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Effect of trans-chalcone on hepatic IL-8 through the regulation of miR-451 in male rats

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Objective. Trans-chalcone is a chalcone with hepatoprotective and anti-inflammatory effects. However, the mechanism of these positive effects, especially on miR-451 as an inflammatory regulator, is poorly understood. In this regard, this microRNA (miRNA) acts by inhibition of hepatic interleukin-8 (IL-8) production in the liver which is one of the main proinflammatory cytokines. This study for the first time examined the effect of trans-chalcone on miR-451/IL-8 pathway.

Methods. In present study, 21 male rats were randomly divided into 3 groups (n=7 per each group): control which received solvent (NS), groups 2 (N2T) and 3 (N6T), which received trans-chalcone for 2 and 6 weeks, respectively. Hepatic level of miR-451 was measured by qRT-PCR. Serum levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) as well as hepatic level of IL-8 protein were measured.

Results. Trans-chalcone decreased hepatic level of IL-8 protein and serum level of ALT after 2 weeks of treatment without significant change in hepatic miR-451. Moreover, it increased hepatic level of miR-451 and reduced hepatic IL-8 as well as AST and ALT after 6 weeks.

Conclusion. Based on the results of present study, miR-451/IL-8 pathway is a possible mechanism for hepatoprotective action of trans-chalcone in long-term.

Key words: trans-chalcone, miR-451, interleukin-8, liver

MicroRNAs (miRNAs) are endogenous, small, and single-stranded non-coding RNAs which have role in the regulation of various biological processes (Ohno et al. 2013; Zarfeshani et al. 2015). In the liver, miRNAs control lipid and glucose metabolism, inflammation, and fibrosis by regulation of their target genes (Szabo and Bala 2013). The role of miRNAs in regulating inflammatory process have been documented (Boldin and Baltimore 2012). In this case, miR-451 has a key role in regulation of NF-κB-mediated inflammation. This miRNA inhibits inflammation through inhibition of NF-κB activity (Sun et al. 2016). Dysregulation of this miRNA is involved in autoimmune arthritis (Murata et al. 2014), hepatocellular carcinoma (Li et al. 2013), non-alcoholic steatohepatitis (Hur et al. 2015), diabetic nephropathy (Sun et al. 2016), and hypertrophic cardiomyopathy (Song et al. 2014). Considering anti-inflammatory role of miR-451 through inhibition of NF-κB activity (Sun et al. 2016) and since NF-κB controls transcription of interleukin-8 (IL-8) (Wang et al. 2001; Karlsen et al. 2007; Hur et al. 2015), it seems that miR-451 has

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inhibitory effect on IL-8. In this regard, it has been shown that miR-451 inhibits inflammation and negatively regulates IL-8 production in palmitate treated hepatocellular carcinoma (HepG2) cells. Therefore, this miRNA may be regarded as a therapeutic target (Hur et al. 2015).

Chalcones have wide distribution in the plant kingdom. These compounds have role in pigmentation of flowers, protection of plants against insects and pathogens, flavonoid biosynthesis, and medicinal importance of plants. Chalcones have two isoforms and trans isoform is thermodynamically more favorable than cis (Batovska and Todorova 2010). Chalcones exert a broad range of biological activities such as anti-infective, anti-inflammatory (Nowakowska 2007), anti-oxidant (Singh et al. 2016a,b), anti-cancer (Katsori and Hadjipavlou-Litina 2009), cytoprotective (Sikander et al. 2011), anti-obesity (Karkhaneh et al. 2016), and anti-diabetic (Najafian et al. 2010). Trans-chalcone is a simple structure chalcone and also an open chain flavonoid (Batovska and Todorova 2010; Singh et al. 2016a) which has anti-fibrotic, antioxidant, and anti-inflammatory effects (Singh et al. 2016a). However, the mechanism of its anti-inflammatory effect is poorly understood and also the effect of this chalcone on miR-451/IL-8 pathway has not been investigated. Therefore, this study examined the impact of trans-chalcone on miR-451 and IL-8 as a possible mechanism for its anti-inflammatory effect.

Materials and Methods

Animals. In this study, 21 male Wistar rats (200–250 g) were obtained and kept under controlled conditions (12-h light–dark cycle and temperature of 22 °C). Animals had free access to standard food and water. All animal handling and experimental procedures were approved by Ethical Committee of Tabriz University of Medical Science (code number: IR.TBZMED.REC.1396.85).

Study design. Animals were randomly divided into 3 groups (n=7 for each). Animals in control group (NS) received 10% Tween 80 (as trans-chalcone solvent) (Okunrobo et al. 2006) for 6 weeks. Animals in groups 2 (N2T) and 3 (N6T) received trans-chalcone (20 mg/kg) (Najafian et al. 2010; Singh et al. 2016a) for 2 and 6 weeks, respectively. Administration of solvent and trans-chalcone were done by daily oral gavage.

At the end of treatments, animals were fasted for 8 h and then were anesthetized through intraperitoneal co-administration of ketamine (60 mg/kg) and xylazine (10 mg/kg) (Yousefzadeh et al. 2016). Then, liver samples immediately removed for additional analysis.

Quantitative Real-time PCR (qRT-PCR). For evaluating the expression levels of miR-451 in the liver samples, total RNA was isolated by miR-amp kit (parsgenome Co, Iran) and complementary DNA (cDNA) was generated from miRNA samples using miR-amp kit (parsgenome Co. Iran). Real-time PCR reactions were performed using SYBR Green PCR Master Mix (Fermentase, Germany) and the housekeeping gene miR-191 was used as previously described (Habibi et al. 2016). Relative quantitative expression of miR-451 was calculated through the $2^{-\Delta\Delta Ct}$ method (Yousefzadeh et al. 2015). Sequences of miR-451 and miR-191 primers were listed in Table 1.

ELISA and biochemical measurements. The level of IL-8 protein in liver samples was measured using ELISA kit (MyBiosource) according to the manufacturer's instruction. For evaluating the effect of trans-chalcone on liver function, serum levels of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using commercial kits (Pars Azmoon, Tehran, Iran) according to the manufacturer's instructions.

Statistical analysis. Data are expressed as mean ± SEM. Data analysis was performed using SPSS 16.0 software (SPSS Inc., IL, USA). Group means were compared by one-way analysis of variance (ANOVA) with post-hoc Tukey's test and $p<0.05$ was considered statistically significant.

Table 1
The primers sequences for genes

Genes	Accession number	Target sequence[a]
rno-miR-451-5p	MIMAT0001633	AAACCGUUACCAUUACUGAGUU
rno-miR-191a-5p	MIMAT0000866	CAACGGAAUCCCAAAAGCAGCUG

[a]Sequences were derived from miRBase (www.mirbase.org)

Results

Long-term oral administration of trans-chalcone (6 weeks) significantly ($p<0.001$) increased miR-451 level in liver (Figure 1). However, short-term administration of this chalcone (2 weeks) had no significant effect on hepatic level of this miRNA (Figure 1).

In next part of the study, we examined the effect of trans-chalcone on hepatic level of IL-8 protein as target of miR-451. Analysis of the data revealed that short-term and long-term treatment with trans-chalcone significantly ($p<0.001$) reduced hepatic levels of IL-8 protein (Figure 2). Regarding the effect of this chalcone on liver function, it significantly ($p<0.001$) decreased the serum levels of AST after 6 weeks (Figure 3A) and also ALT after 2 ($p<0.01$) and 6 ($p<0.001$) weeks of oral administration (Figure 3B).

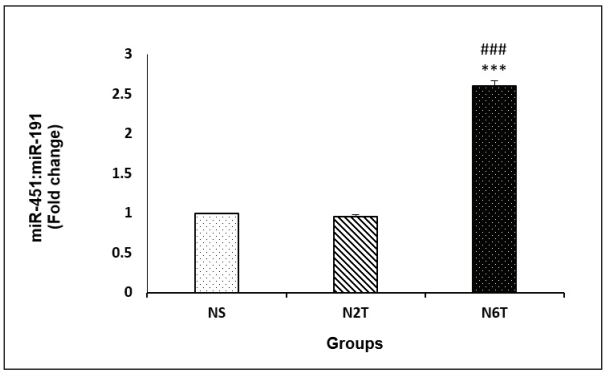

Figure 1. Hepatic expression levels of miR-451 in study groups. NS – control; N2T – trans-chalcone for 2 weeks; N6T – trans-chalcone for 6 weeks. Data were expressed as mean ± SEM. ***p<0.001 vs. NS, ###p<0.001 vs. N2T

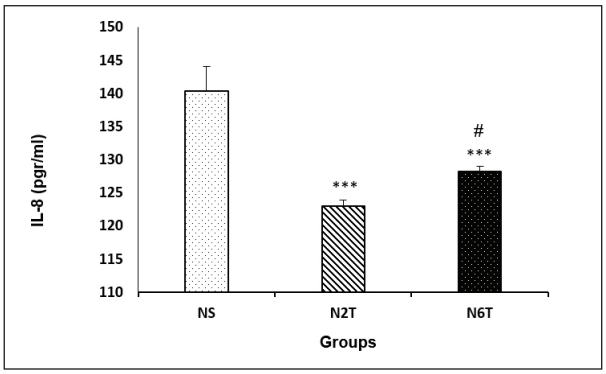

Figure 2. Hepatic protein levels of interleukin-8 (IL-8) in study groups. NS – control; N2T – trans-chalcone for 2 weeks; N6T – trans-chalcone for 6 weeks. Data were expressed as mean ± SEM. ***p<0.001 vs. NS, #p<0.05 vs. N2T

Discussion

This study examined the effect of short and long-term oral gavage of trans-chalcone on hepatic miR-451/IL-8 pathway. In the present study, long-term administration of trans-chalcone, exert anti-inflammatory role through elevation of miR-451 which cause reduction of IL-8 protein levels. Also, this chalcone could reduce serum levels of AST after 6 weeks as well as ALT after 2 and 6 weeks. Hepatoprotective effect of trans-chalcone and its ability to reduce serum levels of AST and ALT has been shown by Karkhaneh et al (2016). Moreover, as shown by Singh et al. (2016a), this chalcone reduced markers of liver injury (AST and ALT) and inflammation (TNF-α) in toxicant induced liver injury. Hence, anti-inflammatory effect of trans-chalcone may be involved in its hepatoprotective effect (Singh et al. 2016a).

It has been known that IL-8 is one of main proinflammatory cytokines, which acts as a key chemoattractant agent and also an activator for basophils, neutrophils, and T cells (Remick 2005; Joshi-Barve

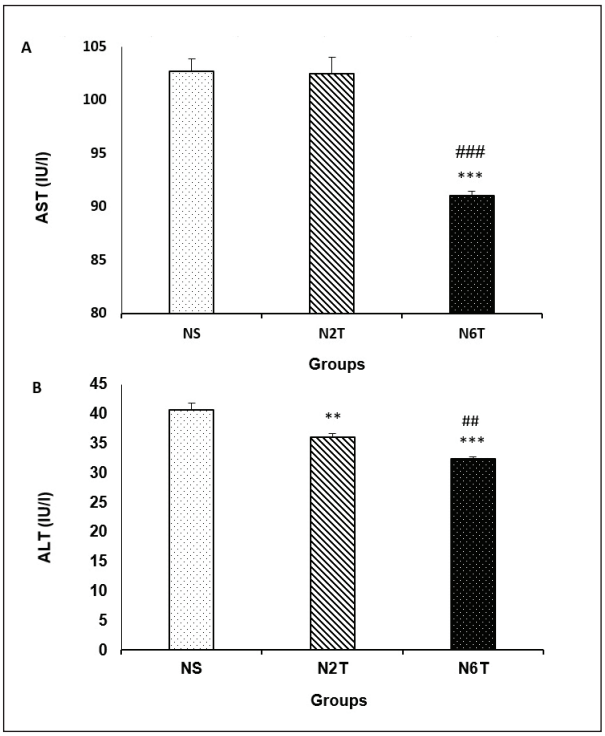

Figure 3. Serum levels of aspartate aminotransferase (AST) (**A**) and alanine aminotransferase (ALT) (**B**) in study groups. NS – control; N2T – trans-chalcone for 2 weeks; N6T – trans-chalcone for 6 weeks. Data were expressed as mean ± SEM. **p<0.01 vs. NS, ***p<0.001 vs. NS, ##p<0.01 vs. N2T, ###p<0.001 vs. N2T

et al. 2007). Hepatocytes produce significant level of this cytokine, which is involved in the hepatic inflammation and injury (Joshi-Barve et al. 2007). Moreover, as shown by Hur et al. (2015), miR-451 exerts anti-inflammatory action by inhibition of IL-8 production as a proinflammatory cytokine in palmitate treated HepG2 cells. They reported that transfection of miR-451 mimics into palmitate treated HepG2 cells could significantly reduce TNF-α and IL-8 expression levels (Hur et al. 2015). Present study suggested that trans-chalcone elevated miR-451 and consequently reduced IL-8 levels after 6 weeks. Furthermore, it could reduce IL-8 after 2 weeks, which was independent of miR-451. Consistent with the present study, Karlsen et al. (2007) have suggested that anthocyanins, belonging to the flavonoid family, exert anti-inflammatory action in health condition. They have shown that anthocyanin supplementation led to a reduction of plasma level of IL-8 as a NF-κB-controlled proinflammatory cytokine in healthy adults (Karlsen et al. 2007). The role of miR-451 in inhibition of NF-κB induced inflammation has been suggested previously (Sun et al. 2016). Therefore, in long-term trans-chalcone inhibits IL-8 through elevation of miR-451. However, it seems that there is other unknown mechanism for reduction of IL-8 level by short-term administration of trans-chalcone. One possible mechanism is elevation of Sirtuin1 (SIRT1) through the inhibition of miR-34a. In this pathway, miR-34a directly decreases the expression of SIRT1 (Choi et al. 2013). Moreover, SIRT1 suppresses expression of IL-8 by deacetylation of histones in its promoter (Hayakawa et al. 2015). However, further studies are needed to clarify this possible mechanism or other unknown mechanisms.

Conclusions

The present study for the first time introduced miR-451/IL-8 pathway as a possible mechanism for hepatoprotective action of trans-chalcone in long-term. Taken together, trans-chalcone has beneficial effect on the liver health and function, which is revealed by reduction of liver injury biomarkers. These positive impacts may be due to inhibitory effect of this chalcone on IL-8 production in liver. These findings suggest the role for the trans-chalcone in the prevention of chronic inflammatory diseases and also are basis of future works about the effects of chalcones on miR-451/IL-8 pathway in pathologic conditions.

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