The effect of Curcuma longa extract and its active component (curcumin) on gene expression profiles of lipid metabolism pathway in liver cancer cell line (HepG2)

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\textbf{ABSTRACT}

\textbf{Objective:} Dyslipidemia is a disorder in lipoprotein metabolism. This disorder could lead to atherosclerosis and it promotes the coronary artery disease and stroke. Turmeric and its active compound, curcumin, modulate the lipid metabolism and also have been used to treat the fatty liver. The present study evaluated the effect of Curcuma longa extract and curcumin on gene expression profiles of lipid metabolism pathway in human HepG2.

\textbf{Methods:} HepG2 cell line was cultured in RPMI-1640 media. The cells in each well were incubated in various concentrations of curcumin and hydro-alcoholic extract of Turmeric (15, 30, 60, 125, 250, 1000, 2000 μg/ml). The cell viability was investigated by MTT assay and the expression of lipid metabolism genes was determined using real-time PCR in IC20 concentration.

\textbf{Results:} The proliferation of HepG2 cells significantly reduced in curcumin-treated and hydro-alcoholic extract of Turmeric-treated groups with increasing the concentrations. The expression of lipogenesis-related genes such as PGC1A, CPT1A and ACOX1 significantly increased in both treatment groups as compared with the control group. Curcumin and hydro-alcoholic extract of Turmeric significantly diminished the expression of lipid synthesis genes such as SCD1, SREBF2, and DGAT1.

\textbf{Conclusion:} According to our study, Turmeric and curcumin reduce the viability of HepG2 cells in a concentration-dependent manner; in addition, these ingredients decrease the lipogenesis genes while they increase the lipogenesis genes. This report suggests that Turmeric could remove the lipid and fatty acids. This herbal substance could be a helpful treatment for obesity and fatty liver.

1. Introduction

Abnormalities in the lipid metabolism are growing throughout the world in recent years. These abnormalities are related to dyslipidemia, diabetes, overweight, cardiovascular disease, and inflammation (Dennis Kasper et al., 2015). Dyslipidemia is an abnormal amount of lipids in blood and could be a disorder in lipoprotein metabolism. This disorder could be a main factor in increasing of total cholesterol (TC), low-density lipoproteins (LDLs), triglycerides (TG) and reducing of high-density lipoproteins (HDLs) (Dennis Kasper et al., 2015). It also could promote atherosclerosis that is a main risk factor for coronary artery disease and stroke (Micah and Nkum, 2012).

Although different treatments have been suggested for dyslipidemia, combination therapy is considered as a new option for this
disorder. This treatment is equally effective in reducing the amount of lipid, drug doses, and side effects (Catapano et al., 2014). Fibrates are a class of amphipathic carboxylic acids which are used in treatment of a wide range of metabolic disorders (Shipman et al., 2016). They decrease plasma triglyceride levels in the liver (Staels et al., 1998) and elevate HDL-C in patients with hyperlipidemia and diabetes type 2 (Takei et al., 2017). Although some factors such as statins and fibrates could moderate the lipid metabolism and improve the insulin resistance, these drugs could lead to develop some adverse effects (Ornato, 2003). Some studies focus on the traditional medicines and have evaluated some medicinal herbs because these medicines are effective low-cost treatments. One of the medical herbs is the root of Turmeric (Curcuma longa) (Araujo et al., 2016).

Turmeric has a wide range of biological properties such as anti-bacterial, anti-inflammatory and antioxidant (Abdel-Tawwab and Abbass, 2017; Nonse et al., 2014; Ramadan et al., 2011; Saccol et al., 2017). Turmeric improves the activities of digestive enzymes and is involved in lipid metabolism (Midun et al., 2016; Seo et al., 2008). This plant contains some main components such as sesquiterpenoids, Tumerona, demethoxycurcumin, bisdemethoxycurcumin, and curcuminoids (Singh et al., 2011). Curcumin is a bioactive curcuminoid in Turmeric (Nguyen et al., 2017). This ingredient is a natural polyphenol which exerts lots of biological activities including antioxidant, anti-inflammatory, anticaner, and neuronal protection (Ding et al., 2015a; Wang et al., 2016). Several studies demonstrated that curcumin could lower the blood sugar by diminishing the hepatic glucose production. In addition, curcumin has some effective result on modulation of lipid metabolism (Ding et al., 2015b; Ghorbani et al., 2014; Liu et al., 2015; Xie et al., 2012). A study showed that this component prevents lipid accumulation in liver and reduces adipose tissue and also can inhibits adipogenesis and differentiation of lipid cells (Zhao et al., 2011). According to another study, curcumin manages the weight in overweight people affirmatively and has a significant reduction effect on hepatic fatty acid biosynthesis (Jang et al., 2008).

Some genes are involved in lipogenesis and fatty-acid oxidation such as ABCA2, PGC1α, CPT1A and ACOX1. ABCA2 is a member of ABC1 subfamily and is highly expressed in the brain tissue and less in the liver. ABCA2 gene is involved in macrophages fat metabolism, drug resistance and neural growth and also it could regulate the intracellular metabolism of sphingolipids (Davis Jr. and Tew, 2018). PGC1α is transcriptional coactivator of the PPAR which interact with PPARγ. This gene interferes in biogenesis of mitochondria, fatty acid oxidation gluconeogenesis and insulin signaling (Petr et al., 2018). CPT1A is a mitochondrial enzyme that has a critical role in beta-oxidation of fatty acids especially long-chain fatty acids. It is involved in synthesis of acylcarnitine through the transfer of the acyl group from coenzyme A to t-carnitine (Guan et al., 2019). The first enzyme in the beta-oxidation of fatty acids pathway is ACOX1. This gene has an important role in metabolism of branched-chain fatty acids and defect on ACOX1 may lead to adrenoleukodystrophy which is characterized by the accumulation of long-chain fatty acids (Vamecq et al., 2018).

SCD1, SREBF2 and DGAT1 genes are related to lipid synthesis. One of the main enzymes in the synthesis of monounsaturated fatty acids is SCD1. There are plenty of the main substrates in cholesterol esters, triglycerides and membrane phospholipids. This gene is highly expressed in liver and adipose tissue and its half-life is 3–5 h then it de-generates in the microsomes (Polonioti et al., 2015). SREBF2 is an important factor in cholesterol metabolism and lead to expression of rate-limiting enzymes and enhancement of LDL-C and triglyceride level. Once the cholesterol reduces inside the cells mature form of SREBF activates the LDLR and HMGCR (Ma et al., 2015). DGAT1 is a metabolic enzyme and crosses the membrane. This protein could catalyze the alteration of diacylglycerol, acyl-CoA into triacylglycerol. DGAT1 is related to obesity and other metabolic disease (Bhatt-Wessel et al., 2018).

With regard to the liver, it has a main role in the lipid metabolism, human hepatocellular carcinoma cell line (HepG2) which include the liver carcinoma cells are suitable for studying the pathway of lipid metabolism (Moriwaki et al., 2014). In the current study, we try to evaluate the effect of Curcuma longa extract and its active compound (curcumin) on gene expression profiles of lipid metabolism pathway in HepG2.

2. Material and methods

Rhizome of Turmeric was purchased from commercial markets and confirmed by staff herbarium, Department of Phytology at Vali-e-Asr University of Rafsanjan. The rhizomes were dried at room temperature, and then they were completely powdered by laboratory blender (Waring Products Division model, USA). The powdered Turmeric (5 g) was extracted with water and ethanol (250 ml, ratio 2:8) at 50 °C for 72 h in a Soxhlet extractor (Bakhshi Industries, Iran) (Rabbani Haghighi et al., 2014). Then alcohol was completely extracted and liquid extracts were dried in freeze drier (VaCo5-D model, Zirbus Technology, Germany) at −55 °C for 48 h. The yellow powder extracted was kept at −20 °C until used (Choi et al., 2014).

In current study, the HepG2 cell line was used. The cells were cultured in RpMI-1640 media supplemented with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin and incubated in a 5% CO2 incubator at 37 °C. When the cells occupied 80% of the flasks, adherent cells were trypsinized with trypsin and cultured in 96-well plates at a density of 5 × 10^3 cells/well (Faedmaleki et al., 2014).

2.1. MTT assay

HepG2 cells in each well were incubated in various concentration of hydro-alcoholic extract of Turmeric (62.5, 125, 250, 500, 1000 μg/ml) and curcumin (5, 10, 20, 40, 80 μg/ml) in a 5% CO2 incubator at 37 °C (Abdel-Lateef et al., 2016). After 24 h, the medium of wells was removed, and 0.5 mg/ml of MTT added to wells then the plates incubated for 4 h at 37 °C. Then the medium containing MTT was removed and 150 μl DMSO (dimethyl sulfoxide) was added to each well to dissolve Formazan Crystals. The optical density (OD) was measured at 492 nm by using a microplate reader (Fan et al., 2014). The MTT assay was performed in triplicate.

The cells viability was defined by following formula (Dai et al., 2013):

Cell viability (%) = OD (test well)/OD (reference well) × 100%.

MTT assay showed that IC20 for hydro-alcoholic extract of Turmeric was 62.5 μg/ml and curcumin was 5 μg/ml which in these concentrations, the cell viability was the most. IC50 for hydro-alcoholic extract of Turmeric was approximately 125 μg/ml, and curcumin was 40 μg/ml, so we chose IC20 concentration for real-time-PCR.

2.2. Real-time-PCR

In the present study, the cells in one group were incubated in concentrations of 62.5 μg/ml hydro-alcoholic extract of Turmeric, and in another group, concentration of curcumin was 5 μg/ml. After 24 h, total RNA of cultured cells was extracted by TRizol reagent according to the manufacturer's protocol of RNA extraction kit (Pars Tous, Iran). cDNA was synthesized from extracted RNA by cDNA synthesis (Pars Tous, Iran). Real-time PCR was undertaken in triplicate for PGC1α, DGAT1, CPT1α, SCD1, SREBP1, ABCA2, ACOX1 and β-Actin genes. β-Actin was used as a housekeeping gene. Primers of each gene were designed by vector NTI software and confirmed in the BLAST database. The sequences of primers are demonstrated in Table 1. The real-time PCR reaction was executed by using real-time SYBR Premix Ex TaqTM (Thermo Fisher ABI). The following protocol was programmed on a CF996 system (Bio-Rad Laboratories Inc., Hercules, CA, USA) for PCR amplification. There was one cycle of 95 °C, 40 cycles of 95 °C, and 40 cycles of 60 °C. Relative quantification was computed by the 2^−ΔΔCt method.
formula, \( \Delta C_t = C_t \text{Gene} - C_t \text{Control} \). Data were analyzed using the Bio-Rad CFX manager.

2.3. Statistical analysis

All data were analyzed by SPSS Statistical software version 21. All the results are presented in statistical indices and graphs. The differences between the average gene expressions of the groups were analyzed through one-way analysis of variance (ANOVA) and t-test. All data were measured as statically significant at \( P \) value of \(<0.05\).

3. Result

3.1. Cell viability

Cell viability was performed using the MTT assay to investigate the effect of curcumin and hydro-alcoholic extract of Turmeric on 24 h of treatment. The result showed that the different concentrations of curcumin reduced the cell proliferation in a dose-dependent manner. The reduction of the viability of HepG2 cells in curcumin-treated groups were statistically significant compared to the control group (\( P \) value = .001) (Fig. 1).

The results revealed that the proliferation of HepG2 cells diminished in the treatment groups that were treated with hydro-alcoholic extract of Turmeric (extract). This diminution was promoted with increasing the concentrations of extract. Compared to the control group, the reduction in the viability of HepG2 cells in the extract-treated groups was statistically significant (\( P \) value = .001) (Fig. 2).

3.2. Real-time PCR

In the present study, we evaluated the expression of important genes in the lipid metabolism such as SCD1, ABCA2, PGC1A, SREBF2, CPT1A, ACOX1 and DGAT1 by real-time PCR assay using the IC20 concentration (a non-toxic concentration in which 80% of Hepg2 cells are able to survive). The expression of fatty-acid oxidation and lipogenesis-related genes such as ABCA2, PGC1A, CPT1A and ACOX1 increased in the curcumin-treated and the extract-treated groups. The levels expression of PGC1A, CPT1A, and ACOX1 were statically significant in both treatment groups as compared with the control group (\( P \) value = .018, .001, and .001, respectively) while the expression of ABCA2 was non-significant (\( P \) value = .08) (Fig. 3).

Analysis of mRNA for the major genes involved in lipid synthesis such as SCD1, SREBF2, and DGAT1 revealed that curcumin and extract significantly reduced the expression of these genes compared to the control group (\( P \) value = .004, .003, and .002, respectively). The expression of SCD1 and SREBF2 genes in the curcumin-treated group was

<table>
<thead>
<tr>
<th>Table 1</th>
<th>The sequence of primers for real-time PCR.</th>
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<tr>
<td>Gene</td>
<td>Forward primer</td>
</tr>
<tr>
<td>ABCA2</td>
<td>TGAGGCAAAACTTTGCGCTG</td>
</tr>
<tr>
<td>ACOX1</td>
<td>CTTCAGCGCTACCAAGGGGCA</td>
</tr>
<tr>
<td>CPT1A</td>
<td>TTTCGTCCCTCCCTCCCTCT</td>
</tr>
<tr>
<td>DGAT1</td>
<td>TGGCGGTTCGCCAATCAC</td>
</tr>
<tr>
<td>PGC1A</td>
<td>CGCAACGCAGCAAGAATTC</td>
</tr>
<tr>
<td>SREBF2</td>
<td>TCCGCGCTTCGAGATGTC</td>
</tr>
<tr>
<td>SCD1</td>
<td>CGGACGTCGCTTCTTTCTCT</td>
</tr>
<tr>
<td>β-actin</td>
<td>GATCAGCAAGCAGGAGATG</td>
</tr>
</tbody>
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Fig. 1. Viability average of Hepg2 cells after 24 h of hydro-alcoholic extract of turmeric treatment.

Fig. 2. Viability average of Hepg2 cells after 24 h of hydro-alcoholic extract of turmeric treatment.

Fig. 3. t-Test for statistical analysis of fatty-acid oxidation and lipogenesis-related genes expression. The results are presented in mean ± SEM. The significance level was considered \( P < .05 \).
studied the effects of curcumin on clozapine-induced lipid disturbance. They found that curcumin alleviated the clozapine-induced disturbance of lipid metabolism through interaction with AMPK-SREBP pathway; however, it did not have any effect on the body weight gain (Liu et al., 2017).

We analyzed the mRNA expressions of main genes involved to lipid metabolism by real time PCR. Our results indicated that curcumin and hydro-alcoholic extract of Turmeric overexpress the PGC1α, CPT1α and ACOX1 genes significantly while they suppress the mRNA expression of SCD1, SREBF2, and DGAT1 genes in HepG2 cell. A study in 2017 was performed on murine HepG2 cell line to determine the anti-nonalcoholic steatohepatitis (NASH) role of *Curcuma longa* and *Artemisia iwayomogi*. Their data which are in line with our study, demonstrated that curcumin can diminish SREBP1 gene expression but elevate PPAR-α gene expression (Kim et al., 2017). Singh et al. (2015) evaluated the anti-atherosclerosis effect of curcumin oil in THP1-derived macrophage. They showed that curcumin inhibited the cholesterol accumulation via increasing the lipid-related genes. Some studies showed that curcumin and extract of Turmeric could regulate the lipid metabolism in liver through the activation of AMPK and suppression of SREBF. As regards to SREBF, they are able to regulate the transcription of the involved genes in triglyceride biosynthesis such as GPAT, SCD1 and FAS, so inhibition of SREBF expression may lead to reduction of SCD1 expression and lipogenesis in liver and adipose tissue (Ding et al., 2016; Um et al., 2013). Activation of AMPK can also inhibit the expression of Acetyl-CoA carboxylase and reduce malonyl-CoA level and it in turn increases the CPT1α and ACOX1 expression (Shin et al., 2014; Shin et al., 2011). Overexpression of PPARα, LXRα, ABCA1 and ABCG1 in macrophages may inhibit the progress of atherosclerosis (Singh et al., 2015). Fan et al. (2014) demonstrated that the expression of fatty acids synthase (FAS) decreased in HepG2 cells with exposure to curcumin. Their data showed that curcumin can prevent fatty acids synthase function (Fan et al., 2014). Another study investigated the effect of KBH-1 on HepG2 cells as an in vitro and on the statotatic liver of high-fat diet-induced obesity rats as an in vivo study. KBH-1, a combination of *Saururus chinensis*, *Curcumalona* and *Polygonatenuifolia*, diminished the main lipogenesis genes such as SREBP-1c, SCD-1, CD36 and ACC, whereas it increased the expression of main lipolysis gene such as ACOX1, CPT-1 and PPARα. These data showed that KBH-1 could modify the fatty acid catabolism. The results of their study confirm the results of our study (Lee et al., 2016). A study revealed that curcumin may have a role in modulation of PPARα and its target genes expression. Therefore, it could enhance the expression of genes which are related to catabolism of the fatty acids (Shin et al., 2011). Several studies explained that curcumin could reduce plasma low-density lipoprotein-cholesterol (LDL) in three ways: it prohibits the uptake of cholesterol in intestine cells, increases the LDL receptor and in turn removes LDL cholesterol from the blood, and enhances the activity of cholesterol-7-hydroxylase (Feng et al., 2010; Ghosh et al., 2014; Peschel et al., 2007). Some studies examined the role of curcumin on hepatic gene expression such as low-density lipoprotein receptor (LDLR). They reported that curcumin in Hep2 cells promoted expression of LDLR gene, but in hepatic stellate cells could suppress the expression of this gene (Peschel et al., 2007; Kang and Chen, 2009).

5. Conclusion

Our result demonstrated that Turmeric and curcumin could reduce the viability of HepG2 cells and also decrease the lipogenesis genes while they increase the lipid synthesis genes. Therefore, Turmeric and its active component could be useful herbal substances to remove the lipid and fatty acids. This report suggests that Turmeric could be a helpful treatment for obesity. In this study, we did not consider positive control testing, so it is better to add some inhibitor of the target genes as the positive control in other studies and also in vivo studies are needed to evaluate the effect of Turmeric and curcumin on humans.

![Fig. 4](image_url)

**Fig. 4.** - Test for statistical analysis of lipid synthesis genes expression. The results are presented in mean ± SEM. The significance level was considered *P* < .05.


