JKMU Research Article

Journal of Kerman University of Medical Sciences, 2017; 24 (1): 16-27

The Effect of Co-administration of Pioglitazone and Simvastatin on Insulin Resistance Parameters and PPAR.γ Expression in Insulin-resistant Rats

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Received: 8 August 2015 Accepted: 9 March 2016

Abstract

Backgrounds: Insulin resistance is a pathological condition associated with metabolic syndrome. In this condition, insulin action in liver, muscles, and adipocytes decreases which leads to hyperglycemia, hyperinsulinemia, and dyslipidemia. Thiazolidinediones (Pioglitazone) have been used to enhance insulin sensitivity but due to dyslipidemia associated with insulin resistance, adult treatment panel III (ATPIII) have suggested statin therapy for ameliorating dyslipidemia in metabolic syndrome.

Method: In this study, 40 rats were randomly divided into 5 groups (8 rats per group). The first group was considered as the healthy control group and fed with regular chow. In other groups, insulin resistance was induced by feeding a high-fructose diet for 6 weeks. Then, the 2nd, 3rd and 4th groups respectively received Pioglitazone, Simvastatin and Simvastatin+Pioglitazone through gavage for 2 weeks and the 5th group (control group) did not receive any drug. At the end of the treatment period, serum samples were collected in fasting condition. The levels of glucose, triglycerides, insulin, and adiponectin were measured by ELISA method, and HOMA-IR was calculated. Animals were anesthetized to remove liver for measuring PPAR.γ expression.

Results: Blood glucose in Pioglitazone group (129.1±5.8 mg/dl) and Simvastatin+Pioglitazone group (137.1±9.9 mg/dl), triglyceride in Simvastatin group (123.6±16.6 mg/dl) and Simvastatin+Pioglitazone group (101.5±7.5 mg/dl), insulin in Pioglitazone group (40.27±2.75 pmol/L), Simvastatin group (70.07±10.35 pmol/L), and Simvastatin+Pioglitazone group (47.62±2.80 pmol/L) and adiponectin in Pioglitazone group (5.90±0.29 µg/ml) and Simvastatin+Pioglitazone group (5.89±0.41 µg/ml) showed significant differences with the corresponding values in the control group [blood glucose (187.5±15.9 mg/dl), triglyceride (217.6±18.5 mg/dl), adiponectin (3.86±0.14 µg/ml), insulin (137.65±34.22 pmol/L) and HOMA-IR (9.7±2.13)]. Pioglitazone significantly increased PPAR.γ expression, but Simvastatin suppressed the effect of Pioglitazone on PPAR.γ expression.

Conclusion: The results show that Simvastatin has beneficial effects on insulin resistance in rats fed with high-fructose diet, but it has no synergistic or antagonistic effect with Pioglitazone.

ARTICLE INFO

Article type:
Original article

Keywords:
Insulin Resistance
Adiponectin
Simvastatin
Pioglitazone

Introduction

Insulin resistance is characterized by abnormal and weak response to insulin. It is diagnosed through reduction of target tissues sensitivity to insulin, increase in glucose concentration and production of atherogenic lipids in the liver (1). Insulin resistance is associated with other metabolic disorders like type 2 diabetes, glucose intolerance, obesity, lipid metabolism disorders, atherosclerosis, and hypertension, which are collectively known as metabolic syndrome (2-4). Thiazolidinediones are a class of medications that are commonly used for increasing insulin sensitivity and function as agonists for peroxisome proliferator activated receptor γ (PPARγ). PPARs are hormone receptors in the cell nucleus that are bound to DNA and regulate the transcription of a wide range of genes. PPAR.γ is one of the most important factors in metabolic syndrome because it functions as an important transcription regulator for adipogenesis and lipogenesis and plays an important role in glucose homeostasis and insulin sensitivity (5, 6). The role of PPAR.γ in insulin sensitivity is confirmed by this finding that Thiazolidinediones (Rosiglitazone and Pioglitazone), a class of anti-diabetic drugs, have a high affinity for PPAR.γ (5).

In insulin resistance, increased hepatic gluconeogenesis and glucose output, elevation of lipolysis in fat tissues and consequently, increased plasma fatty acids and production of VLDL occur which lead to metabolic dyslipidemia and hypertriglyceridemia (7). According to the adult treatment panel III (ATPIII), the treatments that reduce LDL cholesterol and triglycerides and increase HDL cholesterol should be considered in metabolic syndrome (7, 8). Statins are a class of medications that inhibit the production of endogenous cholesterol through inhibiting HMG-CoA reductase, and reduce circulating LDL levels (7, 8). Considering the various and controversial roles reported on the effects of statins on insulin sensitivity, and also co-administration of this medicine with Thiazolidinediones in dyslipidemia associated with insulin resistance, the aim of this study was to evaluate the role of simvastatin when used in combination with pioglitazone on the effect of pioglitazone on insulin sensitivity and PPAR.γ expression.

Materials and Methods

Preparation of High-fructose Food

In order to prepare high-fructose food (containing 60% fructose), according to Shih et al, fructose was mixed with casein (60:40 respectively), multivitamins, minerals, and methionine. The mixture was changed to dough by adding distilled water. Then, this dough was formed as standard pellets and dried in the air (9). The food was kept in the refrigerator for use.

Animals and treatment

For this study, 40 male Wistar rats (250-300 g) were purchased from Afzalipour School of

Medicine. The rats were transferred into a separate room in animal house and randomly grouped in 4-well shelves. To get used to the new environment, animals were kept in this room with standard condition (12 hrs. dark/ light cycle) at 20° C for 2 weeks.

After two weeks, animals were randomly divided into 5 groups (8 rats per group). As a healthy control group, the first group was fed with standard rat food. Other groups were fed with high-fructose diet for 6 weeks, and at the end of 6 weeks, to ensure the development of insulin resistance, glucose tolerance test was performed on animals after a 12-hour fast. Results showed the presence of insulin resistance in all animals compared to the healthy control group (group I, results not showed). Then, treatment with drugs was started and continued for two weeks as follows:

Group II: received Pioglitazone (10 mg/kg/day) (10).

Group III: received Simvastatin (25 mg/kg/day) (11).

Group IV: received Pioglitazone (10 mg/kg/day) and Simvastatin (25 mg/kg/day).

Group V: received no medication (as a control group)

Drugs (dissolved in 1 ml PBS for each rat) were administered through gavage.

Animals were weighed weekly and in the last two weeks, their daily food and water intake were measured and recorded.

All samples were collected in the morning, after a 12-hour fast. For sampling, rats were anesthetized with ether and blood samples were taken from their heart. The blood samples were kept on ice until serum was separated. Samples were immediately centrifuged at 3000 rpm for 10 minutes at 4°C. Liver was removed to study PPAR.γ expression. Liver tissue was immediately frozen in liquid nitrogen. Serum samples and tissues were kept in the freezer at -75°C until the time of experimation.

Measurement of Biochemical Factors

Using enzymatic methods, glucose, triglycerides, cholesterol, and HDL-c were measured by a RA-1000 auto analyzer in Razi Clinical Laboratory (Kerman).

Measurement of Insulin and Adiponectin, and Calculation of HOMA-IR:

Insulin and adiponectin concentations were measured by ELISA (USCN Company) according to manufacturer's protocol. HOMA-IR was calculated according to the following equation: [glucose (mmol/L) × insulin (μU / ml)/22.5].

Measurement of PPAR.γ Transcription

The level of PPAR.γ transcription was determined by measuring the level of its mRNA in liver cells through Real Time PCR. For this purpose, using RNA extraction kit (Rneasy mimi kit, Qiagen), the total RNA was extracted

according to the kit protocol. The purity of extracted RNA was confirmed by calculating the 260nm:280nm absorbance ratio. The quality of extracted RNA was evaluated after electrophoresis on agarose gel and observing the ribosomal RNA bands.

Using cDNA Synthesis Kit (Quanti Tect Reverse Transcription Kit, Qiagen), complementary DNAs (cDNA) were produced according to the kit protocol and used for PCR. Polymerase chain reaction (PCR) was performed using PCR kit (QuantiFast SYBR Green PCR kit, Qiagen). After drawing standard curve charts for target gene (PPAR.γ) and reference gene (GAPDH) as well as calculation and comparing the performance of PCR, the $2^{-\Delta\Delta CT}$ equation was used to compare gene expression in different groups. The sequences of primers used are shown in Table 1.

Table 1. Sequences of primers used in Real Time PCR for PPAR.γ and GAPDH

Gene	Primer Sequences	Product Size (Base Pairs)	Accession Number
	R: TGTCATATTTCTCGTGGTTCA		
Gene	**Primers Sequence**	**Product Size (Base Pairs)**	**Accession Number**
PPAR.γ	F: CATGCTTGTGAAGGATGCAAG R: TTCTGAAACCGACAGTACTGACAT	131	NM_001145367
GAPDH	F: TGGAGTCTACTGGCGTCTT R: TGTCATATTTCTCGTGGTTCA	138	NM_017008

Data Analysis

Data were analyzed using SPSS 18. Statistical analysis was performed using One-way ANOVA and Tukey's post hoc Test. Results are reported as Mean ± SEM. P values <0.05 are considered significant.

Results

Body Weight changes

Animals' initial weighs and their weight measured during the experiment at the beginning of each week showed no significant difference. In addition, mean of weight gain (final weight - initial weight) during eight weeks showed no significant difference between the groups.

Blood Glucose Levels

Blood glucose levels in the pioglitazone group (129.1 ± 5.8 mg/dl) and simvastatin+pioglitazone group (137.1 ± 9.9 mg/dl) compared to that of the control group (187.5 ± 15.9 mg/dl) showed a significant decrease. However, mean blood glucose of simvastatin group (166.6 ± 12.1 mg/dl) showed no significant difference in comparison to the control group. In addition, the level of blood glucose in pioglitazone and simvastatin groups showed no significant difference compared to simvastatin-pioglitazone group. In other words, co-administration of simvastatin and pioglitazone had no synergistic or antagonistic effect with pioglitazone with regard to controls. The results are summarized in Table 2.

Triglyceride and Cholesterol Levels

Triglyceride levels in simvastatin group (123.6± 16.6 mg/dl) and simvastatin-pioglitazone group (101.5±7.5 mg/dl) showed a significant decrease compared to the control group (217.6±18.5 mg/dl). In addition, triglyceride levels in simvastatin and simvastatin+pioglitazone groups compared to the pioglitazone group (200.7 ± 21.6 mg/dl) were significantly lower. However, total cholesterol and HDL-c levels showed no significant difference among groups. The results are summarized in Table 2.

Insulin and Adiponectin Levels

Fasting insulin levels in pioglitazone (40.27±2.75 pmol/L), simvastatin (70.07±10.35 pmol/L) and simvastatin+pioglitazone (47.62±2.80 pmol/L) groups were significantly different compared to the control group (137.65 ± 34.22 pmol/L). Adiponectin level in pioglitazone (5.90±0.29 μg/ml) and simvastatin-pioglitazone (5.89±41 μg/ml) groups showed a significant increase compared to the control group (3.86±0.14μg/ml). Adiponectin level in simvastatin group (3.72 ± 55 μg/ml) showed no significant difference compared to the control and pioglitazone groups. The results are summarized in Table 2.

HOMA-IR Values

Comparison of mean HOMA-IR among the groups revealed that compared to the control group, pioglitazone (2.11 ± 0.13), simvastatin (4.76 ± 0.37), and simvastatin-pioglitazone (2.70 ± 0.29) signigicantly decreased the level of HOMA-IR (9.7 ± 2.13). The level of HOMA-IR in the control group (2.76±0.38) compared to the control group was significantly lower, which confirms the insulin resistance in this group. The results are summarized in Table 2.

Table 2. *Changes in serum parameters and body weight in insulin-resistant rats after the treatment period*

	Studied Groups			
	Control	**Pioglitazone**	**Simvastatin**	**Simvastatin-pioglitazone**
Initial Weight (g)	270±8	268±13	259±17	264±13
P value		1	0.997	0.999
Total Weight (g)	307±8	294±16	287±24	304±17
P value		1	0.991	1
Wight Gain (g)	37±8	26±12	28±19	40±14
P value		0.678	0.998	0.740
Blood Glucose (mg/dl)	187.5±15.9	129.1±5.8	166±12	137.1±9.9
P value		0.002	0.86	0.012
Triglyceride (mg/dl)	217.6±18.5	202±26	123.6±16.6	101.5±7.5
P value		1	0.054	0.06
Total Cholesterol (mg/dl)	59±3	63±4	54±3	61±5
P value		1	0.996	1
HDL (mg/dl)	28±2	29±1	26±1	27±2
P value		1	0.937	0.989
Insulin (pmol/L)	137.65±34.22	40.27±2.75	70.07±10.35	47.62±2.80
P value		<0.0001	<0.0001	<0.0001
Adiponectin (µg/ml)	3.86±0.14	5.90±0.29	3.72±0.55	5.89±0.41
P value		0.006	0.516	0.006
HOMA-IR	9.7±2.13	2.11±0.13	4.76±0.37	2.70±0.29
P value		<0.0001	<0.0001	<0.0001

The values have been reported as Mean ± SEM, and the reported values show difference in comparison to the control group (without any drug therapy)

PPAR.γ Transcription

As shown in Figure 1, the relative expression of PPAR.γ in the pioglitazone group compared to the control group showed a significant difference (p=0.027), but in the simvastatin group no significant difference was observed compared to the control group (p=0.793). It was also found that simvastatin inhibits the effect of pioglitazone on PPAR.γ expression; that is, co-administration of simvastatin and pioglitazone did not increase PPAR.γ expression (p=0.992). The expression level of PPAR.γ in all 4 groups is shown in Figure 1.

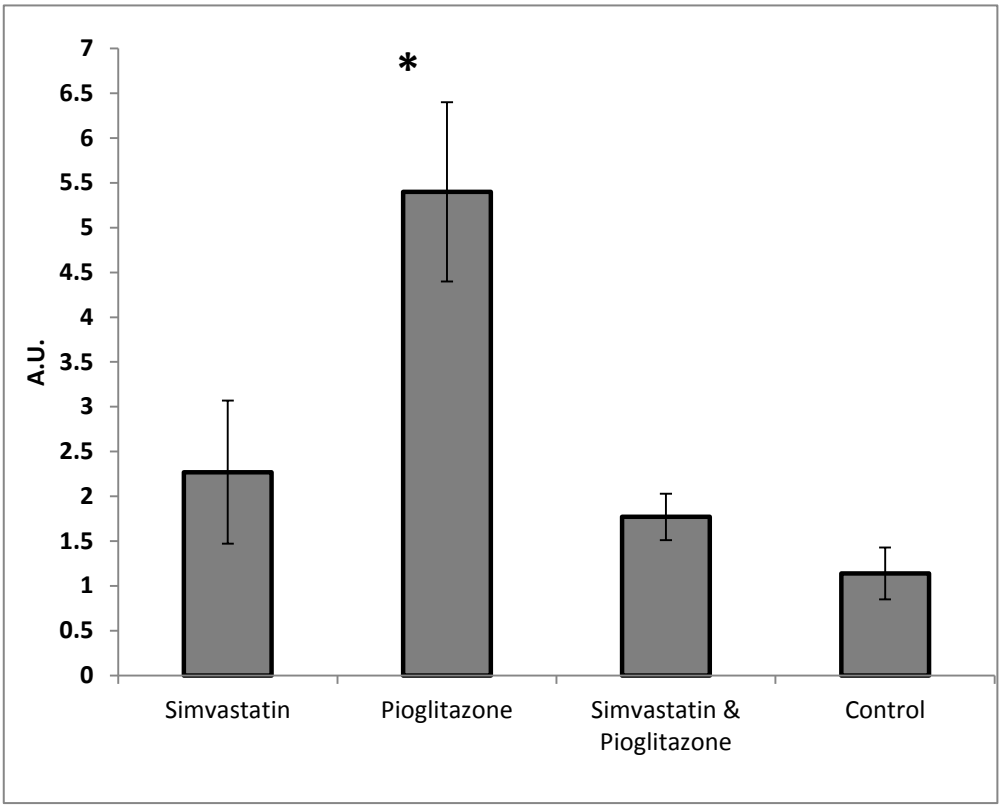

Figure 1. changes in PPAR.γ expression induced by pioglitazone, simvastatin, and simvastatin+ pioglitazone in insulin-resistant rats

Discussion and Conclusion

Insulin resistance is one of the main charactristics of type 2 diabetes that plays an important role in the pathogenesis of this disease (12). Thiazolidinedione's are a class of medications that have been used to increase insulin sensitivity since 1997 (13). On the other hand, due to dyslipidemia which is common in insulin resistance and metabolic syndrome, statins are recommended by the ATPIII for improving dyslipidemia (8). Therefore, in this study the effect of administration of pioglitazone or simvastatin alone and in combination on insulin resistance factors was evaluated.

Results showed that treatment with simvastatin, pioglitazone, and their combination for two weeks, did not have any significant effect on the body weight in insulin-resistant rats. Blood glucose level in pioglitazone (P=0.002) and pioglitazone-simvastatin (P=0.012) groups was significantly decreased, however, this decrease was more significant in pioglitazone group compared to pioglitazone+simvastatin group. Insulin level was significantly decreased in pioglitazone, simvastatin, and pioglitazone+simvastatin groups

(P<0.0001). In addition, HOMA-IR as a marker of insulin resistance, showed a significant reduction in all 3 groups compared to the control group (P <0.0001).

In a study performed on the effect of co-administration of simvastatin and pioglitazone on inflammatory factors, it was reported that simvastatin has no effect on HOMA-IR (14). This difference with our results can be attributed to the difference in the design of two studies. Because the mentioned study was performed on non-diabetic patients at risk of atherosclerosis, but the present study was performed on the insulin resistant rats without clear dyslipidemia.

Adiponectin is the most abundant plasma adipocytokine involved in regulating insulin sensitivity, inflammation, and lipid metabolism. Reducing this adipocytokine leads to increase in insulin resistance and risk of metabolic syndrome (3, 15). The results also show that adiponectin concentration was significantly increased by pioglitazone and pioglitazone+ simvastatin but simvastatin showed no effect on the level of adiponectin. In the study of Bulcao et al (2007), simvastatin did not show any effect on insulin sensitivity in patients with metabolic syndrome and it just improved their dyslipidemia. In addition, no relationship between lipid profile and HOMA-IR has been reported in this study (16).

Ding et al (2009) reported that statin therapy reduces insulin sensitivity and adiponectin secretion (17). In the study of Forst et al (2007) on non-diabetic patients with risk of atherosclerosis, it was revealed that simvastatin reduces adiponectin secretion (18). Consistent with the study of Schaalan et al (2012), the present study indicated that simvastatin has no significant effect on adiponectin level (4).

In the study of Wang et al (2013) on Streptozotocin-induced diabetic rats, it was revealed that simvastatin increases the hyperglycemia (19), this is in contrast with the results of the present study. This inconsistency can be due to the dysfunction of beta cells in animals affected by streptozotocin while in the present study, the animals have were made insulin resistant only by high-fructose diet.

Paolisso et al (2000), in their study on patients with type 2 diabetes, reported that simvastatin and atorvastatin reduce HOMA and also improve dyslipidemia in these patients (20), this is in agreement with the results of the present study. Lalli et al (2008) also reported that statins increase insulin sensitivity in insulin-resistant animals treated with lovastatin. They suggest elevation of tyrosine phosphorylation of IRS as the probable mechanism (21).

In the study of Shen et al, it was reported that simvastatin increases the PPAR.γ activity and inhibits NFκB (22). Since PPAR.γ has a role in insulin sensitivity (23), increase in the activity of this receptor can be considered as one of the main mechanisms through which simvastatin improves insulin resistance.

Forst et al, in their study on the effect of co-administration of pioglitazone and simvastatin in non-diabetic patients at risk of cardiovascular disease, showed that pioglitazone and pioglitazone in combination with simvastatin increase adiponectin secretion and decrease HOMA-IR, but simvastatin alone has no effect on HOMA and reduces adiponectin secretion (18). This is in contrast with the results of the present study. It has been also reported in Forst et al study that co-administration of these drugs improves lipid status (18). In Leonhardt et al study, a synergistic effect of simvastatin and pioglitazone on the reduction of small- dense LDLs (Low-density lipoprotein) has been confirmed (24).

Schaalan et al in their study on the effect of co-administration of simvastatin and pioglitazone in insulin-resistant rats reported that simvastatin can enhance the effect of pioglitazone on elevation of insulin sensitivity and adiponectin secretion; even though, it has no effect on blood glucose level (4).

The present study showed that simvastatin inhibits the effects of pioglitazone on the induction of PPAR.γ expression, although it has no effect on pioglitazone role in increasing insulin sensitivity. Thiazolidinediones (Rosiglitazone, Pioglitazone, and Troglitazone) as PPAR.γ agonists activate these receptors, however according to several studies, these medications increase the expression of PPAR.γ as well. Rosiglitazone increases PPAR.γ expression in the liver of insulin-resistant rats (10) and skeletal muscle cell line (L6) (25).

Pioglitazone also increases PPAR.γ expression in the liver of rats fed with high-fructose diet (26) which is consistent with the results of the present study. Through activating PPAR.γ expression, pioglitazone increases the secretion of adiponectin and consequently reduces insulin resistance (18). It is worth mentioning that pioglitazone, through a PPAR.γ- independent mechanism improves TNF.α- induced insulin resistance by increasing phosphorylation of tyrosine roots in IRS-1 (27). In the inflammatory conditions that SOCS.3 expression increases, pioglitazone reduces the expression of this protein to its basal level (26). Given that the increased mRNA expression??? of a gene does not necessarily lead to an increase in the relevant protein (28), simvastatin, despite pioglitazone-induced mRNA increase, had no effect on insulin sensitivity. It is likely that there is no increase in the PPAR.γ expression. However, further studies are needed to confirm this claim.

Considering the results and as it was expected, it can be concluded that pioglitazone increases the insulin sensitivity in insulin-resistant rats but has no effect on body weight or triglyceride levels. According to the ATPIII, simvastatin monotherapy is recommended in insulin-resistant animals to improve the lipid profile in metabolic syndrome. Although simvastatin has no significant effect on the level of blood glucose, it increases insulin sensitivity. However, co-administration of simvastatin and pioglitazone compared to pioglitazone alone, besides increasing insulin

sensitivity, reduces triglyceride and consequently improves lipid profile. No significant synergistic or antagonistic effect was observed in their co-administration. Therefore, in contrast with some studies suggesting a negative role for statins in insulin resistance, the results of the present study show that co-administration of simvastatin and pioglitazone does not reduce the effect of pioglitazone on developing insulin sensitivity.

References

1. Sathya Bhama C.V, Balaji S, Sithalakshemi A. Analysis of the degree of insulin resistance in post menopausal women by using skin temperature measurements and fasting insulin and fasting glucose levels: a case control study. *J Clin Diagn Res* 2012; 6 (10):1644-7.
2. de Luca C, Olefsky JM. Inflammation and insulin resistance. *FEBS Lett* 2008; 582 (1):97-105.
3. Ding Y, Li S, Ma RL, Guo H, Zhang J, Zhang M, et al. Association of homeostasis model assessment of insulin resistance, adiponectin, and low-grade inflammation with the course of the metabolic syndrome. *Clin Biochem* 2015; 48 (7-8):503-7.
4. Schaalan MF. Effects of pioglitazone and/or simvastatin on circulating TNFalpha and adiponectin levels in insulin resistance. *J Immunotoxicol*. 2012; 9(2): 201-9.
5. He W, Barak Y, Hevener A, Olson P, Liao D, Le J, et al. Adipose-specific peroxisome proliferator-activated receptor gamma knockout causes insulin resistance in fat and liver but not in muscle. *PNAS* 2003; 100 (26):15712-7.
6. Zhang Y, Zhan RX, Chen JQ, Gao Y, Chen L, Kong Y, et al. Pharmacological activation of PPAR gamma ameliorates vascular endothelial insulin resistance via a non-canonical PPAR gamma-dependent nuclear factor-kappa B trans-repression pathway. *Europ J Pharmacol* 2015; 754: 41-51.
7. Chong T, Naples M, Federico L, Taylor D, Smith GJ, Cheung RC, et al. Effect of rosuvastatin on hepatic production of apolipoprotein B-containing lipoproteins in an animal model of insulin resistance and metabolic dyslipidemia. *Atherosclerosis* 2006; 185 (1):21-31.
8. Naples M, Federico LM, Xu E, Nelken J, Adeli K. Effect of rosuvastatin on insulin sensitivity in an animal model of insulin resistance: evidence for statin-induced hepatic insulin sensitization. *Atherosclerosis* 2008; 198 (1):94-103.
9. Shih CC, Lin CH, Lin WL, Wu JB. Momordica charantia extract on insulin resistance and the skeletal muscle GLUT4 protein in fructose-fed rats. *J Ethnopharmacol* 2009; 123 (1):82-90.
10. Yadav H, Jain S, Yadavb M, Sinha PR, Prasad GBKS, Marotta F. Epigenomic derangement of hepatic glucose metabolism by feeding of high fructose

diet and its prevention by Rosiglitazone in rats. *Digestive and Liver Disease* 2009; 41: 500-8.

11. QIN YW, YE P, HE JQ, Seng L, Wang LY, Du J. Simvastatin inhibited cardiac hypertrophy and fibrosis in apolipoprotein E-deficient mice fed a "Western-style diet" by increasing PPAR α and γ expression and reducing TC, MMP-9, and Cat S levels. *Acta Pharmacol Sin* 2010; 31 (10):1350-8.
12. DeFronzo RA, Tripathy D. Skeletal muscle insulin resistance is the primary defect in type 2 diabetes. *Diabetes Care* 2009; 32 Suppl 2: S157-63.
13. Quinn CE, Hamilton PK, Lockhart CJ, McVeigh GE. Thiazolidinediones: effects on insulin resistance and the cardiovascular system. *Br J Pharmacol* 2008; 153 (4):636-45.
14. Hanefeld M, Marx N, Pfutzner A, Baurecht W, Lubben G, Karagiannis E, et al. Anti-inflammatory effects of pioglitazone and/or simvastatin in high cardiovascular risk patients with elevated high sensitivity C-reactive protein: the PIOSTAT Study. *J Am Coll Cardiol* 2007; 49 (3): 290-7.
15. Ding YS, Guo SX, Ma RL, Li SG, Guo H, Zhang JY, et al. Association of Metabolic Syndrome with the Adiponectin to Homeostasis Model Assessment of Insulin Resistance Ratio. *Mediators Inflam* 2015; 607364.
16. Bulcão C, Ribeiro-Filho FF, Sañudo A, Roberta Ferreira SG. Effects of simvastatin and metformin on inflammation and insulin resistance in individuals with mild metabolic syndrome. *Am J Cardiovasc Drugs*2007; 7 (3): 219-24.
17. Ding PY-A, Hsu P-F, Lu T-M. Statin Therapy on Insulin Resistance and Plasma Level of Adiponectin in Non-Diabetic, Hypercholesterolemic Patients. *Acta Cardiol Sin* 2009; 25: 183 9.
18. Forst T, Pfutzner A, Lubben G, Weber M, Marx N, Karagiannis E, et al. Effect of simvastatin and/or pioglitazone on insulin resistance, insulin secretion, adiponectin, and proinsulin levels in nondiabetic patients at cardiovascular risk-the PIOSTAT Study. *Metabolism* 2007; 56 (4): 491- 6.
19. Wang L, Duan G, Lu Y, Pang S, Huang X, Jiang Q, et al. The Effect of Simvastatin on Glucose Homeostasis in Streptozotocin Induced Type 2 Diabetic Rats. *Journal of Diabetes Research* 2013; 1:1-5.
20. Paolisso G, Barbagallo M, Petrella G, Ragno E, Barbieri M, Giordano M, et al. Effects of simvastatin and atorvastatin administration on insulin resistance and respiratory quotient in aged dyslipidemic non-insulin dependent diabetic patients. *Atherosclerosis* 2000; 150 (1):121-7.
21. Lalli CA, Pauli JR, Prada PO, Cintra DE, Ropelle ER, Velloso LA, et al. Statin modulates insulin signaling and insulin resistance in liver and muscle of rats fed a high-fat diet. *Metabolism* 2008; 57 (1): 57-65.
22. Shen Y, Wu H, Wang C, Shao H, Huang H, Jing H, et al. Simvastatin attenuates cardiopulmonary bypass-induced

myocardial inflammatory injury in rats by activating peroxisome proliferator-activated receptor gamma. *Eur J Pharmacol* 2010; 649 (1-3): 255-62.

23. Patterson AD, Peters JM. PPAR action in insulin resistance unraveled by metabolomics: potential clinical implications. *Genome Med* 2011; 3 (8): 54.
24. Leonhardt W, Pfutzner A, Muller J, Pietzsch J, Forst T, Karagiannis E, et al. Effects of pioglitazone and/or simvastatin on low density lipoprotein subfractions in non-diabetic patients with high cardiovascular risk: A sub-analysis from the PIOSTAT study. *Atherosclerosis* 2008; 201:155-62.
25. Kim SH, Hur HJ, Yang HJ, Kim HJ, Kim MJ, Park JH, et al. Citrus junos Tanaka Peel Extract Exerts Antidiabetic Effects via AMPK and PPAR-gamma both In Vitro and In Vivo in Mice Fed a High-Fat Diet. *Evidence-based Complementary and Alternative Medicine*: eCAM. 2013; 1-8.
26. Collino M, Aragno M, Castiglia S, Miglio G, Tomasinelli C, Boccuzzi G, et al. Pioglitazone improves lipid and insulin levels in overweight rats on a high cholesterol and fructose diet by decreasing hepatic inflammation. *Br – Pharmacol* 2010; 160 (8):1892-902.
27. Iwata M, Haruta T, Usui I, Takata Y, Takano A, Uno T, et al. Pioglitazone ameliorates tumor necrosis factor-alpha-induced insulin resistance by a mechanism independent of adipogenic activity of peroxisome proliferator--activated receptor-gamma. *Diabetes* 2001; 50 (5):1083-92.
28. Hayashi AA, Webb J, Choi J, Baker C, Lino M, Trigatti B, et al. Intestinal SR-BI is upregulated in insulin-resistant states and is associated with overproduction of intestinal apoB48-containing lipoproteins. *Am j physiol Gastrointest liver physiol* 2011; 301(2): 326-37.