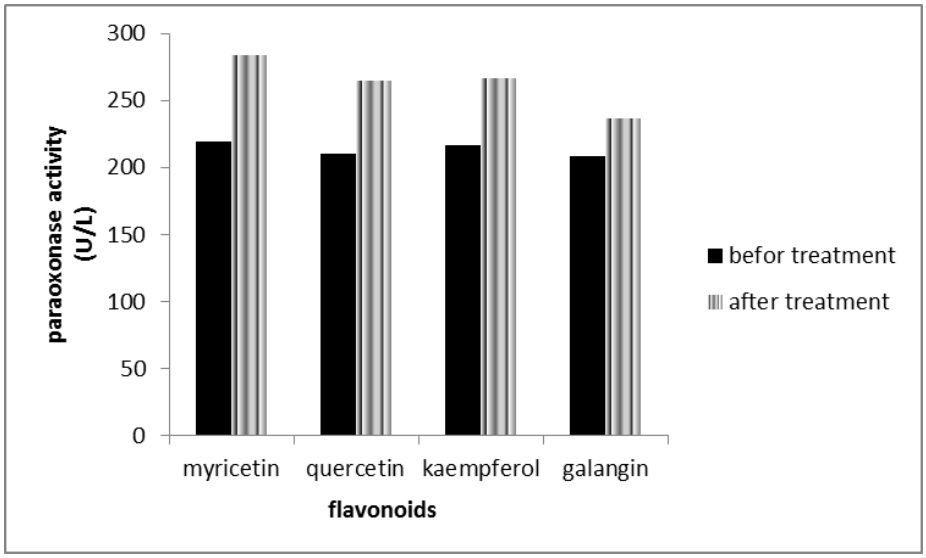

Figure 2: Comparison of serum paraoxonase activity before and after treatment in dosage of 7.5 mg/kg body weight

Our results showed that at a dose of 7.5 mg/kg body weight, there was a significant difference between the activity of serum paraoxonase before treatment and that of four-weeks after gavage treatment with all of the flavonoids ($p<0.043$) (figure 3). The same pattern was obtained with 15 mg dosage. ($p<0.043$) (figure 3). In agreement with our study, are the results of Aldridge (1953) who reported an increased serum paraoxonase activity by galangin. Feese *et al.*, (2002) reported that kaempferol was able to inhibit lipid peroxidation. Administration of quercetin (10 mg/l fluid diet) for four weeks increased the expression of hepatic paraoxonase by 35% and the activity of hepatic paraoxonase by 57% and that of serum paraoxonase by 29% (Leckey *et al.*, 2010). Similarly, 4-week consumption of quercetin by mice devoid of LDL receptors(LDL^{-1}), caused increases of 40% and 90% in hepatic mRNA and serum paraoxonase activity respectively (Rosenblat *et al.*, 2008).

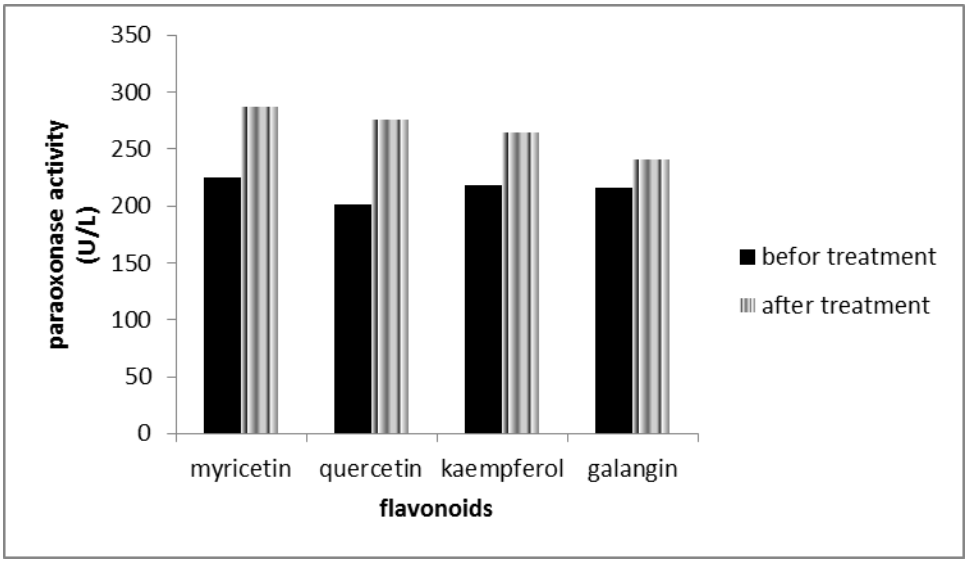

Figure 3: Comparison of serum paraoxonase activity before and after treatment in dosage of 15 mg/kg body weight

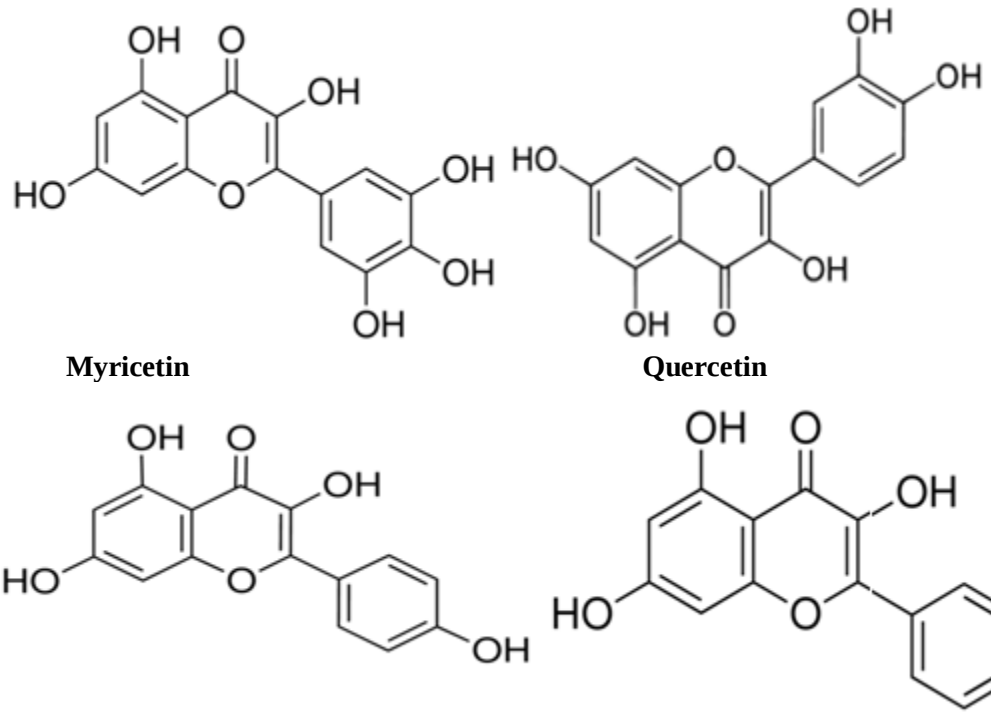

Figure 4: Structure of flavonols

In dosages of 7.5 and 15 mg/kg body weight, the order of increasing serum paraoxonase activity was as follows:

Myricetin > quercetin > kaempferol > galangin (Figure 1 A and B)

It has been shown that there was no significant relationship between dosage of 7.5 and dosage of 15 mg/kg body weight for each of these flavonoids.

Fuhrman and Aviram showed that consumption of wine flavonoids preserved paraoxonase activity. The increased levels of serum paraoxonase in E^0mice have been contributed to the reduction of LDL oxidation in the presence of red wine-derived polyphenolic antioxidants (Fuhrman and Aviram, 2002). Our results about the effect of flavonoids on paraoxonase activity are similar to those of recent studies. Consumption of red wine by 40 E^0mice increased serum paraoxonase activity by 75% during six weeks. Administration of pomegranate to Apo-E deficient rats increased paraoxonase activity by 26% to 43% (Aviram *et al.*, 2000a). Consumption of pomegranate by healthy men caused an increase in serum paraoxonase activity by 20% (Boesch-Saadatmandi *et al.*, 2010b). Also Bosch Saadatmandi *et al.* showed that Flavonols can increase serum paraoxonase activity (Boesch-Saadatmandi *et al.*, 2011a). Rosenblant *et al*. investigated the effect of green tea on serum paraoxonase activity. It was shown that the antioxidants of green tea increased serum paraoxonase activity (Rosenblat *et al.*, 2008). Tas *et al.*, (2011) studied the effect of green tea on streptozotocin-induced diabetic rats for 3 and 6 weeks. They reported that consumption of green tea which is rich in flavonoids had antihyperlipidemic and antioxidative effects and it could prevent progression of atherogenesis by preserving paraoxonase activity. Similarly, in other investigations flavonoids of citrus increased serum paraoxonase activity (Jung *et al.*, 2006).

Figure 4 shows the structure of the flavonoids used in our study. The basic structure of these four flavonoids is the same and the difference is mainly due to the number and location of hydroxyl groups. Paraoxonase activating potential of these flavonoids is probably due to the different numbers and locations of hydroxyl groups on these compounds. Myricetin, quercetin, kaempferol and galangin contain

6, 5, 4 and 3 hydroxyl groups, respectively. The underlying mechanism by which these flavonoids increased serum paraoxonase activity in rats has not yet been fully elucidated.

Conclusion

In this study it was shown that flavonoids can enhance serum paraoxonase activity. Also, we studied the relationship between hydroxyl groups and their effects on paraoxonase activity. However, the mechanism by which flavonoids could increase serum paraoxonase activity is still unknown.

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REFERENCES

ALDRIDGE WN (1953). Serum esterases. I. Two types of esterase (A and B) hydrolysing p-nitrophenyl acetate, propionate and butyrate, and a method for their determination. *Biochemical Journal* **53** 110-117.

Arslan M, Arslan M, and Demir D (2011). Comparison of serum paraoxonase 1 (PON1) activities among different sheep breeds in Turkey. *Journal of Animal and Veterinary Advances* **10** 489-494.

Aviram M, Dornfeld L, Rosenblat M, Volkova N, Kaplan M, Coleman R, Hayek T, Presser D and Beltowski J, Wojcicka G and Jamroz A (2003). Leptin decreases plasma paraoxonase 1 (PON1) activity and induces oxidative stress: the possible novel mechanism for proatherogenic effect of chronic hyperleptinemia. *Atherosclerosis* **170** 21-29.

Beltowski J, Wojcicka G and Jamroz A (2004). Effect of 3-hydroxy-3-methylglutarylcoenzyme A reductase inhibitors (statins) on tissue paraoxonase 1 and plasma platelet activating factor acetylhydrolase activities. *Journal of Cardiovascular Pharmacology* **43** 121-127.

Billecke S, Draganov D, Counsell R, Stetson P, Watson C, Hsu C and La Du BN (2000). Human serum paraoxonase (PON1) isozymes Q and R hydrolyze lactones and cyclic carbonate esters. *Drug Metabolism and Disposition* **28** 1335-1342.

Boesch-Saadatmandi C, Loboda A, Wagner AE, Stachurska A, Jozkowicz A, Dulak J, Doring F, Wolffram S and Rimbach G (2011). Effect of quercetin and its metabolites isorhamnetin and quercetin-3-glucuronide on inflammatory gene expression: role of miR-155. *The Journal of Nutritional Biochemistry* **22** 293-299.

Boesch-Saadatmandi C, Niering J, Minihane AM, Wiswedel I, Gardeman A, Wolffram S and Rimbach G (2010). Impact of apolipoprotein E genotype and dietary quercetin on paraoxonase 1 status in apoE3 and apoE4 transgenic mice. *Atherosclerosis* **211** 110-113.

Costa LG, Cole TB, Jarvik GP and Furlong CE (2003). Functional genomics of the paraoxonase (PON1) polymorphisms: effects on pesticide sensitivity, cardiovascular disease, and drug metabolism. *Annual Review of Medicine* **54** 371-392.

Costa LG, Giordano G and Furlong CE (2011). Pharmacological and dietary modulators of paraoxonase 1 (PON1) activity and expression: the hunt goes on. Biochemical Pharmacology **81** 337-344.

Draganov DI, Stetson PL, Watson CE, Billecke SS and La Du BN (2000). Rabbit serum paraoxonase 3 (PON3) is a high density lipoprotein-associated lactonase and protects low density lipoprotein against oxidation. *Journal of Biological Chemistry* **275** 33435-33442.

Freese R, Alfthan G, Jauhiainen M, Basu S, Erlund I, Salminen I, Aro A and Mutanen M (2002). High intakes of vegetables, berries, and apples combined with a high intake of linoleic or oleic acid only slightly affect markers of lipid peroxidation and lipoprotein metabolism in healthy subjects. *American Journal of Clinical Nutrition* **76** 950-960.

Fuhrman B and Aviram M (2002). Preservation of paraoxonase activity by wine flavonoids: possible role in protection of LDL from lipid peroxidation. *Annals of the New York Academy of Sciences* **957** 321-324.

Gil F, Gonzalvo MC, Hernandez AF, Villanueva E and Pla A (1994). Differences in the kinetic properties, effect of calcium and sensitivity to inhibitors of paraoxon hydrolase activity in rat plasma and microsomal fraction from rat liver. Biochemical Pharmacology **48** 1559-1568.

Hayek T, Fuhrman B, Vaya J, Rosenblat M, Belinky P, Coleman R, Elis A and Aviram M (1997). Reduced progression of atherosclerosis in apolipoprotein E-deficient mice following consumption of red wine, or its polyphenols quercetin or catechin, is associated with reduced susceptibility of LDL to oxidation and aggregation. *Arteriosclerosis, Thrombosis, and Vascular Biology* **17** 2744-2752.

Jakubowski H (2000). Calcium-dependent human serum homocysteine thiolactone hydrolase. A protective mechanism against protein N-homocysteinylation. *The Journal of Biological Chemistry* **275** 3957-3962.

Jung UJ, Lee MK, Park YB, Kang MA and Choi MS (2006). Effect of citrus flavonoids on lipid metabolism and glucose-regulating enzyme mRNA levels in type-2 diabetic mice. *The International Journal of Biochemistry and Cell Biology* **38** 1134-1145.

Juretic D, Tadijanovic M, Rekic B, Simeon-Rudolf V, Reiner E and Baricic M (2001). Serum paraoxonase activities in hemodialyzed uremic patients: cohort study. *Croatian Medical Journal* **42** 146-150.

Kosaka T, Yamaguchi M, Motomura T and Mizuno K (2005). Investigation of the relationship between atherosclerosis and paraoxonase or homocysteine thiolactonase activity in patients with type 2 diabetes mellitus using a commercially available assay. *Clinica Chimical Acta* **359** 156-162.

La Du BN, Aviram M, Billecke S, Navab M, Primo-Parmo S, Sorenson RC and Standiford TJ (1999). On the physiological role(s) of the paraoxonases. *Chemico-Biological Interactions* **119-120** 379-388.

Leckey LC, Garige M, Varatharajalu R, Gong M, Nagata T, Spurney CF and Lakshman RM (2010). Quercetin and ethanol attenuate the progression of atherosclerotic plaques with concomitant up regulation of paraoxonase1 (PON1) gene expression and PON1 activity in LDLR-/- mice. *Alcoholism: Clinical and Experimental Research* **34** 1535-1542.

Lee MK, Bok SH, Jeong TS, Moon SS, Lee SE, Park YB and Choi MS (2002). Supplementation of naringenin and its synthetic derivative alters antioxidant enzyme activities of erythrocyte and liver in high cholesterol-fed rats. *Bioorganic and Medicinal Chemistry* **10** 2239-2244.

Lusis AJ, Fogelman AM and Fonarow GC (2004). Genetic basis of atherosclerosis: part II: clinical implications. *Circulation* **110** 2066-2071.

Perez-Vizcaino F and Duarte J (2010). Flavonols and cardiovascular disease. *Molecular Aspects of Medicine* **31** 478-494.

Primo-Parmo SL, Sorenson RC, Teiber J and La Du BN (1996). The human serum paraoxonase/arylesterase gene (PON1) is one member of a multigene family. *Genomics* **33** 498-507.

Rosenblat M, Volkova N, Coleman R, Almagor Y and Aviram M (2008). Antiatherogenicity of extra virgin olive oil and its enrichment with green tea polyphenols in the atherosclerotic apolipoprotein-E-deficient mice: enhanced macrophage cholesterol efflux. *The Journal of Nutritional Biochemistry* **19** 514-523.

Tapas AR, SDM and Kakde RB (2008). Flavonoids as nutraceuticals: A Review. *Tropical Journal of Pharmaceutical Research* **7** 1089-1099.

Tas S, Celikler S, Ziyanok-Ayvalik S, Sarandol E and Dirican M (2011). Ulva rigida improves carbohydrate metabolism, hyperlipidemia and oxidative stress in streptozotocin-induced diabetic rats. *Cell Biochemistry and Function* **29** 108-113.

Van Himbergen TM, Van Tits LJ, Roest M and Stalenhoef AF (2006). The story of PON1: how an organophosphate-hydrolysing enzyme is becoming a player in cardiovascular medicine. *Netherlands Journal of Medicine* **64** 34-38.

Vaya J, Mahmood S, Goldblum A, Aviram M, Volkova N, Shaalan A, Musa R and Tamir S (2003). Inhibition of LDL oxidation by flavonoids in relation to their structure and calculated enthalpy. *Phytochemistry* **62** 89-99.

Yeung DT, Josse D, Nicholson JD, Khanal A, McAndrew CW, Bahnson BJ, Lenz DE and Cerasoli DM (2004). Structure/function analyses of human serum paraoxonase (HuPON1) mutants designed from a DFPase-like homology model. *Biochimica et Biophysica Acta* **1702** 67-77.