

Trans-chalcone enhances insulin sensitivity through the miR-34a/SIRT1 pathway

Elham Karimi-Sales¹, Sajad Jeddi², Abbas Ebrahimi-Kalan³, Mohammad Reza Alipour^{1*}

¹ Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

² Endocrine Physiology Research Center, Research Institute for Endocrine Sciences, Shahid Beheshti University of Medical Sciences, Tehran, Iran

³ Neurosciences Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

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ABSTRACT

Objective(s): Trans-chalcone as the parent member of the chalcone series reduces circulating levels of insulin and glucose. However, the cellular mechanism of these effects is poorly understood. Sirtuin 1 (SIRT1) as a direct target of miR-34a controls homeostasis of glucose, and also improves insulin sensitivity. Therefore, the present study for the first time investigated the influence of trans-chalcone on the miR-34a/SIRT1 pathway as a possible mechanism for its hypoglycemic and hypoinsulinemic effects.

Materials and Methods: In this study, thirty male rats were randomly divided into three groups (n=10): solvent control (NS), oral administration of trans-chalcone for 2 (N2T) and 6 weeks (N6T) groups. Then, hepatic levels of miR-34a and SIRT1 were measured through the qRT-PCR method.

Results: Trans-chalcone reduced food intake, body weight gain, and serum glucose as well as insulin levels. Also, this chalcone inhibited hepatic miR-34a expression and significantly elevated SIRT1 mRNA level.

Conclusion: Trans-chalcone as an insulin-sensitizing chalcone partly acts through the miR-34a/SIRT1 pathway.

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Introduction

Chalcones are the main class of natural compounds which are plentiful in edible plants. These compounds have been subject of attention because of their wide biological activities such as anti-diabetic, antioxidant, anti-inflammatory, and hepatoprotective effects (1-5). Trans-chalcone with a simple structure is a flavonoids precursor and a parent of chalcone series (3, 6-8). It has been shown that trans-chalcone reduces the body weights and circulating levels of glucose and insulin in healthy and diabetic rats. Therefore, it is possible that trans-chalcone may have an insulin-like influence in these animals (9). Hyperinsulinemia and hyperglycemia are characteristics of insulin resistance, which is the main feature of metabolic syndrome (10, 11).

Micro-ribonucleic acids (miRNAs) are small noncoding RNAs that negatively control the expression of their target genes in post-transcriptional level. Certain miRNAs regulate insulin signalling and affect insulin resistance (12). One of the best-studied miRNAs which has important roles in glucose homeostasis and insulin production is miR-34a (12). This miRNA directly targets and inhibits expression of Sirtuin 1 (SIRT1) as a NAD-dependent protein deacetylase with anti-aging properties. It has been shown that SIRT1 protects mammals against metabolic disease (13-16). The main physiologic effects of this deacetylase are regulation of metabolic pathways in the metabolic tissues (liver, adipose tissue, and pancreas) and reaction to oxidative stress (14, 15, 17). In this case, SIRT1 regulates insulin secretion, lipid metabolism and homeostasis of glucose,

and also improves insulin sensitivity (10, 14, 16).

The liver has a key role in the insulin-dependent regulation of glucose homeostasis. In the insulin resistance conditions, low hepatic response to the insulin hormone leads to hyperglycemia (18). Some previous studies have suggested that flavonoids exert their positive effects on health and metabolic diseases by regulation of hepatic miRNAs (19-25). Therefore, hypoglycemic and hypoinsulinemic effects of trans-chalcone may be partly due to its effect on the miR-34a/SIRT1 pathway. This study for the first time investigated effects of trans-chalcone on miR-34a and SIRT1 in male rats.

Materials and Methods

Animals

Male Wistar rats (200–250 g) were obtained and kept in a room under standard conditions (22 °C, 12 hr light:12 hr dark cycle). Animals had free access to water and normal chow. Animal studies were directed according to the Regulations for the Use and Care of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA) and Animal Care Review Committee of Tabriz University of Medical Sciences (IR.TBZMED.REC.1395.582).

Experimental protocol

After the acclimatization period (1 week), thirty male rats were randomly divided into three groups (n=10): NS (10% Tween 80 as trans-chalcone solvent (26), for 6 weeks by daily oral gavage), N2T (trans-chalcone 20

*Corresponding author: Mohammad Reza Alipour. Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. Tel/Fax: +98-4133364664; Email: alipourmr@tbzmed.ac.ir; alipourmr52@gmail.com

mg/kg (3, 9) in 5th and 6th weeks by daily oral gavage), and N6T (trans-chalcone 20 mg/kg for 6 weeks by daily oral gavage) (Figure 1). At the beginning and the end of the treatment period, the body weights and the food intakes were measured in all groups.

After 8 hr fasting, rats were deeply anesthetized by intraperitoneal injection of a combination of ketamine (60 mg/kg) and xylazine (10 mg/kg) (27). Then, the blood samples were obtained from inferior vena cava for biochemical measurements and the liver samples were immediately excised for gene expression analysis. Biochemical measurements (serum insulin and glucose levels) were done by ELISA kit (Rat insulin ELISA; Mercodia, Uppsala, Sweden) and a commercial kit (Pars Azmoon, Tehran, Iran), respectively. Also, gene expressions (miR-34a and SIRT1 mRNA) were assessed by real-time PCR.

Real-time quantitative PCR

To determine the expression levels of hepatic miR-34a and SIRT1, total RNA including miRNA and mRNA was extracted from liver samples with the miR-amp kit (Parsgenome Co, Iran) and RNX-Plus solution kit (Fermentase, Cinagen Co Iran). Complementary DNA (cDNA) synthesis from mRNA samples, was done using Thermo Fisher Scientific Revert Aid Reverse Transcriptase in accordance with manufacturer’s instructions. In brief, total RNA (3 µg) was reversed to cDNA using 1 µl of M-MuLV RevertAid Reverse Transcriptase (200 U/µl), 1 µl of random hexamer primers (100 µM), 2 µl of dNTPs (10 mM), and 0.5 µl of Thermo Fisher Scientific RiboLock RNase-inhibitor (40 U/µl), incubated for 10 min at 25 °C, then 60 min at 42 °C in a final volume of 20 µl. The reaction was ended by heating at 70 °C for 5 min. Moreover, synthesis of cDNA from miRNA samples was performed using the miR-amp kit (Parsgenome Co Iran). Real-time PCR was done by means of SYBR Green PCR Master Mix (Fermentase, Cinagen Co Iran) on a real-time PCR machine (Rotor-Gene 3000) (28). Housekeeping (reference) genes for SIRT1 mRNA and miR-34a were β-actin and miR-191, respectively. Relative quantitative expressions of these genes were determined through the 2^{-ΔΔCt} method as described previously (27). Sequences of miR-34a and SIRT1, as well as β-actin and miR-191 primers, are listed in Table 1.

Statistical analysis

All data are expressed as mean±SEM. The SPSS 16.0 software (SPSS Inc., IL, U.S.A.) was used for analysis of data. Because of the normal distribution of data, which was confirmed by Shapiro-Wilk analysis, the differences between groups were assessed by one-way analysis of variance (ANOVA) followed by Tukey’s *Post hoc* test and *P*<0.05 was considered statistically significant.

Table 1. Primers set list for mRNAs and miRNAs

Gene	Primers Sequence ^a	Gene bank Accession number	Product length (bp)
SIRT1	F: AGG GAACCTCTGCTCATCTAC R: GGCAATCTGGCACTAA	XM_008772947.2	100
β-actin	F: TAGAGCTTCAGCAGCAGC R: AT GCC ACA GGA TTC CAT ACC	NM_031144.3	190
Gene name	Accession number	Target sequence ^b	
rno-miR-34a-5p	MIMAT0000815	UGGCAGUGUCUAGCUGGUUGU	
rno-miR-191-5p	MIMAT0000866	CAACGGAAUCCAAAAGCAGCUG	

^a Sequences were derived from NCBI (www.ncbi.nlm.nih.gov)
^b Sequence was derived from miRBase (www.mirbase.org)

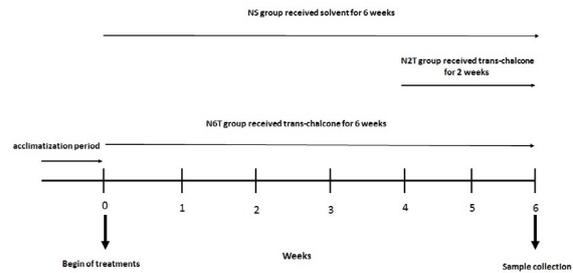


Figure 1. Study design and experimental groups

Results

The effect of trans-chalcone on food intake and body weight gain

Analysis of the food intake data revealed that in the initiation of the study, this variable was not significantly different between different groups (Data are not shown), but trans-chalcone significantly reduced (*P*<0.05) food consumption after 2 and 6 weeks of treatment (Figure 2) compared with the NS group. Consistent with reduction of food intake, trans-chalcone significantly decreased (*P*<0.001) body weight gain in the N6T group compared with NS (Figure 3). However, there was no significant change in body weight gain of N2T compared with NS group.

The effect of trans-chalcone on serum glucose and insulin levels

As shown in Figure 4, trans-chalcone significantly diminished (*P*<0.001) serum glucose levels after 2 and 6 weeks of oral administration. Also, trans-chalcone significantly reduced (*P*<0.001) serum insulin levels in N2T and N6T groups compared with the NS group

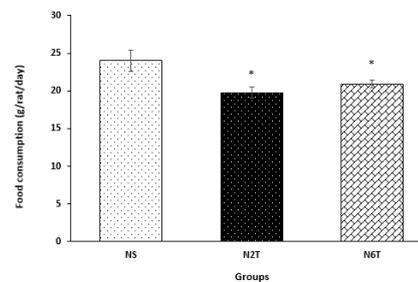


Figure 2. Last day food consumption in the study groups. Food consumption was significantly reduced in N2T and N6T groups compared with the NS group (NS: solvent control, N2T: trans-chalcone for 2 weeks, N6T: trans-chalcone for 6 weeks). Data were expressed as mean±SEM. * *P*<0.05 versus NS

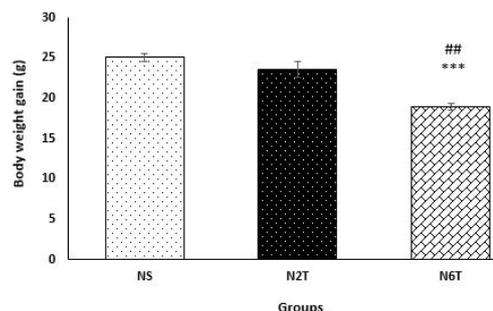


Figure 3. Body weight gain in the study groups. Body weight gain was significantly reduced in the N6T group compared with the NS group. There was a significant difference between body weight gain in N2T and N6T groups (NS: solvent control, N2T: trans-chalcone for 2 weeks, N6T: trans-chalcone for 6 weeks). Data were expressed as mean±SEM. *** *P*<0.001 versus NS, ## *P*<0.01 versus N2T

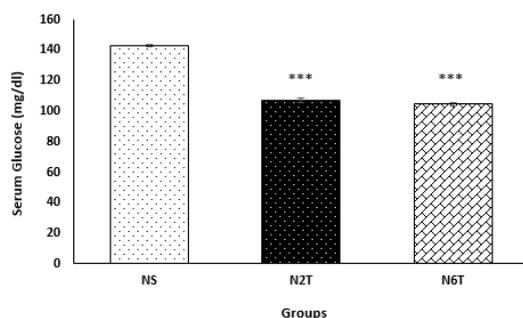


Figure 4. Serum glucose levels in the study groups. Serum levels of glucose were significantly reduced in N2T and N6T groups compared with the NS group (NS: solvent control, N2T: trans-chalcone for 2 weeks, N6T: trans-chalcone for 6 weeks). Data were expressed as mean \pm SEM. *** $P<0.001$ versus NS

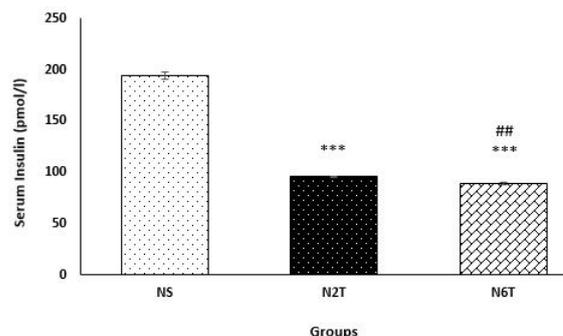


Figure 5. Serum insulin levels in the study groups. Serum levels of insulin hormone were significantly reduced in N2T and N6T groups compared with the NS group. There was a significant difference between serum insulin levels in N2T and N6T groups (NS: solvent control, N2T: trans-chalcone for 2 weeks, N6T: trans-chalcone for 6 weeks). Data were expressed as mean \pm SEM. *** $P<0.001$ versus NS, ## $P<0.01$ versus N2T

(Figure 5).

The effect of trans-chalcone on miR-34a/SIRT1 pathway

For examining the hypothesis that trans-chalcone exerts its insulin-sensitizing effect through the miR-34a/SIRT1 pathway, this study measured the trans-chalcone induced changes in the expression of miR-34a and SIRT1 mRNA. As expected, trans-chalcone significantly decreased ($P<0.001$) hepatic miR-34a in N2T (by 39%) and N6T (by 56%) groups compared with the NS group (Figure 6). Also as shown in Figure 7, chalcone upregulated mRNA levels of SIRT1 in N2T and N6T groups, which was significant ($P<0.05$) in the N2T group compared with NS.

Discussion

The present study evaluated the effects of oral administration of trans-chalcone on insulin sensitivity and the miR-34a/SIRT1 pathway as its possible mechanism in healthy rats. As described in the results section, treatment with trans-chalcone decreased food intake, body weight gain, and serum insulin as well as glucose levels. Consistent with our finding, some previous studies showed that flavonoids and trans-chalcone improved glucose homeostasis and insulin sensitivity (9, 29-31). In this regard, Knekt *et al.* (30) showed that some flavonoids (quercetin and myricetin) diminished type 2 diabetes occurrence in human subjects. Another study suggested that there is

an association between consumption of anthocyanins and low risk of type 2 diabetes in adult persons (29). Also, it has been shown by two previous works that intake of flavonoid-enriched chocolate reduced insulin resistance (32, 33). Furthermore, Karkhaneh *et al.* (31) demonstrated that trans-chalcone diminished body weight and insulin resistance in cholesterol-fed mice. A similar study reported body weight, food intake, insulin, and glucose-lowering effects of this flavonoids precursor in healthy and type 1 diabetic rats (9). Taken together, beneficial effects of trans-chalcone on insulin sensitivity have been shown by present and previous studies.

As mentioned in the introduction, direct regulation of SIRT1 by miR-34a and its important role in insulin sensitivity have been documented (13, 14, 34). This study investigated the effect of trans-chalcone on this pathway as a possible mechanism for its insulin-sensitizing action. In this regard, main findings of this study showed the inhibitory influence of trans-chalcone on hepatic miR-34a in parallel with elevation of hepatic SIRT1 expression. Consistent with the present study, previous works reported that miR-34a exerted pro-diabetic and pro-obesity functions through repression of its target, SIRT1 (13, 35). A study (16) suggested that miR-34a overexpression reduced SIRT1 expression and elevated free fatty acid induced apoptosis in rat hepatocytes. Also, Choi *et al.* (13) revealed that overexpression of this miRNA in the lean mice caused reduction of SIRT1 activity and obesity-associated

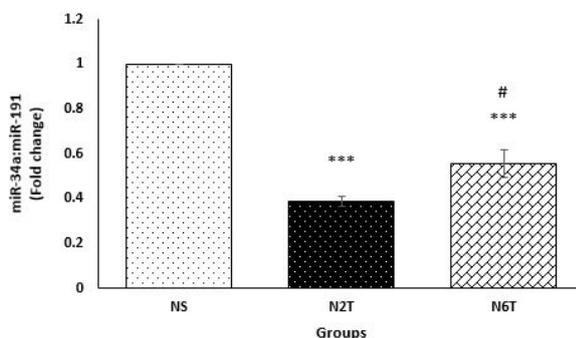


Figure 6. Hepatic expression levels of miR-34a in the study groups. Hepatic levels of miR-34a were significantly reduced in N2T and N6T groups compared with the NS group. There was a significant difference between hepatic levels of miR-34a in N2T and N6T groups (NS: solvent control, N2T: trans-chalcone for 2 weeks, N6T: trans-chalcone for 6 weeks). Data were expressed as mean \pm SEM. *** $P<0.001$ versus NS, # $P<0.05$ versus N2T

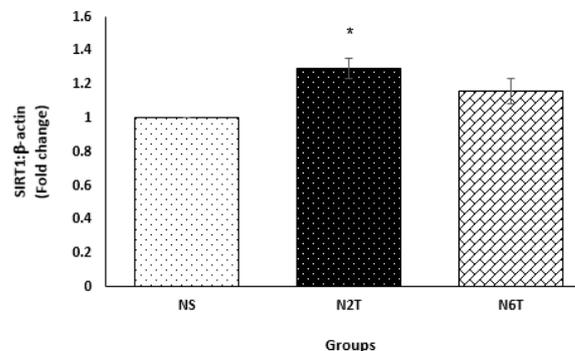


Figure 7. Hepatic expression levels of SIRT1 mRNA in the study groups. Hepatic level of SIRT1 was significantly elevated in the N2T group compared with the NS group (NS: solvent control, N2T: trans-chalcone for 2 weeks, N6T: trans-chalcone for 6 weeks). Data were expressed as mean \pm SEM. * $P<0.05$ versus NS

outcomes such as glucose intolerance and hepatic fat deposition. Moreover, they suggested that elevation of miR-34a diminished hepatic SIRT1 activity in obesity. On the contrary, silencing of miR-34a alleviated steatosis and glucose intolerance in diet-induced obese mice (13). In this regard, another study indicated that miR-34a was dysregulated during metabolic syndrome (obesity and diabetes) (36). Therefore, miR-34a is a possible therapeutic target for steatosis and type 2 diabetes treatment (13).

In agreement with the present study, it has been shown that flavonoids could increase SIRT1 expression (37, 38). Quercetin as a natural flavonoid enhanced expression of SIRT1 in mice (37). Also, troxerutin (a flavonoid with hepatoprotective effect) elevated hepatic SIRT1 protein expression in 2,2',4,4'-tetrabromodiphenyl ether-induced liver injury (38). However, 3,2',3',4'-tetrahydroxychalcone (a synthesized chalcone) inhibited the SIRT1 activity and could be considered an anti-cancer agent (39). This disagreement may be due to the different structures of 3,2',3',4'-tetrahydroxychalcone and trans-chalcone. To sum up, the present study suggested a new mechanism for hypoglycemic and hypoinsulinemic effects of trans-chalcone. Here, we showed that this simple chalcone inhibited miR-34a, which led to elevation of SIRT1 expression as its target gene.

Conclusion

This study proposed that trans-chalcone exerts its insulin-sensitizing effect through the miR-34a/SIRT1 pathway. Also, appetite lowering and anti-obesity effects of this chalcone may be other mechanisms of its insulin-sensitizing action. However, these effects were observed in normal healthy state and the influence of trans-chalcone on the miR-34a/SIRT1 pathway in pathologic conditions remains to be investigated.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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