J Biochem Tech (2014) 5(2): 708-717
ISSN: 0974-2328

Possible Molecular Targets of Cinnamon in the Insulin Signaling Pathway

Sana Eijaz, Asmat Salim*, Mohammed Anwar Waqar

Received: 25 June 2013 / Received in revised form: 04 August 2013, Accepted: 20 August 2013, Published online: 30 April 2014

Abstract

Cinnamon (CN) is known for its anti-diabetic activities in traditional medicine. CN extracts are reported to have beneficial effects on normal and impaired glucose tolerance, insulin resistance and type-2 diabetes. The aim of this study is to observe the effect of CN extract on certain diabetogenes involved in insulin signaling. Streptozotocin (STZ) induced type-2 diabetic rats were given CN extract for one month and its effect was observed on blood glucose levels, body weights and gene expression levels of protein tyrosine phosphatase-1B (PTP-1B), insulin receptor (INSR), insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI3K), protein kinase B (PKB), protein kinase C-theta (PKCθ) and phosphoinositide-dependent protein kinase-1 (PDK1) in skeletal muscle and adipose tissue. Statistically significant difference was found in the glucose levels and body weights of test and diabetic control groups. In muscle, statistically significant difference was observed in gene expression levels of PTP-1B, IRS-1, PKB, PDK1, PI3K and PKCθ between test and diabetic control groups and PTP-1B, IRS-1, PKB, PDK1 and PKCθ between normal and diabetic control groups. In adipose tissue, statistically significant difference was found in gene expression levels of PTP-1B, PKCθ, IRS-1 between test and diabetic control groups and PTP-1B, PDK1, PI3K, PKCθ and IRS-1 between normal and diabetic control groups. These results suggest that cinnamon normalizes blood glucose level and body weight and affect certain molecular targets in the insulin signaling pathway and therefore, possess strong anti-diabetogenic and hypoglycemic action in STZ-induced type-2 diabetic rat model. The consistent and / or variable pattern of these genes in skeletal muscle and adipose tissue indicates that cinnamon acts differently by affecting some but not all of these genes and that their expressions are tissue specific.

Keywords: Type-2 diabetes; cinnamon; streptozotocin; insulin resistance; skeletal muscle; adipose tissue.

Sana Eijaz

Dr. Panjwani Center for Molecular Medicine and Drug Research, International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi-75270, Pakistan.

Asmat Salim*

Dr. Panjwani Center for Molecular Medicine and Drug Research, ICCBS, University of Karachi, Karachi-75270, Pakistan.

Tel.: 00919450358872
*Email: asmat.slim@iccs.edu

Muhammad Anwar Waqar

Dr. Panjwani Center for Molecular Medicine and Drug Research, ICCBS, University of Karachi, Karachi-75270, Pakistan.

Introduction

Insulin is the principal hormone that regulates uptake of glucose from the blood into most cells, including skeletal muscle cells and adipocytes. Insulin resistance which refers to suppressed or delayed responses to insulin, is the major pathway that leads to type-2 diabetes (Lin and Sun 2010). There is a considerable reduction in the insulin induced glucose disposal in skeletal muscle which is the most prominent tissue for the utilization of glucose in insulin dependent manner (Defronzo and Tripathy 2009). This triggers disturbances in glucose homeostasis throughout the body ultimately leading to impaired insulin sensitivity and disease development (Oberg et al. 2011). A number of protein molecules are involved in the insulin signal transduction cascade which includes protein kinases and phosphatases. Insulin receptor (INSR) is hetero-tetrameric in nature (Frodea and Medeirosb 2008; Kim et al. 2012). Its kinase activity is reduced in type-2 diabetes (Frojdo et al. 2008). Insulin receptor substrate-1 (IRS-1) which is significantly expressed in adipocytes, is the first intermediate in the insulin signal transduction pathway. Several downstream proteins docks IRS-1 and initiate certain metabolic pathways (Waqar et al. 2009). In adipose tissue, IRS-1 acts as a critical factor for the translocation of GLUT-4 by phosphoinositide 3-kinase (PI3K) which induces downstream phosphorylation and dephosphorylation events (Chawla et al. 2011). It is suggested that the insulin-induced PI3K activity become lowered in type-2 diabetic patients that may lead to abnormal GLUT-4 translocation and insulin resistance (Choi and Kim 2010). Phosphoinositide-dependent protein kinase-1 (PDK1)

stimulates the phosphorylation and activation of protein kinase B (PKB) at the plasma membrane (Bayascas et al. 2008). Studies suggest that in the adipose tissue of type-2 diabetic subjects, the insulin-induced activation of PKB become moderate (Bayascas 2008; Waugh et al. 2009). Protein kinase C-theta (PKCθ) is significantly associated with insulin resistance in muscle and adipose tissue and thus responsible for type-2 diabetes (Wang et al. 2009). PKCθ is a serine-threonine kinase and induces the abnormal phosphorylation of IRS-1 that weakens its potential to activate PI3K. High plasma concentration of free fatty acids leads to the higher diacyl glycerol (DAG) levels which trigger the plasma activation of PKCθ lowering the tyrosine phosphorylation of IRS-1 (Nowotny et al. 2013). Protein tyrosine phosphatase-1B (PTP-1B) is a protein tyrosine phosphatase which catalyzes the de-phosphorylation of INSR and IRS-1, followed by the modification of insulin action (Pessin and Saltiel 2000; Tsuruzoe et al. 2001). The down regulation of PTP-1B improves insulin sensitivity and glucose tolerance as well as decreases the chances of obesity triggered by high fat diet (Lantz et al. 2010). PTP-1B knockout mice showed enhancement in insulin sensitivity. PTP-1B is therefore, one of the strong candidates to target insulin resistance (Lian et al. 2007; Tsou and Bence 2012).

Anti-diabetic herbs have been used since long but its specific mechanism of action is not completely understood. *Cinnamomum cassia*, commonly known as cinnamon has long been known to have anti-diabetic activity (Qin et al. 2003). It enhances the expression of proteins involved in glucose transport, insulin signaling, and regulates dislipidemia (Cao et al. 2010). In this study**,** we observed the effect of cinnamon extract containing diet on the blood glucose levels and body weights of type-2 diabetic rats and analyzed the expression of certain diabetogenes that play important role in insulin signaling. The data presented here is yet another attempt in elucidating the role of cinnamon herb in the insulin signaling pathway.

Materials and Methods

Experimental animals

Wistar male rats, weighing 180-200 g were housed in the Animal House Facility of the International Center for Chemical and Biological Sciences (ICCBS), University of Karachi, Karachi, Pakistan. Temperature was maintained at 21 ± 1°C and humidity around 57% at 12:12 hour standard light and dark cycle. International guidelines were followed for the care and use of laboratory animals. Animal study was endorsed by the 'Institutional Animal Care and Use Committee' of ICCBS. Animals were grouped into Test, Diabetic Control and Normal. The number of animals was 6 in each group (n = 6).

To prepare High Fat Diet (HFD), butter was mixed with normal diet ingredients in the ratio of 4:6 respectively. HFD was given to two groups (Table 1) for six months. Rats had free access to HFD and water. One group was fed normal diet throughout the experiment and was considered as the normal group. These rats were non-diabetic as compared to the rats in the test and diabetic control groups. During the administration of HFD, weights of all the rats were recorded twice a month to observe the effect of HFD on body mass. Before the analysis of insulin resistance, animals were fasted overnight. Weights of all the rats in each group were recorded. In order to determine the effect of HFD, oral glucose tolerance test (OGTT) of both the test and diabetic control groups were carried out. Fasting glucose levels were first recorded. OGTT was performed by oral administration of glucose (1 gm/ml/kg). Blood samples were collected by venipuncture from the tail at 30, 60 and 120 minutes after the oral glucose administration and readings were recorded with glucometer (Roche).

Intravenous administration of streptozotocin

Streptozotocin (STZ) was prepared in 0.1 M citrate buffer (pH 4.3) and administered intravenously as (35mg/kg/ml) to the test and diabetic control groups while rats were still in fasting condition. Normal group was administered citrate buffer only.

Table 1: Experimental groups used in this study

Experimental Groups	High Fat Diet (HFD)	Streptozotocin (STZ)	Cinnamon (CN) Extract
Diabetic Control	+	+	-
Test	+	+	+
Normal	-	-	-

(+ given, - not given)

Determination of type-2 diabetes

Onset of type-2 diabetes was monitored by OGTT as described in case of insulin resistance.

Administration of cinnamon diet

Cinnamomum cassia, commonly known as cinnamon (CN) was purchased in the dry form from a local distributor. It was washed and dried under sunlight. Ethanolic extracts of cinnamon was prepared by soaking in 80% ethanol for 72 hours. The extracts were filtered and mixed with normal diet ingredient. The extract was administered as 1g/kg/day and continued for one month. During the treatment, weights of all rats were recorded twice a month to see its effect on body mass. The diabetic control group was not given the cinnamon extract.

Effect of cinnamon extract on blood glucose level and body weight

The effect of cinnamon extract on blood glucose level was analyzed by performing OGTT. Body weights of all the treated rats were also recorded.

Effect of cinnamon on PTP-1B, IRS-1, INSR, PI3K, PKB, PKCθ and PDK1 genes

After the completion of the experimental period, rats of all three groups were sacrificed and skeletal muscle and adipose tissues were dissected out. After isolation, muscle and adipose tissues were preserved immediately in RNA stabilization reagent (Qiagen, Germany) and stored at -20°C until later use. RNA isolation was done with SV Total RNA Isolation System (Promega, USA) and quantified at 260nm. 0.5µg total RNA was subjected to cDNA synthesis by RevertAid^{TM} First Strand cDNA Synthesis Kit (Fermentas, USA). RT-PCR was done using GoTaq Green Master Mix (Promega, USA). cDNA samples corresponding to PTP-1B, IRS-1, INSR, PI3K, PKB, PKCθ and PDK1 genes were subjected to denaturation for 5 min at 94°C, followed by 30 cycles of amplification (denaturation at 94°C, annealing at 50-61°C and extension at 72°C for 1 min each) and a final extension at 72°C for 10 min. GAPDH was used as internal standard. The primers were designed using the primer

design program, primer 3 online software (http://frodo.wi.mit.edu/). The primer sequences and their product sizes are: (i) PTP-1B gene 5' CCACACCATCTCCCAGAAGT 3' (forward); 5' CGGAACAG GTACCGAGATGT 3' (reverse); product size: 174 base pairs; (ii) INSR gene 5' GGATGGTCAGTGTGTGGAGA 3' (forward); 5' TCGTGAGGTTGTGCTTGTTC 3' (reverse), product size: 563 base pairs; (iii) IRS-1 gene 5' CACCCAGTTTTTCGACAC 3'(forward); 5' GAGTTGAGCTT CACAAAG 3' (reverse); product size: 600 base pairs; (iv) PI3K gene 5' AGCCACAGGTGAAAATACGG' 3 (forward); 5' TTTTCTTTCCGCAACAGCTT' 3 (reverse); product size: 199 base pairs; (v) PKB gene 5' ACCTCATGCTGGACAAGGAC 3'(forward); 5' TGAGCTCGAACAGCTTCTCA 3'(reverse); product size: 246 base pairs; (vi) PKC-θ gene: 5' CCGAGAAGGACCAATTGAAA 3'(forward); 5' AAACTTCC TTTCCCCAGCAT 3'(reverse); product size: 240 base pairs; (vii) PDK1 gene: 5' CTCACAGAAGGGCCACATTT 3' (forward); 5' AGCATCTGGACTGCTCTGGT 3' (reverse); product size: 228 base pairs; (viii) GAPDH: 5' GGAAAGCTGTGGCGTGATGG 3' (forward); 5' GTAGGCCATGAGGTCCACCA 3' (reverse); product size: 414 base pairs. Each PCR product was electrophoretically resolved on 3% agarose gel. Bands were visualized under UV light in the FluorChem Imaging System (Alpha Innotech, USA). The relative expression ratio of each gene was calibrated with GAPDH and comparison was done between test, diabetic control and normal groups.

Statistical analysis

Statistical analysis was performed by using the Sigma plot 11.2.0. Student's t-test was used to compare the test, diabetic control and normal groups. The differences were considered significant at a value of $p < 0.05$. Data are presented as mean ± SEM.

Results

Effect of high fat diet on blood glucose level

High Fat Diet (HFD) was given to rats for six months. After the completion of the six month period, OGTT of all rats were performed. Readings were recorded at fasting (0 min) and at 30, 60 and 120 min after the administration of oral glucose. There was a significant increase in the blood glucose levels of overnight fasted rats and during all time periods after glucose administration when normal group was compared with diabetic control and test groups (Fig. 1; Table 2). Overall differences between the blood glucose levels among diabetic control and normal groups (p= 0.045*) and test and normal group (p= 0.004**) were recorded and found to be significant (Fig. 1; Table 2).

Effect of streptozotocin on blood glucose level

After the completion of the six month period, STZ was administered intravenously. The normal group was administered citrate buffer only. After one month, OGTT was performed. Readings were recorded at fasting (0 min) and at 30, 60 and 120 min after the administration of oral glucose. There was a significant increase in the blood glucose levels of overnight fasted rats and during all time periods after glucose administration when normal group was compared with diabetic control and test groups (Fig. 2; Table 3). Overall differences between the blood glucose levels among diabetic control and normal groups (p= 0.045*) and test and normal group (p= 0.004**) were recorded and found to be significant (Fig. 2; Table 3). There was a marked overall difference between the blood glucose

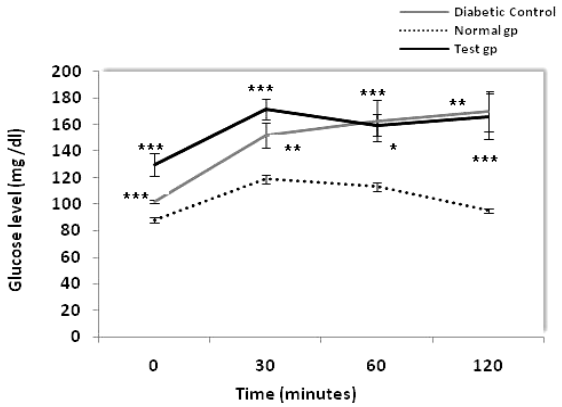


Figure 1: Effect of High Fat Diet (HFD) on blood glucose levels of diabetic control, test and normal groups

levels of diabetic control and normal group (p = <0.001***) and between the test and normal group (p = <0.001***) (Fig. 2; Table 3).

Table 2: Blood glucose levels after administration of high fat diet (HFD)

Experimental Groups	Time			
	0 min	30 min	60 min	120 min
Diabetic Control#	102 ± 1.31***	152 ± 9.71**	163 ± 15.78*	170 ±15.44***
Test##	130 ± 8.38***	172 ± 7.89***	160 ± 8.1***	166 ± 17.17**
Normal	88 ± 2.16	119 ± 3.36	113 ± 3.48	99 ± 1.58

Comparison at individual time periods: Normal group was compared with diabetic control and test groups (*p = 0.01; **p = 0.009 and ***p = <0.001), Overall comparison: Blood glucose levels of test and diabetic control were compared with that of normal group (#p = 0. 045 and ##p = 0.004)

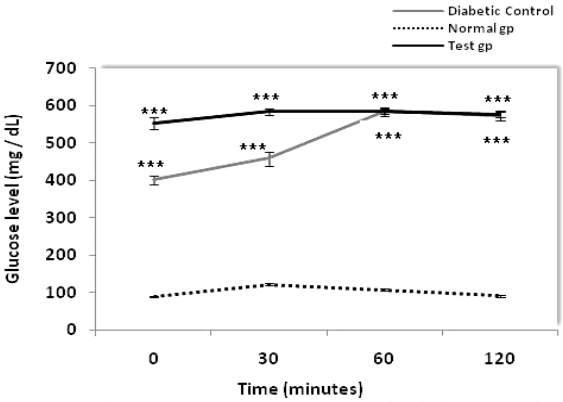


Figure 2: Effect of Streptozotocin (STZ) on blood glucose levels of diabetic control, test and normal groups

Effect of cinnamon extract on blood glucose level

After the development of type-2 diabetes, test group was given cinnamon (CN) extract containing diet while the diabetic control and normal group was given normal diet. After one month, OGTT was performed. Readings were recorded at fasting (0 min) and at 30, 60 and 120 min after the administration of oral glucose. There was a significant difference in the blood glucose levels of overnight fasted rats

and during all time periods after glucose administration when test

Table 3: Blood glucose levels after STZ treatment

Experimental Groups	Time			
	0 min	30 min	60 min	120 min
Diabetic Control###	401 ± 11.38***	458 ± 18.97***	585 ± 6.2***	572 ± 12.1***
Test###	554 ± 15.88***	584 ± 8.76***	584 ± 11.94***	577 ± 9.47***
Normal	89 ± 2.18	122 ± 3.64	108 ± 3.17	91 ± 2.27

Comparison at individual time periods: Normal group was compared with diabetic control and test group (***p = <0.001), Overall comparison: Blood glucose levels of test and diabetic control were compared with that of normal group (### p = <0.001)

group was compared with diabetic control group (Fig 3; Table 4). However, statistical difference was also observed at all time periods between test and normal groups except after 120 min of glucose administration (Fig 3; Table 4). Overall statistically significant difference was observed in blood glucose levels of the test and diabetic control groups (p= <0.001) (Fig 3; Table 4).

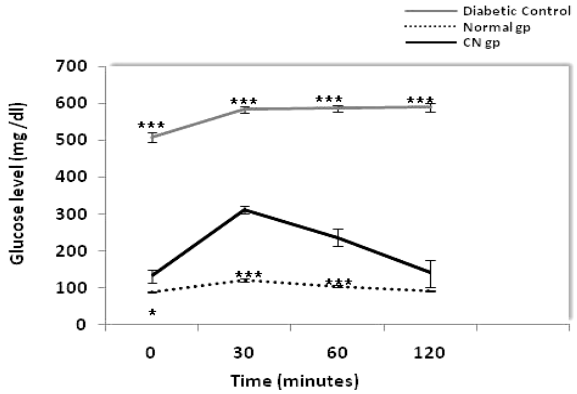


Figure 3: Effect of Cinnamon (CN) extract on blood glucose levels of diabetic control, test and normal groups

Table 4: Blood glucose levels after cinnamon administration

Experimental Groups	Time			
	0 min	30 min	60 min	120 min
Diabetic Control###	507 ± 13.23***	584 ± 8.84***	585 ± 8.8***	589 ± 10.9***
Test###	132 ± 18.79	312 ± 9.00	236 ± 23.35	140 ± 37.05
Normal	89 ± 2.18*	122 ± 4.64***	105 ± 3.02***	92 ± 2.37

Comparison at individual time periods: Test group was compared with diabetic control and normal groups (*p = 0.01 and ***p = <0.001) Overall comparison: Blood glucose levels of test group were compared with that of and diabetic group (### p = <0.001)

Effect of high fat diet, streptozotocin and cinnamon on body weight

During the six months period of HFD administration, body weights of rats belonging to all groups were recorded every month till 6 months. After the administration of HFD, all rats gained weight gradually. In the test group, weight gain was gradual with HFD administration. As the STZ was administered to rats of both diabetic control and test groups, there was a marked decrease in the body weight. After the development of type-2 diabetes, test group was given cinnamon extract for one month. At the end of the treatment, weights of all rats belonging to this group were recorded before and after treatment.

Effect of cinnamon on body weight

Gradual weight gain was observed in the normal group. Decrease in the body weight was observed in the diabetic control and test groups after the administration of STZ. Weight gain was normalized in the test group after treatment with cinnamon while no change was observed in the diabetic control group (Fig 4; Table 5). Statistically significant change was observed in the body weights within the test group before and after CN treatment (p= <0.001). There was also a statistically significant difference in the body weights of the test and diabetic control groups (p= 0.002**) (Table 5).

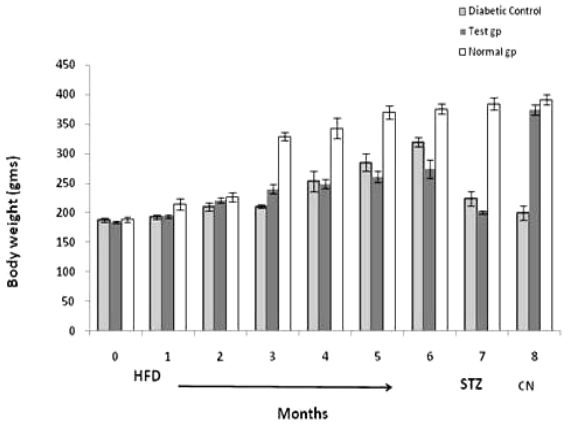


Figure 4: Effect of High Fat Diet (HFD), streptozotocin (STZ) and cinnamon extract (CN) on body weights of diabetic control, test and normal groups: The weights were recorded every month, before the start of any treatment or HFD administration (month 0), during HFD administration (months 1-6), STZ treatment (month 7) and cinnamon treatment (month 8).

Effect of cinnamon on PTP-1B, IRS-1, INSR, PI3K, PKB, PKCθ and PDK1 genes in skeletal muscle

The expression levels of PTP-1B, IRS-1, INSR, PI3K, PKB, PKCθ, and PDK1 genes were analyzed in the skeletal muscle of diabetic control, test and normal groups (Table 6). Statistically significant difference was observed in the gene expression levels of PTP-1B, IRS-1, PKB, PDK1, PI3K and PKCθ between diabetic control and test groups and PTP-1B, IRS-1, PKB, PDK and PKCθ between diabetic control and normal groups (Fig. 5). In case of normal and test groups statistically significant difference was only observed in expression level of PDK1 (p= 0.004***) (Table 7).

Effect of cinnamon on PTP-1B, IRS-1, INSR, PI3K, PKB, PKCθ and PDK1 genes in adipose tissue

The expression levels of PTP-1B, IRS-1, INSR, PI3K, PKB, PKCθ, and PDK1 genes were analyzed in the adipose tissue of diabetic control, test and normal groups (Table 8). Statistically significant difference was found in the gene expression levels of PTP-1B, PKCθ and IRS-1 between diabetic control and test groups and PTP-1B, PDK1, PI3K, PKCθ and IRS-1 between diabetic control and normal groups (Fig. 6). In case of normal and test groups statistically significant difference was observed in the expression level of PTP-1B, PDK1, PI3K, PKCθ and INSR (Table 9).

Table 5: Body weights recorded during the course of study

Groups	Months								
	0 HFD	1 HFD	2 HFD	3 HFD	4 HFD	5 HFD	6 HFD	7 STZ	8 CN
Diabetic control	188±3.17	193±3.37	210±7.08	211±3.24	253±17.7	285±14.2	319±7.88	224±11.9	200±11.9
Test#	185±1.85	194±2.02	221±4.13	240±8.02	249±7.27	261±10.1	274±15.48	201±2.74	374±8.44
Normal	189±4.02	215±9.64	227±7.42	329±7.16	343±16.9	370±11.5	376±8.34	384±10.1	392±8.48

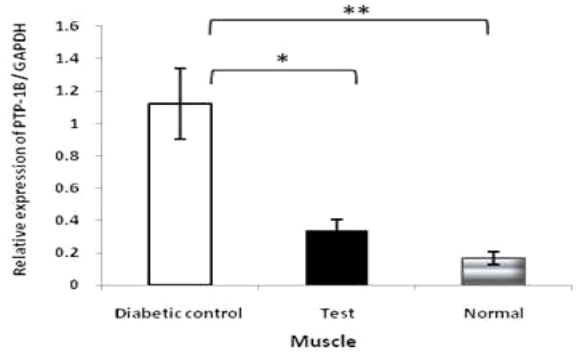
HFD was given to all rats; STZ to diabetic control and test groups while CN only to the test group, # p = <0.001*** and = 0.002** respectively when values were compared within test group and with the diabetic control group after cinnamon treatment.

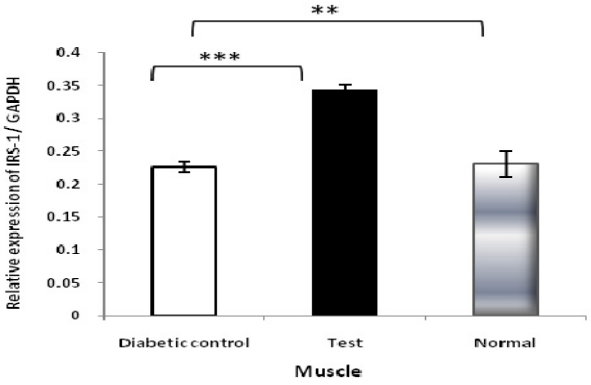
Table 6: Expression of dibeteogenes in skeletal muscle

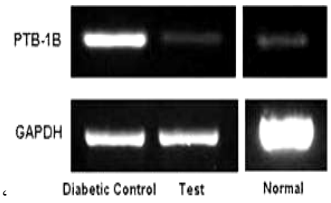
Genes	Groups		
	Test	Diabetic Control	Normal
PTP-1B	0.337 ± 0.07	1.123 ± 0.22	0.169 ± 0.04
IRS-1	0.344 ± 0.006	0.226 ± 0.008	0.230 ± 0.02
INSR	0.137 ± 0.05	0.105 ± 0.01	0.087 ± 0.03
PDK1	1.454 ± 0.14	0.255 ± 0.06	0.592 ± 0.02
PKB	5.717 ± 1.603	0.165 ± 0.05	1.237 ± 0.04
PI3K	0.489 ± 0.05	0.210 ± 0.03	0.334 ± 0.06
PKCθ	0.311 ± 0.03	0.800 ± 0.02	0.194 ± 0.04

Table 7: Statistical difference among groups with respect to expression of dibeteogenes in skeletal muscle

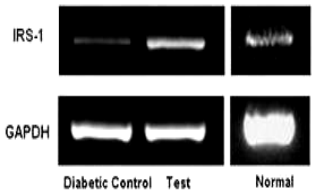
Genes	Groups		
	Diabetic Control and Test	Diabetic Control and Normal	Normal and Test
PTP-1B	0.03*	0.01**	0.06
IRS-1	<0.001***	0.01**	0.14
INSR	0.45	0.64	0.38
PDK1	0.001***	0.01**	0.004***
PKB	0.02*	0.03*	0.24
PI3K	0.01**	0.20	0.28
PKCθ	<0.001***	<0.001***	0.12



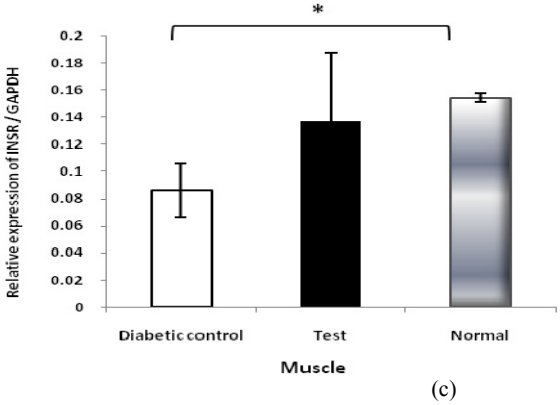


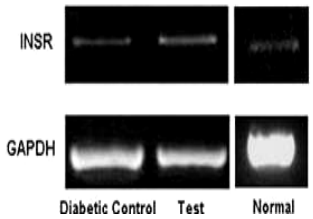


(a)



(b)





(c)

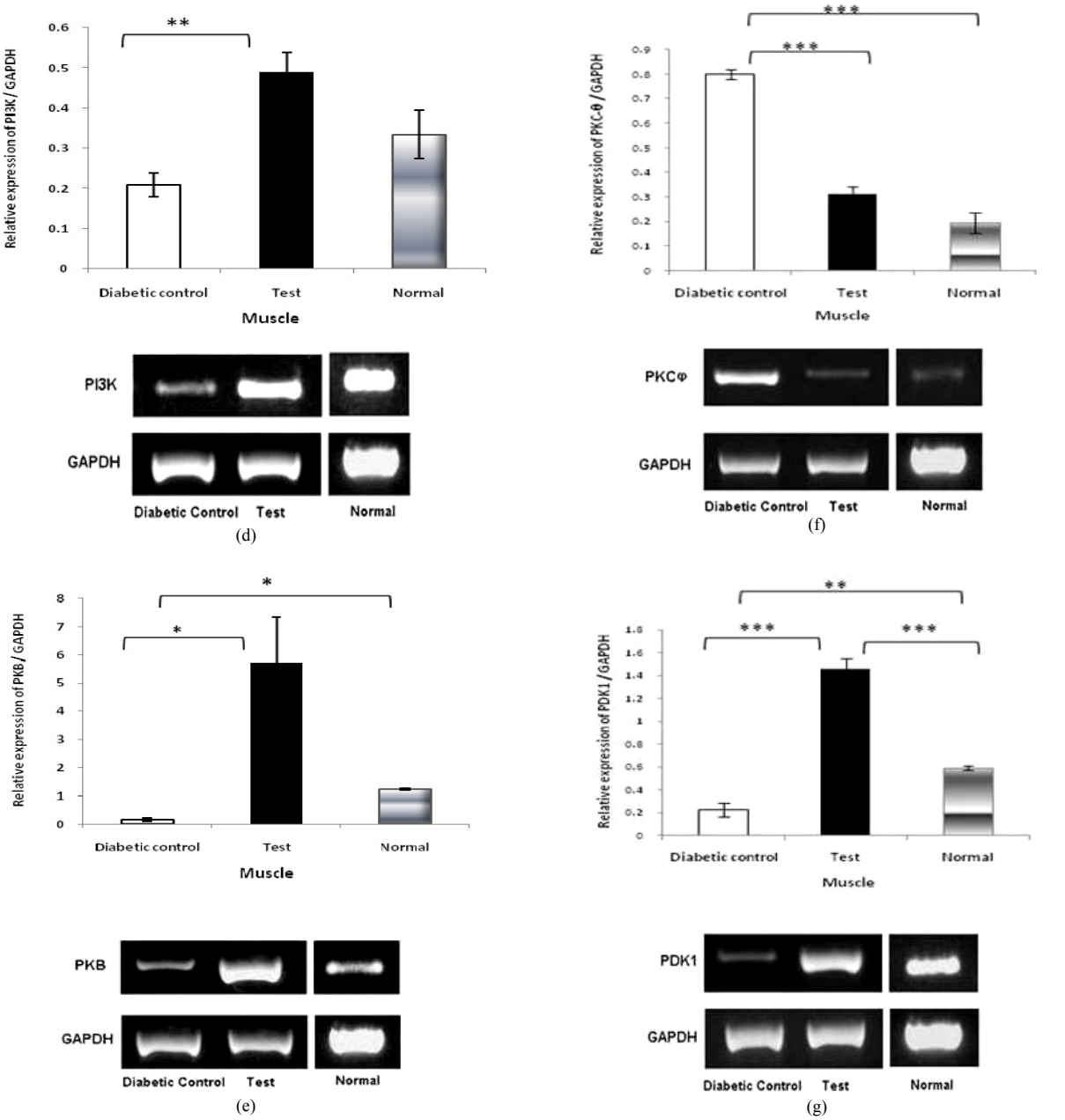


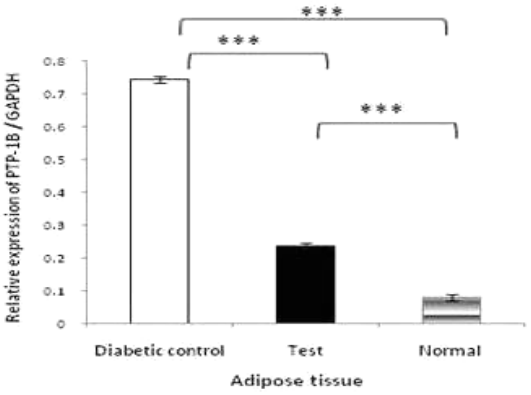
Figure 5: Effect of cinnamon (CN) extract on the expression levels of (a) PTP-1B, (b) IRS-1, (c) INSR, (d) PI3K (e) PKB (f) PKCθ and (g) PDK1 genes in skeletal muscle: The density of each band was measured as "integrated density values (IDVs)". Graphs are showing expression of genes relative to the expression of GAPDH. Data are expressed as means ± SEM, indicating the significant levels of difference in the expression profile of these genes in the diabetic control, test and normal groups.

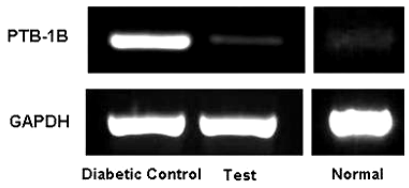
Table 8: Expression of dibeteogenes in adipose tissue

Genes	Groups		
	Test	Diabetic Control	Normal
PTP-1B	0.238 ± 0.007	0.742 ± 0.009	0.080 ± 0.01
IRS-1	0.403 ± 0.05**	0.153 ± 0.02	1.167 ± 0.01
INSR	0.055 ± 0.01	0.045 ± 0.01	0.109 ± 0.02
PDK1	0.676 ± 0.01	0.689 ± 0.005	0.485 ± 0.05
PKB	0.211 ± 0.09	0.197 ± 0.006	0.207 ± 0.03
PI3K	1.582 ± 0.21	0.261 ± 0.06	1.361 ± 0.31
PKC-θ	0.441 ± 0.04	0.727 ± 0.08	0.112 ± 0.006

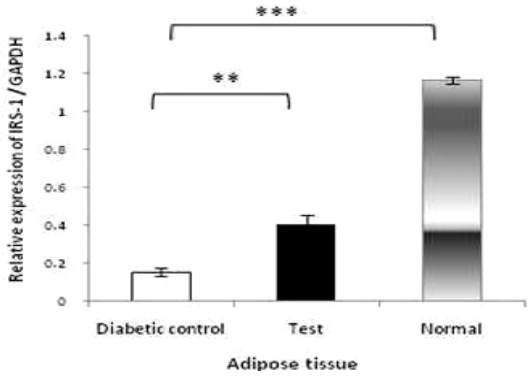
Table 9: Statistical difference among groups with respect to expression of dibeteogenes in adipose tissue

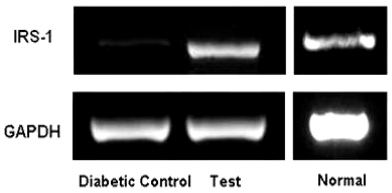
Genes	Groups		
	Diabetic Control and Test	Diabetic Control and Normal	Normal and Test
PTP-1B	<0.001***	0.002***	<0.001***
IRS-1	0.01**	<0.001***	0.55
INSR	0.61	0.21	0.05*
PDK1	0.45	0.02*	<0.001***
PKB	0.88	0.79	0.96
PI3K	0.59	0.02*	0.005**
PKCθ	0.04*	0.002***	0.002***


***
***
***
Relative expression of PTP-1B / GAPDH
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Diabetic control
Test
Normal
Adipose tissue

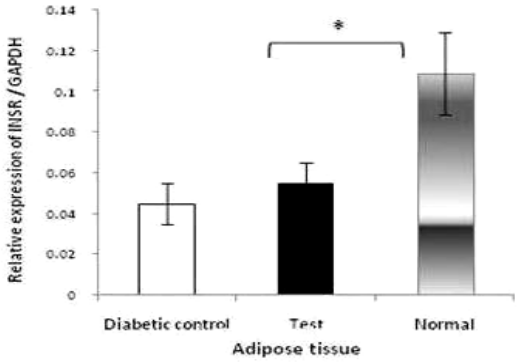

PTB-1B
GAPDH
Diabetic Control
Test
Normal

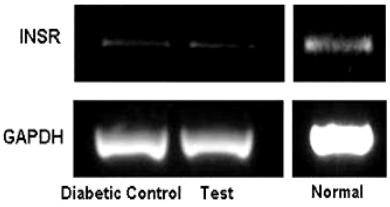
(a)


***
**
Relative expression of IRS-1 / GAPDH
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Diabetic control
Test
Normal
Adipose tissue

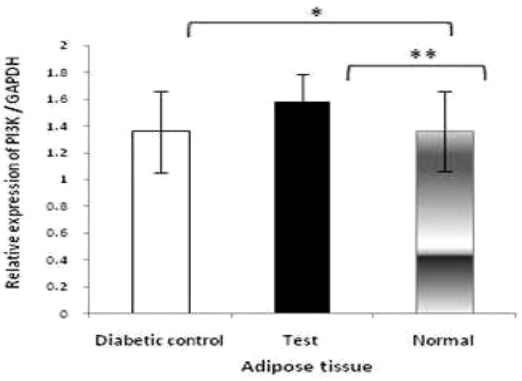

IRS-1
GAPDH
Diabetic Control
Test
Normal

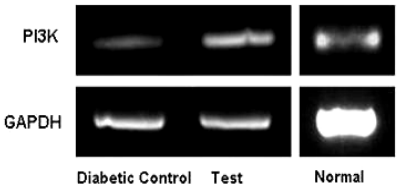
(b)


*
Relative expression of IINSR / GAPDH
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
Diabetic control
Test
Normal
Adipose tissue

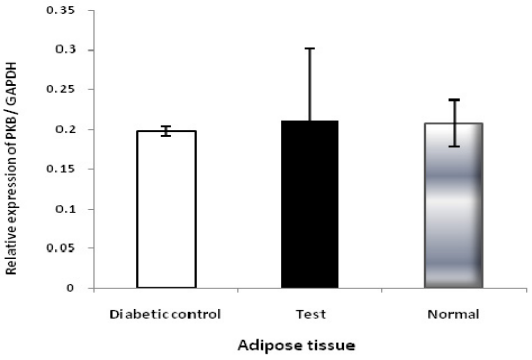

INSR
GAPDH
Diabetic Control
Test
Normal

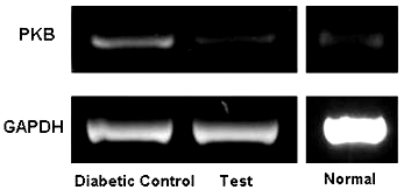
(c)


*
**
Relative expression of PI3K / GAPDH
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
Diabetic control
Test
Normal
Adipose tissue


PI3K
GAPDH
Diabetic Control
Test
Normal

(d)


Relative expression of PKB / GAPDH
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
Diabetic control
Test
Normal
Adipose tissue


PKB
GAPDH
Diabetic Control
Test
Normal

(e)

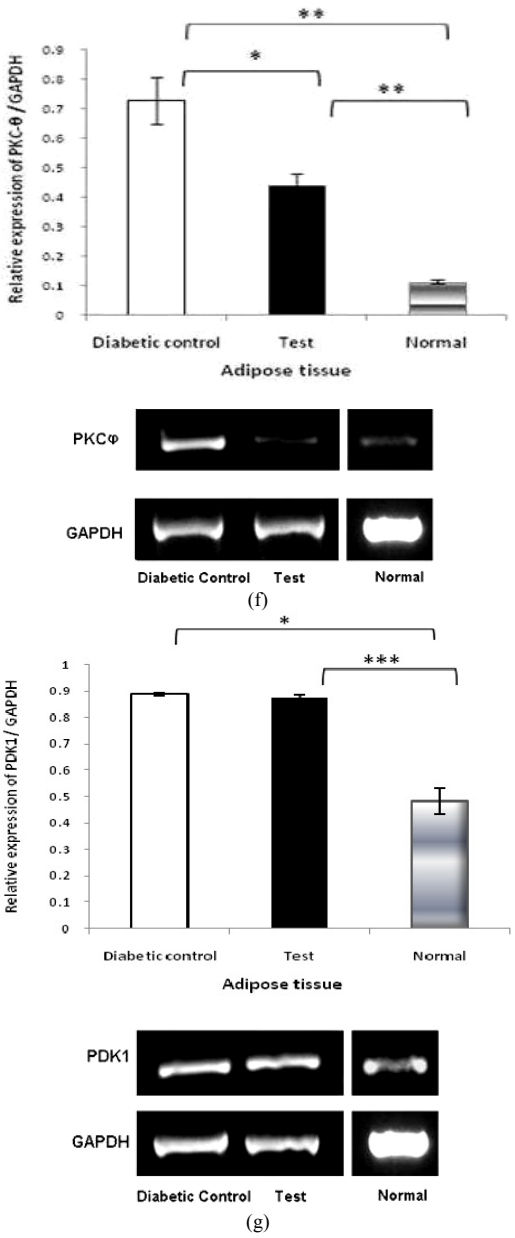


Figure 6: Effect of cinnamon (CN) extract on the expression levels of (a) PTP-1B, (b) IRS-1, (c) INSR, (d) PI3K (e) PKB (f) PKCθ and (g) PDK1 genes in adipose tissue. The density of each band was measured as "integrated density values (IDVs)". Graphs are showing expression of genes relative to the expression of GAPDH. Data are expressed as means ± SEM, indicating the significant levels of difference in the expression profile of these genes in the diabetic control, test and normal groups.

Discussion

Type-2 diabetes mellitus is the major metabolic disorder throughout the world. As conventional drugs are associated with a number of side effects, there is a need to search for safe alternative drugs. In this respect, natural products hold great promise and are expected to have similar efficacy with no side effects (Anand et al. 2010). Some patients who experienced insulin resistance do not respond well to the conventional drugs but show good response to natural products (Waqar et al. 2009). A number of anti-diabetic herbs have been used for a long period of time. However, their exact mechanism of action is not known. Earlier studies have suggested that cinnamaldehyde, an active component of cinnamon acts by enhancing release of insulin through direct insulin releasing effect on β-cells (Bolkent et al. 2000; Sharma et al. 2006; Patel et al. 2012). The present study was conducted to see the effect of cinnamon extract on blood glucose level, body weight and expression level of some diabetogenes in the STZ-induced diabetic rats. Insulin resistance was developed by means of High Fat Diet (HFD) which critically affects insulin signaling pathway (Hancock et al. 2008). Adipose tissues and skeletal muscles are drastically affected by HFD and show increased insulin resistance (Gong et al. 2012; Higashida et al. 2013). The high fat-fed animal models therefore aid in understanding the patho-physiological mechanisms in association with insulin resistance but their phenotype differ substantially in various studies (Henriksen et al. 2008).

In our study, experimental animals showed impaired glucose tolerance and marked increase in the body weight during the course of HFD administration. OGTT confirmed that all rats with HFD developed insulin resistance which is the pre-diabetic state. We used streptozotocin (STZ) treatment to develop type-2 diabetes in insulin resistant rats. After one month of STZ administration, the OGTT results showed marked increase in blood glucose level in the STZ-administered groups. The blood glucose level remained elevated even after two hours of oral glucose administration, whilst the blood glucose level of normal group returned back to normal value after two hours. The body weight of STZ-administered groups also reduced significantly as compared to the normal group. As the STZ-administered rats became diabetic, there was reduced glucose uptake and the body tissues fail to utilize glucose as energy source. To tackle with this critical situation, diabetic rats start to use surplus body fat as energy source which was accumulated around their tissues. After a certain period of time, the fat stores began to deplete so the obese diabetic rats lose their body weight.

After the confirmation of the onset of diabetes, we treated one of the diabetic groups with cinnamon extract (test group) while the other one was left untreated (diabetic control group). The normal group did not receive cinnamon extract. After one month, blood glucose level was monitored by OGTT and it was found that the test group showed reduced blood glucose levels after two hours. This shows that the cinnamon extract has improved glucose uptake. On the other hand, blood glucose level of the diabetic control group did not return to normal levels even after two hours showing that the glucose uptake process is still impaired. The results of both the groups were statistically significant. We also analyzed the body weights after the cinnamon extract administration. The results showed statistically significant increase in the body weights of the test group. This means that as the glucose uptake becomes normal, body has started to use glucose instead of fats.

In the present study, the effect of cinnamon extract on the expression of some diabetogenes in skeletal muscle and adipose tissue was also analyzed. Most of the studied diabetogenes in our study are the components of insulin signaling pathway. These include protein tyrosine phosphatase-1B (PTP-1B), insulin receptor (INSR), insulin receptor substrate-1 (IRS-1), phosphoinositide 3-kinase (PI3K), protein kinase B (PKB), protein kinase C-theta (PKCθ) and phosphoinositide-dependent protein kinase-1 (PDK1) genes in skeletal muscle and adipose tissue. In our study, we hypothesized that cinnamon may improve insulin resistance by

ameliorating some of the impaired insulin signaling genes in skeletal muscle and adipose tissue. Our results showed increased expression of PTP-1B and PKCθ in the skeletal muscle and adipose tissue of the diabetic control group as compared to normal group while cinnamon extract significantly reduced the expression of these genes in the test group. The reduced expression is significant in both skeletal muscle and adipose tissues in case of PTP-1B and while PKCθ expression was markedly reduced mainly in the skeletal muscle. Decreased expression of PKB, PDK1 and IRS-1 genes was observed in diabetic control and normal groups. This decrease is significant in both skeletal muscle and adipose tissue for PDK1 and IRS-1 genes while PKB showed significant decrease only in case of skeletal muscle. Cinnamon markedly increased the expression of PKB and PDK1 in skeletal muscle and that of IRS-1 in both skeletal muscle and adipose tissue of the test group. Decreased expression was also observed in case of PI3K in diabetic control as compared to normal group both in skeletal muscle and adipose tissue while the test group showed remarkable increase in the expression of PI3K in the skeletal muscle in comparison with diabetic control after the administration of cinnamon extract. We did not observe any significant different both in the skeletal muscle and adipose tissue of diabetic control and the normal group and there was no change observed in the test group as well.

Conclusion

The present study demonstrates that cinnamon has strong anti-diabetogenic and hypoglycemic action in HFD and STZ-induced type-2 diabetic rat models. Cinnamon affected the expression of PTP-1B, PKB, PDK1, PI3K, PKCθ, IRS-1 and INSR which accounts for the onset of insulin resistance and type-2 diabetes. It was interesting to note that the effect of cinnamon is consistent in both skeletal muscle and adipose tissue in case of some genes while for others it showed variable pattern. This shows that cinnamon acts differently in these tissues by affecting some of these genes while at the same time other genes are not affected. Taken together, the results suggest that amelioration of hyperglycemia and insulin resistance by cinnamon in type-2 diabetic rats is possibly due to the effective normalization of the expression of insulin signaling genes. Moreover, there is an inhibitory effect on these genes which negatively influence the insulin signaling pathway. In our study, we attempted to see the effect of cinnamon herb on genes of insulin regulating pathway. These findings may help to understand the possible molecular mechanism of action of cinnamon and to elucidate its precise role as an anti-diabetic herb.

Acknowledgement

We would like to acknowledge Mr. Ghulam Abbas (PCMD) for his help regarding development diabetic rat model.

References

Anand P, Murali KY, Tandon V, Murthy PS, Chandra R (2010) Insulinotropic effect of cinnamaldehyde on transcriptional regulation of pyruvate kinase, phosphoenolpyruvate carboxykinase, and GLUT4 translocation in experimental diabetic rats. Chemico-Biological Interactions 186: 72-81

Bayascas JR (2008) Dissecting the role of the 3- phosphoinositide-dependentprotein kinase-1 (PDK1) signalling pathways. Cell Cycle 7: 2978-2982

Bayascas JR, Wullschleger S, Sakamoto K, Garcia-Martinez JM, Clacher C, Komander D, Van ADM, Boini KM, Lang F, Lipina C, Logie L, Sutherland C, Chudek JA, Van DJA, Voshol PJ, Lucocq JM, Alessi DR (2008) Mutation of the PDK1 PH domain inhibits protein kinase B/Akt, leading to small size and insulin resistance. Molecular and Cellular Biology 28: 3258-3272

Bolkent S, Yamardag R, Tabakogluoguz A, Ozsoy-Sacon O (2000) Effects of chard (B. vulgaris L. var. cicla) extract on pancreatic B-cells in streptozotocin-diabetic rats: a morphologic and biochemical study. Journal of Ethnopharmacology 73: 251-259

Chawla S, Gupta D, Tiwari A (2011) Type-2 diabetes in the wake of insulin resistance: Molecular etiology and therapeutics. Journal of Pharmacy Research 4: 1118-1123

Choi K and Kim Young-Bum (2010) Molecular mechanism of insulin resistance in obesity and type 2 diabetes. Korean J Intern Med 25:119-129

Cao H, Polansky MM, Anderson RA (2010) Cinnamon extract regulates plasma levels of adipose-derived factors and expression of multiple genes related to carbohydrate metabolism and lipogenesis in adipose tissue of fructose-fed rats. Hormone and Metabolic Research 42: 187-193

Defronzo RA, Tripathy D (2009) Skeletal muscle insulin resistance is the primary defect in type-2 diabetes. Diabetes care 32: 157-163

Frodea TS, Medeirosb YS (2008) Animal models to test drugs with potential antidiabetic activity. Journal of Ethnopharmacology 115: 173-183

Frojdo S, Vidal H, Pirola L (2009) Alterations of insulin signalling in type 2 diabetes: A review of the current evidence from humans. Biochim Biophys Acta 1792: 83-92

Gong Y, Li J, Li C, Mu Y, Xiao Y, Tian H, Pan C, Liu Y (2012) The adipose tissue endocrine mechanism of the prophylactic protective effect of pioglitazone in high-fat diet-induced insulin resistance. Journal of International Medical Research 40: 1304-1316

Hancock CR, Han Dong-Ho, Chen M, Terada S, Yasuda T, Wright DC, Holloszy JO (2008) High-fat diets cause insulin resistance despite an increase in muscle mitochondria. PNAS 105: 7815-7820

Henriksen EJ, Teachey MK, Lindborg KA, Diehl CJ, Beneze AN (2008) The high-fat-fed lean Zucker rat: a spontaneous isocaloric model of fat-induced insulin resistance associated with muscle GSK-3 overactivity. Am J Physiol Regul Integr Comp Physiol 294: 1813-1821

Higashida K, Fujimoto E, Higuchi M, Terada S (2013) Effects of e-day fasting on high-fat diet induced insulin resistance in rat skeletal muscle. Life Science 93: 208-13

Kim D, Cho SY, Yeau S-H, Park SW, Sohn YB, Kwon M-J, Kim J-Y, Ki C-S, Jin D-K (2012) Two novel insulin receptor gene mutations in a patient with Rabson-Mendenhall Syndrome: The first Korean case confirmed by biochemical, and molecular evidence. J Korean Med Sci 27: 565-568

Lantz KA, Hart SG, Planey SL, Roitman MF, Ruiz-White IA, Wolfe HR, McLane MP (2010) Inhibition of PTP1B by Trodusquemine (MSI-1436) Causes Fat-specific Weight Loss in Diet-induced Obese Mice. Obesity 18: 1516-1523

Lian JH, Guo L, Hu WR, Wu GL, Ji W, Gong BQ (2007) Establishment of a cell-based drug screening model for identifying down-regulators of protein tyrosine phosphatase 1B expression. Experimental and Clinical Endocrinology & Diabetes 115: 24-28

Lin Y, Sun Z (2010) Current views on type 2 diabetes. Journal of Endocrinology 204: 1-11

Nowotny B, Zahiragic L, Krog D, Nowotny PJ, Herder C, Carstensen M, Yoshimura T, Szendroedi J, Phielix E, Schadewaldt P, Schloot NC, Shulman G, Roden M (2013)

Mechanisms underlying the onset of oral lipid-induced skeletal muscle insulin resistance in humans. Diabetes 62: 2240-2248

Oberg AI, Yassin K, Csikasz RI, Dehvari N, Shabalina IG, Hutchinson DS, Wilcke M, Ostenson CG, Bengtsson T (2011) Shikonin increases glucose uptake in skeletal muscle cells and improves plasma glucose levels in diabetic Goto-Kakizaki rats. PLoS one 6: 1-10

Patel DK, Prasad SK, Kumar R, Hemalatha S (2012) An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pac J Trop Biomed 2: 320-330

Pessin JE, Saltiel AR (2000) Signaling pathways in insulin action: molecular targets of insulin resistance. Journal of Clinical Investigation 106: 165-169

Qin B, Nagasaki M, Ren M, Bajotto G, Oshida Y, Sato Y (2003) Cinnamon extract (traditional herb) potentiates in vivo insulin regulated glucose utilization via enhancing insulin signaling in rats. Diabetes Research and Clinical Practice 62: 139-148

Sharma SB, Nasir A, Prabhu KM, Murthy PS (2006) Antihyperglycemic effect of the fruitpulp of Eugenia jambolana in experimental diabetes mellitus. Journal of Ethnopharmacology 104: 367-373

Tsou RC and Bence KK (2012) The Genetics of PTPN1 and Obesity: Insights from Mouse Models of Tissue-Specific PTP1B Deficiency. Journal of Obesity 2012: 1-8

Tsuruzoe K, Emkey R, Kriauciunas KM, Ueki K, Kahn CR (2001) Insulin receptor substrate 3 (IRS-3) and IRS-4 impair IRS-1- and IRS-2-mediated signaling. Molecular and Cellular Biology 21: 26-38

Wang C, Liu M, Riojas RA, Xin X, Gao Z, Zeng R, Wu J, Dong LQ, Liu F (2009) Mechanisms of Signal Transduction: Protein Kinase C theta (PKC theta) dependent Phosphorylation of PDK1 at Ser504 and Ser532 Contributes to Palmitate-induced Insulin Resistance. J Biol Chem 284: 2038-2044

Waqar MA, Aijaz S, Shaukat S, Saleem A (2009) Malfunctioning of genes contributing towards diabetes mellitus type-2. Journal of Chemical Society of Pakistan 31: 339-356

Waqar MA, Aijaz S, Shaukat S, Waqar A (2009) Herbs can effectively talk with diabetogenes. Journal of Chemical Society of Pakistan 31: 677-687

Waugh C, Sinclair L, Finlay D, Bayascas JR, Cantrell D (2009) Phosphoinositide (3,4,5)-Triphosphate Binding to Phosphoinositide-Dependent Kinase 1 Regulates a Protein Kinase B/Akt Signaling Threshold That Dictates T-Cell Migration, Not Proliferation. Molecular and Cellular Biology 29: 5952-5962