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Inhibitory Effect of Tea (*Camellia Sinensis* (L.) O. Kuntze, Theaceae) Flower Extracts on Oleic Acid-Induced Hepatic Steatosis in Hepg2 Cells

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Abstract Tea (*Camellia sinensis* (L.) O. Kuntze, Theaceae) flowers possess many physiological functions and have been used in traditional medicines for deodorization, skin care, cough suppressant and expectorant in China. However, there is little information about its effect on nonalcoholic fatty liver disease (NAFLD). In this study, an oleic acid-induced HepG2 cell model of steatosis was established, and the anti-NAFLD effects of tea flowers and the related mechanisms were investigated. Three tea flower extracts, 40% TFE, 80% TFE and TFRE couldn't prevent triglyceride (TG) accumulation in oleic acid-treated HepG2 cells ($p > 0.05$), but significantly decreased the TG level in lipid-overloaded HepG2 cells after 48 h treatment ($p < 0.05$). RT-PCR analysis revealed that three tea flower extracts did not affect the mRNA levels of peroxisome proliferator-activated receptors α (PPARα) and Acyl-CoA oxidase-1 (ACOX-1) ($p > 0.05$), but up-regulated the mRNA level of carnitine palmitoyl-CoA transferase-1A (CPT-1A) ($p < 0.05$). Moreover, tea flower extracts could significantly reduce intracellular reactive oxygen species (ROS) level ($p < 0.05$). These results indicated tea flowers may be a potential natural resourse for the treatment of NAFLD.

Keywords: *tea flowers, NAFLD, triglyceride, CPT-1A, ROS*

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most common liver diseases in the world [1]. NAFLD encompasses a broad clinicopathologic spectrum ranging from simple steatosis to nonalcoholic steatohepatitis (NASH), and may advance to cirrhosis and end-stage liver disease [2]. The development and progression of NAFLD have been explained according to a "two-hit" hypothesis. Briefly, the "first hit" also called steatosis, is the deposition of triglyceride (TG) in hepatocytes. Once steatosis has been established, the "second hit" is required for hepatocyte injury, which is accompanied by mitochondrial dysfunction, lipid peroxidation and production of inflammatory cytokines in hepatic cells [3,4].

So far, hepatoprotective drugs and weight reduction are the only two strategies for the treatment of NAFLD. However, both of them have poor long-term success rates [1,5]. Bioactive compounds derived from plant foods have aroused a great interest in ameliorating adverse metabolic disorders because of their low toxicity and few side effects [6]. Numerous studies demonstrated that green tea (*Camellia sinensis* (L.) O. Kuntze, Theaceae) have hypolipidemic, thermogenic, antioxidant and anti-inflammatory activities that may mitigate the occurrence and progression of NAFLD [7]. Compared with tea leaves, tea flowers have many similar chemical compounds and bioactivities, and are valuable resource too [8]. Tea flowers have been used in traditional medicines for deodorization, skin care, cough suppressant and expectorant in China [9]. The ethanolic extract of tea flowers was found to possess the potent antioxidant activity determined by 2, 2-diphenyl-1-picrylhydrazyl (DPPH) free radical-scavenging assay [10]. The polysaccharides from tea flowers showed antioxidant activity *in vitro* and hepatoprotective activity *in vivo* [11]. Oral administration of tea flower powder could effectively prevent high-fat diet-induced body weight increase and hyperlipidmemia [12].

Although the activities of tea flowers on some metabolic syndromes have been reported, little is known about its beneficial effect on NAFLD. In this study, an oleic acid-induced HepG2 cell model of steatosis was established to investigate the role of different tea flower extracts in attenuating NAFLD and the related mechanisms *in vitro*. Intracellular TG was measured to determine the ability of tea flowers for reducing intracellular lipid accumulation. The mRNA levels of peroxisome proliferator-activated receptors α (PPARα),

carnitine palmitoyl-CoA transferase-1A (CPT-1A) and Acyl-CoA oxidase-1 (ACOX-1) were evaluated to observe the effect of tea flowers on the expression of key genes involved in fatty acid β-oxidation. In addition, the effect of tea flower extracts on intracellular reactive oxygen species (ROS) generation was investigated.

2. Materials and Methods

2.1. Preparation and Characterization of Tea Flower Extracts

Dried Tea flowers were milled into powder by a pulverizer (DFY-500, Linda Machinery Co., Ltd., Zhejiang, China). A 10 g sample was extracted with 150 mL of 40% or 80% ethanol under reflux at 80ºC for 45 min, and two fractions (40% TFE and 80% TFE) were obtained. Furthermore, the residues after 80% ethanol extraction were boiled with 150 mL of distilled water under reflux for 1 h, and the third fraction TFRE was obtained. All decoctions were filtered, frozen at−80ºC and then lyophilized (Christ ALPHA 1-4 LD plus, Germany). The extraction process is presented in Fig 1. The contents of saponins, polyphenols, flavonoids, polysaccharides, proteins, amino acids and total sugar in tea flower extracts were determined by a spectrophotometer as described previously [13-19]. The content of caffeine was analyzed using a high-performance liquid chromatography (HPLC) system (SHIMADZU LC-2010A, Kyoto, Japan) [20].

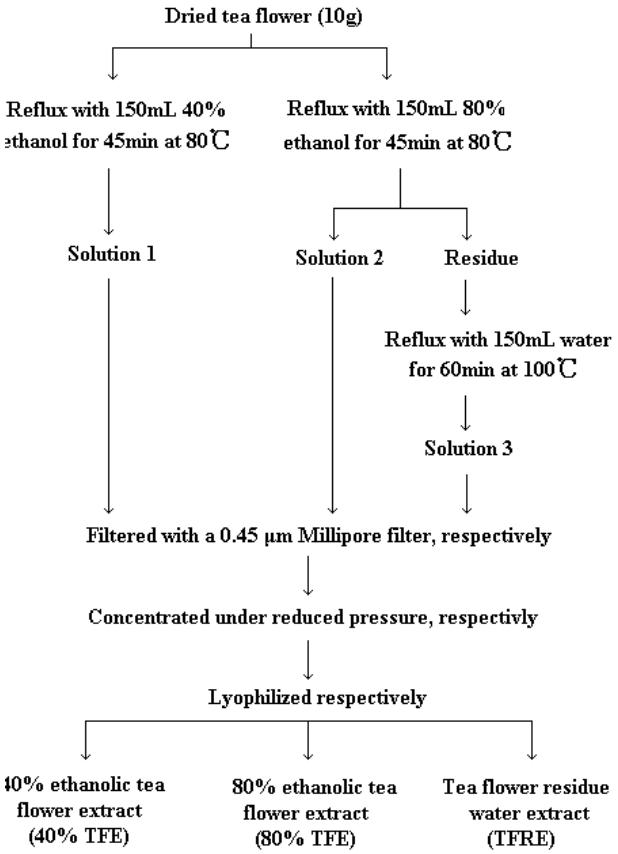

Figure 1. Preparation process of three tea flower extracts

2.2. Cell Culture

Human hepatocellular carcinoma cell line HepG2 were cultured in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT), 1% non-essential amino acid (Gibco, Grand Island, NY, USA) and 1% penicillin/streptomycin (Gibco, Grand Island, NY, USA) at 37°C in a humidified incubator with 5% CO_2.

2.3. Cell Viability Assay

Cell viability was measured with the methylthiazolyldiphenyl-tetrazolium bromide (MTT) cell proliferation and cytotoxicity detection kit (Nanjing KeyGen Biotech, China). Briefly, HepG2 cells were seeded in 96-well plates at a density of 2×10^4 cells/well and incubated for 24h. Then cells were treated with different concentrations of oleic acid (0, 0.25, 0.5, 0.75 mM) or tea flower extracts (0, 50, 75, 100, 150, 300 μg/mL) with 1% fatty acid-free bovine serum albumin (BSA) or dimethyl sulfoxide (DMSO) as vehicle control, respectively. After treatment for 24 h, the medium was removed and fresh medium containing 0.5 mg/mL MTT was added to each well, followed by incubation for 4 h at 37°C. Finally, the medium containing MTT was removed, and cells were lysed with DMSO. The absorbance at 492 nm was measured using a microplate reader (Thermo, Multiscan MK3).

2.4. Measurement of the Intracellular TG Level

TG concentration was analyzed using an enzymatic kit (Beijing BHKT Clinical Reagent Co.,Ltd.). The total protein levels were measured with a BCA assay kit (Beyotime, Jiangsu, China). Intracellular TG level was expressed as micrograms of TG per milligram of cellular protein.

2.5. Induction of Hepatic Steatosis in HepG2 Cells

HepG2 cells were seeded in 6-well plates at a density of 5×10^5 cells/well. After attached to the plates, cells were treated with various concentrations 0, 0.25, 0.5, 0.75 mM) of oleic acid (containing 1% fatty acid-free BSA, Sigma, USA) to induce steatosis in HepG2 cells. After incubation for 24h, intracellular TG level was measured as described in section 2.4, and the optimal oleic acid concentration for steatosis induction was determined.

2.6. Total RNA Preparation and RT-PCR Analysis

Total RNA was extracted from HepG2 cells using RNAiso Plus (Takara, Japan). Complementary DNA (cDNA) was synthesized from 1 μg total RNA using PrimeScript RT reagent kit (Takara, Japan). The levels of mRNA transcripts were quantified by quantitative real-time PCR (RT-PCR) using SYBR Premix Ex Taq II (Takara, Japan) and the 7500 Real Time PCR system (Applied Bio, Foster City, USA) according to the manufacturer's protocol. Sequences of the primers used in the study are shown in Table 1. Relative mRNA expression was calculated with the ΔΔCT method. GAPDH mRNA levels were quantified in each sample and were used as a normalization control.

Table 1. Sequences of the primers used in the RT-PCR analysis

Gene		Sequence (5′→3′)
PPARα	Forward:	CCTCCTCGGTGACTTATCCTG
	Reverse:	CAATACTGGCATTTGTTTCTGTTC
CPT-1A	Forward:	ACAAGGACATGGGCAAGTTTTG
	Reverse:	TTCAGCCTCTGTTCCACCGT
ACOX-1	Forward:	CAGGAAAGTTGGTGTGTGGC
	Reverse:	AATCTGGCTGCACGGAGTTT
GAPDH	Forward:	TGCACCACCAACTGCTTAGC
	Reverse:	GGCATGGACTGTGGTCATGAG

2.7. Measurement of Intracellular ROS

Reactive oxygen species (ROS) production was monitored by microplate reader using a Reactive Oxygen Species Assay Kit (Beyotime, Jiangsu, China). Briefly, cells were incubated with dichlorofluorescin diacetate (DCFH-DA) at a final concentration of 10 μM in medium at 37°C for 20 min. Cells were washed twice with ice-cold PBS, and the fluorescence was recorded at excitation and emission wavelengths of 488 and 525 nm, respectively.

2.8. Statistical Analysis

A minimum of three independent experiments were performed. The data were presented as means ± standard deviations (SD). Multiple comparisons were performed by one-way analysis of variance (ANOVA) followed with Least Significant Difference test (LSD). A probability level of 5% ($p < 0.05$) was considered as significant. All computations were made by employing the SAS system for windows V8.

3. Results

3.1. Chemical Compositions of Tea Flower Extracts

The main compositions of each tea flower extract are shown in Table 2. All three tea flower extracts contained saponins, polyphenols, caffeine, flavonoids, proteins, amino acids, sugar and polysaccharides. The contents of polyphenols, caffeine and amino acids in three tea flower extracts were similar. Compared with the other two extracts, 80% TFE contained more saponins and flavonoids, while TFRE contained more polysaccharides, total sugar and proteins. The first two principal components (except for total sugar) in three tea flower extracts were polyphenols and proteins in 40% TFE, saponins and polyphenols in 80% TFE, proteins and polyphenols in TRFE, respectively.

Table 2. The chemical composition of tea flower extracts

Component (%)	40% TFE	80% TFE	TFRE
Saponins	8.49	21.70	5.45
Polyphenols	15.04	14.80	13.17
Caffeine	2.93	2.49	2.06
Flavonoids	6.08	9.18	5.25
Proteins	10.98	8.10	16.70
Amino acids	1.56	1.43	2.02
Total sugar	20.44	19.08	26.84
Polysaccharides	5.45	4.24	9.64

3.2. Induction of Hepatic Steatosis in HepG2 Cells by Oleic Acid

To determine the optimal concentration of oleic acid for induction of steatosis *in vitro*, HepG2 cells were cultured with oleic acid at a concentration of 0.25, 0.5 and 0.75 mM for 24 h, respectively. Table 3 shows treatment with 0.25, 0.5 and 0.75 mM oleic acid had no cytotoxic effects on HepG2 cells compared with the control as determined by the MTT assay ($p > 0.05$). As shown in Table 4, oleic acid increased intracellular TG level in a dose-dependent manner ($p < 0.05$). Therefore, 0.75 mM of oleic acid was chosen as the optimal concentration to induce steatosis in HepG2 cells for the subsequent experiments.

Table 3. Cytotoxic effect of oleic acid on HepG2 cells

Concentration of oleic acid (mM)	Viability (%)
0	100.00±4.44 [a]
0.25	94.60±3.82 [a]
0.5	103.51±6.99 [a]
0.75	101.30±5.68 [a]

Values are expressed as mean ± SD (n = 5).
Values in a column followed by different letters are significantly different ($p < 0.05$)

Table 4. Effect of oleic acid on TG accumulation in HepG2 cells

Concentrations of oleic acid (mM)	Intracellular TG content (μg/mg)
0	16.96±0.69 [d]
0.25	187.87±16.36 [c]
0.5	329.40±23.96 [b]
0.75	429.65±19.11[a]

Values are expressed as mean ± SD (n = 6).
Values in a column followed by different letters are significantly different ($p < 0.05$).

3.3. Prevention Effects of Tea Flower Extracts on Oleic Acid-induced TG over Accumulation in HepG2 Cells

HepG2 cells were pretreated with each tea flower extract (50 μg/mL, non-cytotoxic concentration) for 2 h, respectively, and then co-treated with oleic acid for 24h. As shown in Table 5, all three tea flower extracts had no effects on TG over accumulation in HepG2 cells induced by oleic acid ($p > 0.05$), indicating tea flower extracts had no effects on inhibition of lipid absorption and biosynthesis.

3.4. Inhibitory Effects of Tea Flower Extracts on Oleic Acid-induced TG over Accumulation in HepG2 Cells

Lipid-overloaded HepG2 cells were treated with each tea flower extract (50 μg/mL) for 24 and 48h, respectively. As shown in Table 6, all the tea flower extracts had little effects on TG accumulation in lipid-overloaded HepG2 cells after 24 h treatment ($p > 0.05$), but significantly decreased intracellular TG level after 48h incubation ($p < 0.05$). Among the three samples, TFRE had the most potential to reduce TG accumulation, and the activity was similar with the positive control bezafibrate (a drug used for the treatment of hyperlipidemia). These results implied that tea flower extracts were able to decrease the deposited fat in lipid-overloaded HepG2 cells.

Table 5. Prevention effects of tea flower extracts on oleic acid-induced TG over accumulation in HepG2 cells

Treatment	Intracellular TG content (μg/mg)
Normal control	16.76±0.55 [b]
Model control	441.77±27.59 [a]
40% TFE	453.72±37.28 [a]
80% TFE	426.82±13.15 [a]
TFRE	412.47±15.73 [a]

Values are expressed as mean ± SD (n = 3).
Values in a column followed by different letters are significantly different ($p < 0.05$).
Normal control: Treatment with vehicle without oleic acid induction.
Model control: Pretreatment with 0.75 mM of oleic acid for 24 h.

3.5. Effects of tea flower extracts on PPARα, CPT-1A and ACOX-1 mRNA expresion

To elucidate the possible molecular mechanisms of the lipid-lowering effect of tea flower extracts, the mRNA levels of genes involved in fatty acid β-oxidation (PPARα, CPT-1A and ACOX-1) were determined by RT-PCR. Figure 2 showed that oleic acid had no effect on the PPARα mRNA expression ($p > 0.05$, Fig 2A), but could significantly decreased the CPT-1A mRNA level ($p < 0.05$, Fig 2B), and reduced the mean mRNA level of ACOX-1 ($p > 0.05$, Fig 2C). Three tea flower extracts had no effects on the mRNA expression of PPARα and ACOX-1, but all elevated the mean mRNA level of CPT-1A. These results suggested that tea flower extracts probably decreased intracellular TG content by up-regulating CPT-1A mRNA expression.

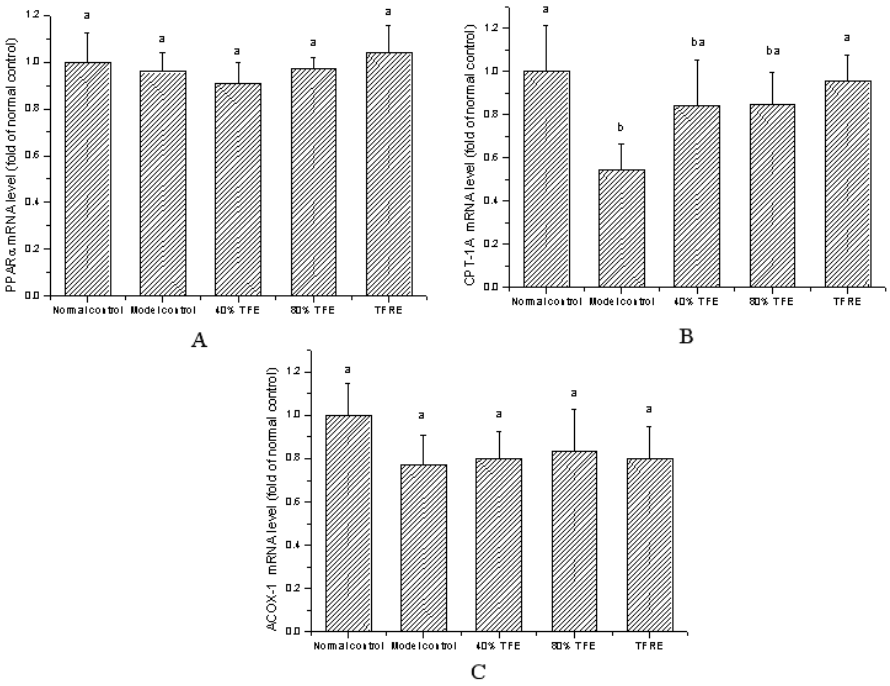

Figure 2. Effect of tea flower extracts on PPARα, CPT-1A and ACOX-1 mRNA expression in lipid-overloaded HepG2 cells. Lipid-overloaded HepG2 cells were treated with 50 μg/mg tea flower extracts for 48h. (A) PPARα, (B) CPT-1A and (C) ACOX-1 mRNA expression was quantified by real-time RT-PCR. Data represent means ± SD from three independent experiments. Significant differences among different treatments are indicated by different letters ($p < 0.05$). Normal control: Treatment with vehicle without oleic acid induction. Model control: Pretreatment with 0.75 mM of oleic acid for 24 h

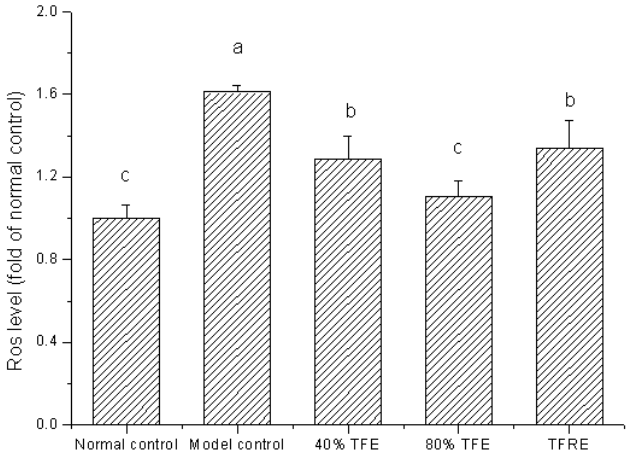

Figure 3. Effect of tea flower extracts on intracellular ROS level in lipid-overloaded HepG2 cells. Lipid-overloaded HepG2 cells were treated with 50 μg/mg tea flower extracts for 48h. Data represent means ± SD from four independent experiments. Significant differences among different treatments are indicated by different letters ($p < 0.05$). Normal control: Treatment with vehicle without oleic acid induction. Model control: Pretreatment with 0.75 mM of oleic acid for 24 h

3.6. Tea flower extracts reduce intracellular ROS level

To investigate the effect of tea flower extracts on ROS generation in lipid-overloaded HepG2 cells, intracellular ROS level was determined by the DCFH-DA probe. As shown in Figure 3, oleic acid significantly increased the oxidative stress in cells ($p<0.05$). 40% TFE, 80% TFE and TFRE obviously decreased intracellular ROS level ($p<0.05$), and 80% TFE had the most potential antioxidant activity.

4. Discussion

NAFLD is one of the most common forms of liver disease worldwide. Hepatic steatosis can either be a benign, noninflammatory condition, or can be associated with NASH, a condition that can result in end-stage liver disease. Intracellular lipid accumulation is the main pathological characteristic of a human liver with NAFLD [21]. Currently, no drug has been approved for the

treatment of NASH, or has been included for use in the clinical practice. As a result, novel strategies are needed for the treatment of NAFLD [22].

Natural medicines for the treatment of NAFLD have a long and successful history of controlling disease without prominent side effects [23]. OA-induced steatosis in HepG2 cells may serve as an *in vitro* model for studying fatty liver disease [24]. Tea flowers possess many physiological functions and have been used in traditional medicines in China. In this study, our results showed three tea flower extracts, 40%TFE, 80% TFE and TFRE couldn't prevent TG accumulation in oleic acid-treated HepG2 cells (Table 5), but significantly decreased the TG level in lipid-overloaded HepG2 cells after 48 h treatment (Table 6). These results indicated tea flower extracts couldn't inhibit lipid absorption or lipogenesis, but could promote fatty acid catabolism, and therefore, may prevent hepatic fat deposition.

Table 6. Inhibitory effects of tea flower extracts on oleic acid-induced TG over accumulation in HepG2 cells

Treatment	Intracellular TG content (μg/mg)	
	24h	48h
Normal control	16.653±0.288 ^{c}	16.76±0.55 ^{d}
Model control	520.23±54.93 ^{a}	472.14±29.99 ^{a}
Positive control	384.67±24.52 ^{b}	316.17±27.44^{c}
40% TFE	501.73±32.46 ^{a}	393.53±15.03^{b}
80% TFE	477.70±40.97 ^{a}	401.62±27.86 ^{b}
TFRE	501.93±15.69 ^{a}	343.86±12.57^{c}

Values are expressed as mean ± SD (n = 6).
Values in a column followed by different letters are significantly different ($p < 0.05$).
Normal control: Treatment with vehicle without oleic acid induction.
Model control: Pretreatment with 0.75 mM of oleic acid for 24 h.
Positive control: Treatment with bezafibrate (100 μg/mL) after 24 h preincubation with 0.75 mM of oleic acid.

TFRE contained higher contents of polysaccharides and proteins compared with 40% TFE and 80% TFE (Table 2), and was the most potential among the three samples to reduce the TG level in HepG2 cells after 48 h treatment (Table 6). Previous studies suggested that tea polysaccharides could significantly decrease the levels of serum TG, total cholesterol and low density lipoprotein in hyperlipedemic mouse and rat models [25,26]. Deng et al. [27] demonstrated that water-soluble protein extract from tea flowers had hypolipidemic effect *in vitro*. In addition, pharmacological studies indicated that floratheaA-C from tea flowers had an inhibitory effect on serum triglyceride (TG) elevation in olive oil-treated mice [28]. In sucrose-rich diet-induced hyperlipidemic rats, tea catechins exhibited strong activity in reducing plasma cholesterol and triglyceride concentrations [29]. These results indicated polysaccharides, proteins, flavonoids and saponins may account for the reduction of TG accumulation by tea flowers.

Fatty acid β-oxidation is a multi step process by which fatty acids are broken down by various tissues to produce energy, and occurs in both mitochondria and peroxisomes. CPT-1A and ACOX-1 are the rate-limiting enzymes for fatty acids β-oxidation in mitochondria and peroxisomes, respectively. Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that function astranscription factors associated with lipid metabolism in various tissues and cells, and have three isoforms including PPARα, PPARδ and PPARγ. PPARα is mostly responsible for the metabolism of fatty acid in the liver, and affects fatty acids β-oxidation by regulating the expressions of CPT-1A and ACOX-1 [30,31]. It was reported that Fuzhuan brick tea water extract had anti-obesity and hypolipidemic functions, and could increase gene expressions of PPARα, CPT-1A and LDL receptor [32]. Polyphenol-rich extract from walnut was found to possess hypotriglyceridemic activity via enhancement of peroxisomal fatty acid beta-oxidation in the liver. The up-regulation of PPARα CPT-1A and ACOX-1 mRNA expression were involved in this activity [33]. In this study, quantitative RT-PCR analysis showed that oleic acid treatment lowered the mRNA level of CPT-1A and ACOX-1. Tea flower extracts could reverse the mRNA level of CPT-1A, but had no effect on that of ACOX-1 and PPARα (Figure 2). These results indicated CPT-1A play an important role in the lipid lowing effect of tea flowers.

Oxidative stress is one of the major pathogenic mechanisms for progression of NAFLD. Free fatty acid increases the oxidative stress with formation of free radicals, including hydrogen peroxide and superoxide. Abnormal lipid peroxidation leads to direct hepatocyte injury and advancing NAFLD [34]. There has been a growing evidence for antioxidant therapy in the treatment of NAFLD [2]. Previous study showed tea flowers could reduce the formation of malondialdehyde and enhance the activities of superoxide dismutase and glutathione peroxidase in carbon tetrachloride-induced liver injury mice [11]. Our results showed tea flowers extracts could reduce intracellular ROS level in lipid-overloaded HepG2 cells, and 80% TFE had the strongest pential among the three samples (Figure 3). This result was consistent with the previous report that 70% ethanol extract of tea flowers showed higher antioxidant activity than the water extracts for their inhibitory effect on hydroxyl radicals and DPPH radicals (Yang, Xu, Jie, He, & Tu, 2007). Component analysis indicated 80% TFE contained more flavonoids and saponins than 40% TFE and TFRE (Table 2). It was reported epigallocatechin gallate (EGCG) and epicatechin gallate (ECG) were the major active components responsible for the antioxidant activity of tea flowers. Besides catechins, myricetin, quercetin, and kaempferol mono-, diglycosides were identified [35]. Sasanquasaponin, a saponin from *Camellia oleifera* Abel., could effectively scavenge ROS *in vitro*, and significantly reduce the serum malondialdehyde level in obese rats [36]. These results suggested tea flowers could protect HepG2 cells from hepatocyte injury via reduction of intracellular oxidative stress, and flavonoids and saponins may response for this activity.

In conclusion, this study demonstrated the anti-NAFLD effects of tea flowers and the related mechanisms. Tea flowers had no effects on lipid absorption and lipogenesis, but could promote lipid degradation via increasing CPT-1A gene expression. In addition, tea flowers might protect the liver from NAFLD through the reduction of oxidative stress. These results indicated tea flowers may be a potential natural resourse for the treatment of NAFLD.

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References

[1] Ekstedt, M., Franzén, L. E., Mathiesen, U. L., Thorelius, L., Holmqvist, M., Bodemar, G., & Kechagias, S. (2006). Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology, 44,* 865-873.

[2] Yu, A. S., & Keeffe, E. B. (2002). Nonalcoholic fatty liver disease. *Reviews in gastroenterological disorders, 2,* 11-19.

[3] Day, C. P., & James, O. F. W. (1998). Steatohepatitis: a tale of two "hits"? *Gastroenterology, 114,* 842-845.

[4] Liu, Y., Wang,D., Zhang, D., Lv, Y., Wei, Y., Wu, W., Zhou, F., Tang, M., Mao, T., Li, M., & Ji, B. (2011). Inhibitory effect of blueberry polyphenolic compounds on oleic acid-induced hepatic steatosis *in vitro*. *Journal of Agricultural and Food Chemistry, 59,* 12254-12263.

[5] Ayyad, C., & Andersen, T. (2000). Long-term efficacy of dietary treatment of obesity: a systematic review of studies published between 1931 and 1999. *Obesity Review, 1,* 113-119.

[6] Müller, C., Gardemann, A., Keilhoff, G., Peter, D., Wiswedel, I., & Schild, L. (2012). Prevention of free fatty acid-induced lipid accumulation, oxidative stress, and cell death in primary hepatocyte cultures by a *Gynostemma pentaphyllum* extract. *Phytomedicine, 19,* 395-401.

[7] Masterjohn, C., & Bruno, R. S. (2012). Therapeutic potential of green tea in nonalcoholic fatty liver disease. *Nutrition Reviews, 70,* 41-56.

[8] Li, B., Jin,Y., Xu, Y., Wu, Y., Xu, J., & Tu, Y., 2011. Safety evaluation of tea (*Camellia sinensis* (L.) O. Kuntze) flower extract: Assessment of mutagenicity, and acute and subchronic toxicity in rats. *Journal of Ethnopharmacology, 133,* 583-590.

[9] Yoshikawa, M., Wang, T., Sugimoto, S., Nakamura, S., Nagatomo, A., Matsuda, H., & Harima, S. (2008). Functional saponins in tea flower (flower buds of *Camellia sinensis*): gastroprotective and hypoglycemic effects of floratheasaponins and qualitative and quantitative analysis using HPLC (in Japanese). *Yakugaku Zasshi, 128,* 141-151.

[10] Yang, Z. Y., Tu, Y. Y., Baldermann, S., Dong, F., Xu, Y., & Watanabe, N. (2009). Isolation and identification of compounds from the ethanolic extract of flowers of the tea (*Camellia sinensis*) plant and their contribution to the antioxidant capacity. *LWT-Food Science and Technology, 42,* 1439-1443.

[11] Xu, R. J., Ye, H., Sun, Y., Tu, Y. Y., & Zeng, X. X. (2012). Preparation, preliminary characterization, antioxidant, hepatoprotective and antitumor activities of polysaccharides from the flower of tea plant (*Camellia sinensis*). *Food and Chemical Toxicology, 50,* 2473-2480.

[12] Ling, Z. J., Xiong, C. Y., Han, Q., & He, P. M. (2011). Study on preventive effect of tea plant flower on obesity and hyperlipidemia of rats (in Chinese). *Journal of Chinese Institute of Food Science and Technology, 11,* 50-54.

[13] Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (A.Q.S.). (2003a). Determination of amino acids in foods. *National Standard of China*, GB/T 5009.124-2003.

[14] Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (A.Q.S.). (2008). Determination of total polyphenols and catechins content in tea. *National Standard of China*, GB/T8313-2008.

[15] Dini, I., Tenore, G. C., & Dini, A. (2009). Saponins in *Ipomoea batatas* tubers: isolation, characterization, quantification and antioxidant properties. *Food Chemistry, 113,* 411-419.

[16] Dreywood, R. (1946). Qualitative test for carbohydrate material. *Industrial and Engineering Chemistry, Analytical Edition 18,* 499.

[17] Masuko, T., Minami, A., Iwasaki, N., Majima, T., Nishimura, S. I., & Lee, Y. C. (2005). Carbohydrate analysis by a phenol-sulfuric acid method in microplate format. *Analytical Biochemistry, 339,* 69-72.

[18] Pourmorad, F., Hosseinimehr, S. J., & Shahabimajd, N. (2006). Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *African Journal of Biotechnology, 5,* 1142-1145.

[19] Sedmak, J. J., & Grossberg, S. E. (1977). A rapid, sensitive, and versatile assay for protein using Coomassie brilliant blue G250. *Analytical Biochemistry, 79,* 544-552.

[20] Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China (A.Q.S.). (2003b). Determination of caffeine content-Method using high-performance liquid chromatography. *National Standard of China*, GB/T19182-2003.

[21] Browning, J.D., & Horton, J.D. (2004). Molecular mediators of hepatic steatosis and liver injury. *Journal of Clinical Investigation, 114,* 147-152.

[22] Li, X., Wang, R., Zhou, N, Wang, X., Liu, Q., Bai, Y., Bai, Y., Liu, Z., Yang, H., Zou, J., Wang, H., & Shi, T., 2013. Quercetin improves insulin resistance and hepatic lipid accumulation in vitro in a NAFLD cell model. *Biomedical Reports, 1,* 71-76.

[23] Kim, M.S., Kung, S., Grewal, T., & Roufogalis, B.D. (2012). Methodologies for investigating natural medicines for the treatment of nonalcoholic fatty liver disease (NAFLD). *Current Pharmaceutical Biotechnology, 13,* 278-291.

[24] Cui, W., Chen, S. L., & Hu, K. Q. (2010). Quantification and mechanisms of oleic acid-induced steatosis in HepG2 cells. *American Journal of Translational Research, 2,* 95-104.

[25] Hou, Y. F., Wang, D. F., Zhou, X. L., Zhang, L., Wang, J. F., & Bucheli, P. (2008). Effect of tea polysaccharides on blood lipids and liver trace elements in hyperlipidemic rats. *Acta Nutrimenta Sinica, 30,* 269-272.

[26] Wu, W. H., & Wu, W. J. (2006). Anti-hyperlipidemic effect of polysaccharides from Pu'er tea (in Chinese). *Tea in Fujian, 2,* 42-43.

[27] Deng, X., & Huang, H. H. (2013). *In vitro* binding of bile salts by water-soluble protein extract from tea flower (in Chinese). *Modern Food Science and Technology 29,* 63-67.

[28] Yoshikawa, M., Morikawa, T., Yamamoto, K., Kato, Y., Nagatomo, A., & Matsuda, H. (2005). Floratheasaponins A-C, acylated oleanane-type triterpene oligoglycosides with anti-hyperlipidemic activities from flowers of the tea plant (*Camellia sinensis*). *Journal of Natural Products, 68,* 1360-1365.

[29] Lee, S.M., Kim, C.W., Kim, J.K., Shin, H.J., & Baik, J.H. (2008). GCG-rich tea catechins are effective in lowering cholesterol and triglyceride concentrations in hyperlipidemic rats. *Lipids, 43,* 419-429.

[30] Chinetti, G., Fruchart, J. C., & Staels, B. (2000). Peroxisome proliferator-activated receptors (PPARs): Nuclear receptors at the crossroads between lipid metabolism and inflammation. *Inflammation Research, 49,* 497-505.

[31] Dreyer, C., Keller, H., Mahfoudi, A., Laudet, V., Krey, G., & Wahli, W. (1993). Positive regulation of the peroxisomal β-oxidation pathway by fatty acids through activation of peroxisome proliferator-activated receptors (PPAR). *Biology of the Cell, 77,* 67-76.

[32] Li, Q., Liu, Z. H., Huang, J. N., Luo, G. A., Liang, Q. L., Wang, D., Ye, X. Y., Wu, C. B., Wang, L.L., & Hu, J. H., 2013. Anti-obesity and hypolipidemic effects of Fuzhuan brick tea water extract in high-fat diet-induced obese rats. *Journal of the Science of Food and Agriculture, 93,* 1310-1316.

[33] Shimoda, H., Tanaka, J., Kikuchi, M., Fukuda, T., Ito, H., Hatano, T., & Yoshida, T., (2009). Effect of polyphenol-rich extract from walnut on diet-induced hypertriglyceridemia in mice via enhancement of fatty acid oxidation in the liver. *Journal of Agricultural and Food Chemistry, 57,* 1786-1792.

[34] Madan, K., Bhardwaj, P., Thareja, S., Gupta, S.D., & Saraya, A. (2006). Oxidant stress and antioxidant status among patients with nonalcoholic fatty liver disease (NAFLD). *Journal of Clinical Gastroenterology, 40,* 930-935.

[35] Yang, Z., Xu, Y., Jie, G., He, P., & Tu, Y. (2007). Study on the antioxidant activity of tea flowers (*Camellia sinensis*). *Asia Pacific Journal of Clinical Nutrition, 16 (Suppl 1),* 148-152.

[36] Lv, X. L., Qiu, S. S., Sun, X. X., & Li, Z. J. (2005). Preliminary study on the capability of antioxidation and scavenging free radicals of sasanquasaponins. *Food Science, 26,* 86-90.