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Fermented *Camellia sinensis*, Fu Zhuan Tea, regulates hyperlipidemia and transcription factors involved in lipid catabolism

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ABSTRACT

Emerging evidence supports health-promoting properties of post-fermented Chinese Brick Tea. Fu Zhuan Tea, fermented with the fungus, *Erotium cristatum*, contains a unique phytochemical profile attributed to its unique method of processing. Fu Zhuan Tea has been shown to activate pancreatic enzymes and regulate blood lipids in laboratory models. Regulation of blood lipid levels by Fu Zhuan Tea consumption was examined in an observational pilot study of volunteers with elevated LDL cholesterol that were not taking any prescription lipid lowering medications. Significant changes in blood lipids were detected after 120 days of daily consumption. Fu Zhuan Tea fractionation led to the investigation of six compounds for regulation of transcription factors involved in lipid metabolism, including Farnesoid X receptor (FXR), Liver X-activated Receptor (LXR) and two isoforms of the Peroxisome Proliferator-Activated Receptors (PPARγ and PPARδ). Reporter gene assays with liver cells revealed dose dependent differences in regulation of transcription factor activation. These data reveal potential therapeutic efficacy and candidate molecular targets for Fu Zhuan Tea, and provide rationale for chemical characterization of bioactive fractions and investigation of therapeutic efficacy in cardiovascular disease and type 2 diabetes.

1. Introduction

A tremendous body of literature suggests that consuming tea (*Camellia sinensis* L.) provides important disease fighting activities, yet results from clinical and epidemiological studies to support a relationship between tea consumption and chronic disease risk are not clear (Frank et al., 2009; Steptoe et al., 2007; Stote & Baer, 2008). Socio-economic demographics and lifestyle differences among human populations are important factors contributing to inconsistent results, as well as variation in tea constituents, which are mainly a result of processing rather than botanical varieties. While the purported benefits and phytochemicals in green and black teas have been extensively reviewed (Khan & Mukhtar, 2007; Saito, Gosmann, Pungartnik, & Brendel, 2009; Sharma & Rao, 2009; Wang & Ho, 2009; Zaveri, 2006), post-fermented teas have received considerably less research attention even though they are widely consumed in China. Similar to green tea, the oxidation of post-fermented teas is halted by steaming, which inactivates enzymes responsible for breaking down chlorophyll and producing tannins. This is followed by one or more microbial fermentation steps that give the leaves a darker color and changes their chemical composition (Xie et al., 2009). Recent metabolic profiling studies also reveal that processing of post-fermented teas changed the chemical contents when aged for 1–10 years (Jeng, Chen, Fang, Hou, & Chen, 2007). Fungal fermentation of Fu Zhuan tea resulted in changes in total tea polyphenols, catechins, amino acids, polysaccharides, and organic acids during the fermentation process and these changes corresponded with stimulation of pancreatic enzymes (Wu et al., 2010).

Fu Zhuan Tea (aka PHatea®), is a unique post-fermented tea product from the Hunan Province in China that is distinguished from other post-fermented teas by deliberate fermentation with the fungus, *Eurotium cristatum* (Huang, 2007). Fu Zhuan Tea was recently introduced for consumption in the United States because of the purported human health benefits and findings from animal studies showing Fu Zhuan Tea mediated regulation of blood lipids (Xiao, 2007a). Epidemiological translations from Chinese literature support

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low prevalence of hyperlipidemia among individuals that co-consume high levels of dietary saturated animal fats with Fu Zhuan Tea. However, the bioactive components and mechanisms responsible for regulating blood lipids are not yet well understood.

Results from a human study conducted in China of daily Fu Zhuan Tea consumption for reduced blood LDL cholesterol prompted an observational study in a small group of US volunteers with elevated cholesterol that were not taking lipid lowering medications. Findings from this pilot cohort reported herein support the feasibility of studying Fu Zhuan Tea consumption for changes in blood lipid parameters. These findings prompted further fractionation and isolation of functional compounds from Fu Zhuan Tea to test the hypothesis that microbial fermented tea derived compounds exhibit bioactivity on transcriptional regulators of lipid catabolism. Regulation of Farnesoid X receptor (FXR, activating and restraining), Liver X-activated Receptor (LXR) and Peroxisome Proliferator-Activated Receptors (PPARγ and PPARδ) were identified as candidate molecular targets that can be screened using reporter gene assays, and may advance identification of bioactive compounds in microbial fermented tea. The findings reported herein reveal bioactivity of Fu Zhuan Tea consumption for regulation of blood lipids in humans and led to identification of transcriptional targets for future investigations. Metabolite profiling studies and high throughput screening of Fu Zhuan Tea fractions compared to other teas is a nascent field and may reveal novel components as recently identified for antimicrobial functions (Ling et al., 2010).

2. Materials and methods

2.1. Volunteers

Ten individuals with baseline LDL between 100 and 200 mg/dl and hemoglobin A1c (HbA1c) ≥6% volunteered to participate in a prospective observational study that took place in the Advanced Integrative Medicine clinic supervised by Dr. Randy Snook. The main objective was to assess changes in blood lipid panel parameters and HbA1c after 120 days of daily Fu Zhuan Tea consumption. A control cohort-receiving medical oversight was randomly selected from the same clinic and followed for the same time period. Volunteer demographics and blood parameters were provided to researchers in a de-identified manner and in accordance with human subjects review board policies. Both tea consuming individuals and controls were not prescribed medications for management of blood cholesterol or Type II diabetes. The intervention involved daily consumption of 1 l of tea, prepared by adding 1 l of boiling water to a pre-weighed 5 g Fu Zhuan Tea brick coin stored at room temperature. Participant compliance was monitored by weekly phone calls and monthly check up visits. Fasting blood was collected at baseline and on the last day of tea consumption. Volunteers were provided a copy of their blood lipid panel results at baseline and at the end of the observational period. Body weight and BMI were recorded at baseline and 120 days post-consumption. Volunteers consuming the tea during or after the 120-day period reported no adverse effects.

2.2. Post fermented tea supply

Fu Zhuan Tea used in all studies was supplied by Hunan Yiyang Tea Factory (Hunan, Yiyang, China), and using their standard quality control procedures. Each 5 g coin was individually wrapped and a 120-day supply was provided to study volunteers. The aqueous preparations consumed by participants were the same as those extracted for chemical identification of compounds and for cell culture treatments.

2.3. Reagents and chemical extraction

Organic solvents including ethyl acetate, methanol, ethanol, 1-butanol and chloroform were purchased from Changsha Chemical Company (Changsha, China). Standards were purchased from Sigma Chemicals, USA.

Fu Zhuan Tea was extracted sequentially using chloroform, 1-butanol and ethyl acetate (Fig. 1). The ethyl acetate extract was dissolved in 60% ethanol and separated using a poly-amidoamine column (Nankai University Chemical Plant, Tianjin, China) with a flow rate of 2 BV per hour. Ethanol extract was vacuum-dried and further separated using Sephadex LH-20 column (Pharmacia, Stockholm, Sweden) at a flow rate of 0.2 BV to yield six fractions. Compounds in each of the fractions were detected using a Shimadzu SCL-10ATVP system equipped with a model LC-10ATVP pump, SPD-M20A diode array detector, a LC-solution data system and a Kromasil™ C_{18} column (5 μm, 4.6 mm × 200 mm, Shimadzu, Japan). The column was operating at ambient temperature (≅40 °C). The mobile phase consisted of water (Solvent A) and methanol (Solvent B), and an isocratic elution of mobile phase A:mobile phase B (60:40, v/v) at a flow rate of 1.0 mL min^{-1}.

2.4. Compound identification

The ultraviolet (UV) spectra of the separated individual peaks were measured using a UV–vis spectrophotometer (model UV-2550, Shimadzu, Japan). The infrared spectra were measured using Fourier-transform infrared spectrometer (WQF-310, Beijing, China) with the samples measured as potassium bromide disks. Purified compounds were submitted for characterization by thin layer chromatography on polyamide plates (TZSHSL, Taizhou, China) using a mobile phase of 95% ethanol. Chromatograms were evaluated under visible light to detect the presence of each compound.

LC/MS spectra were measured with Agilent 1100 LC/MSD SL (Agilent Inc., USA) equipped with an atmospheric pressure chemical ionization (APCI) interface. LC/MS was performed on a Zarbax C_8 column, 200 × 4.6 mm i.d. (Agilent Inc., USA) at flow-rate of 1.0 mL min^{-1}. The elution buffer was a mixture of methanol and water (70:30, v/v) containing 4 g L^{-1} ammonium formate. The effluent from the LC column was delivered to the ion source (150 °C) through heated nebulizer probe (400 °C) using nitrogen as drying gas (5 L min^{-1}, 350 °C) and nebulizer pressure was set to 60 psi. The mass spectrometer m/z ratio was 50 to 1000 in full scan mode.

Nuclear magnetic resonance (NMR) spectrometry was carried out using Varian Unity INOVA 300 NMR. Chemical shifts (δ) are reported in mg kg^{-1} relative to the residual solvent signals (δH 3.35 and δC 49.0 mg kg^{-1}) and coupling constants (J) in Hz.

2.5. Cell culture

Human liver cancer (SMMC-7721) cells were maintained in RPMI 1640 supplemented with 15% heat inactivated fetal bovine serum (FBS), gentamicin (40 mg mL^{-1}), penicillin (100 units mL^{-1}) and streptomycin (10 mg mL^{-1}). Cells were grown at 37 °C in a humidified atmosphere of 5% CO_2/95% air. All Fu Zhuan Tea isolated compounds were resuspended in DMSO (final concentration 0.1%) prior to addition to cell culture medium.

2.6. Cell viability

Cytotoxicity of purified compounds from Fu Zhuan Tea was assessed using the MTS reduction assay (CellTiter 96® Aqueous, Promega, WI, USA). Liver cell line SMMC-7721 (2×10^5/mL) was incubated in 96-well plates and treated with a series of concentrations of each compound for 24 h. Positive controls and blanks were run in parallel under the same conditions. Cell viability was measured according to the manufacturer's guidelines at an absorbance of 490 nm and cytotoxicity was calculated by $(A_s - A_b)/(A_c - A_b)$, where

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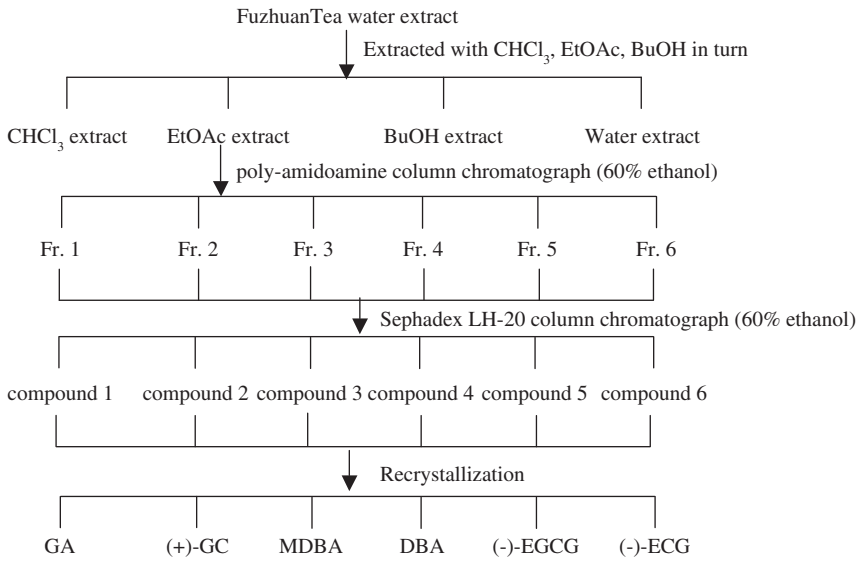

Fig. 1. Schematic of Fu Zhuan Tea extraction for isolation of bioactive compounds. The six compounds that were isolated from bioactive tea extracts were 1) GA = gallic acid, 2) (+)-GC = (+)-gallocatecin, 3) MDBA = 3-methoxy-4,5-dihydroxy-benzoic acid, 4) DBA = 3,4-dihyddroxy-benzoic acid, 5) (−)EGCG = (−)-epigallocatecingallate, and 6) (−)ECG = (−)-epicatechingallate.

A_s, A_b and A_c are the Absorbance at 490 nm in the presence of sample, blank, or control.

2.7. Reporter gene assay

Nuclear receptor activation by Fu Zhuan Tea compounds in human liver cells was measured using reporter gene constructs. DNA response elements for PPARγ, PPARδ, LXR and FXR were inserted upstream of the luciferase reporter gene, such that the regulation of nuclear receptor activation by tea extracts would correlate with luciferase gene expression. Liver cells (2×10^5) were plated in 96-well plates with a series of concentrations of each compound for up to 24 h. Positive controls and blank were run parallel under the same conditions. Irradiant intensity of oxidated luciferin was used as a measure of luciferase gene expression. In order to account for transfection efficiency, the cell inoculation number, isolated tea compounds, and a green fluorescent protein (GFP) plasmid were added together, and the irradiance value was normalized to GFP.

The activation multiple was calculated from the following equation:

$$= (LUC_s / GFP_s) / (LUC_b / GFP_b)$$

LUCs = the output of the luciferase regulated by sample, GFPs = the output of the green fluorescence protein regulated by sample, s = sample and b = blank. The agonists rosiglitazone, 2-bromohexadecanoic acid, chenodeoxycholic acid, and 22-(R)-hydroxycholesterol were used as positive controls of PPARγ, PPARδ, FXR and LXR activation respectively.

2.8. Statistical analysis

Descriptive statistics are presented as means. Differences between tea consuming individuals and non-intervention controls were examined using student's *t* tests. Statistical significance was ascribed to 2-tailed p values<0.05. Analysis was performed using SAS windows, version 9.1. All experiments were performed in triplicate. The significance of the inhibition rate against human liver cancer (SMMC-7721) cells was tested using one-way ANOVA under the SPSS software package (SPSS 13.0 for windows, SPSS Inc. 2004). A probability level of 5% ($p<0.05$) or 1% ($p<0.01$) was considered significant.

3. Results and discussion

3.1. Regulation of blood lipids by Fu Zhuan Tea intake

Given the safety profile for Fu Zhuan Tea consumption from prior toxicity and clinical studies (Xiao, 2007a; Xiao, 2007b), a daily dose of 5 g brick tea/l of water was used in a pilot observational study of tea consumption in adults with elevated LDL cholesterol compared to controls. There were 6 males and 4 females in the intervention group and consisted of seven white Caucasians, with three non-Caucasians (one Hispanic, one African American and one Indian) in the intervention group. The control group contained 7 males and 3 females of the entire white Caucasian race. The mean age of participants in the tea-consuming group was 50.7 ± 7.1 years old and 54.3 ± 5.9 years old in the controls. The study participants in the intervention group were an average of 3 years younger and had a significantly higher baseline LDL and HbA1C than non intervention controls. Table 1 shows the parameters and values that were examined at baseline and 120 days post tea consumption and those for controls. Significant differences were detected in HDL, LDL,

Table 1
Parameters examined at baseline and after 120 days of daily Fu Zhuan Tea intake compared to controls.

Study parameters	Study arms	Baseline	120 days	p value
HDL (mg/dl)	Control	56.8 ± 18.88	55.6 ± 17.66	<0.0009
	Intervention	43.0 ± 8.84	49.6 ± 9.11	
LDL (mg/dl)	Control	122.4 ± 20.82	123.8 ± 22.01	<0.0001
	Intervention	145.7 ± 20.45	113.1 ± 20.82	
HbA1C (%)	Control	5.47 ± 0.24	5.47 ± 0.25	<0.0034
	Intervention	7.25 ± 0.96	6.65 ± 0.84	
Weight (lbs)	Control	216.0 ± 38.76	216.8 ± 39.90	<0.0041
	Intervention	242.3 ± 47.72	233.6 ± 50.56	
Cholesterol (mg/dl)	Control	201.5 ± 37.70	207.4 ± 42.72	<0.0005
	Intervention	230.4 ± 30.15	202.5 ± 22.13	
Triglycerides (mg/dl)	Control	122.7 ± 33.91	123.9 ± 33.68	<0.028
	Intervention	167.2 ± 70.12	153.4 ± 65.76	
Body Mass Index (BMI)	Control	31.43 ± 2.81	32.35 ± 4.47	<0.0637
	Intervention	35.76 ± 6.34	34.44 ± 6.99	

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cholesterol, triglycerides and HbA1C between the control group and the intervention group ($p<0.05$). Improvement in the blood lipid parameters and the decrease in HbA1C suggest that multiple bioactive components and unique mechanisms exist for Fu Zhuan tea bioactivity. There was a small, but non-significant decrease in the body weight and BMI in 6 out of the 10 individuals consuming the tea. Clinic staff asked participants about changes in appetite at each monthly visit, and 6/10 tea consuming individuals verbally reported a decrease in appetite. While future investigations are required to validate this finding, we judge that changes in appetite may be an important factor to consider for some individuals.

These findings demonstrate pilot feasibility testing in humans and support current trends of Fu Zhuan Tea efficacy in hypercholesterimic Wistar rats and Chinese populations (Xiao, 2007a; Xiao, 2007b). Elevated blood cholesterols have also been associated with increased blood HbA1c (Allen et al., 2011; Chao et al., 2010), which is an advanced glycation end product and biomarker for altered glucose homeostasis and diabetes risk. Decreased HbA1c levels are generally associated with pharmacologic management of Type II diabetes (e.g. metformin) (Fonseca et al., 2010), and these data from an observational cohort first demonstrate significant reductions after 120 days in people consuming Fu Zhuan Tea over baseline and compared to control group. These data provide critical information regarding an achievable effect size following daily Fu Zhuan Tea consumption and will be useful for sample size and power calculations needed to assess tea efficacy in a larger study cohort. Future investigations should be directed towards examining the safety and efficacy of consuming the Fu Zhuan Tea/PHatea® daily in combination with other prescription drug medications.

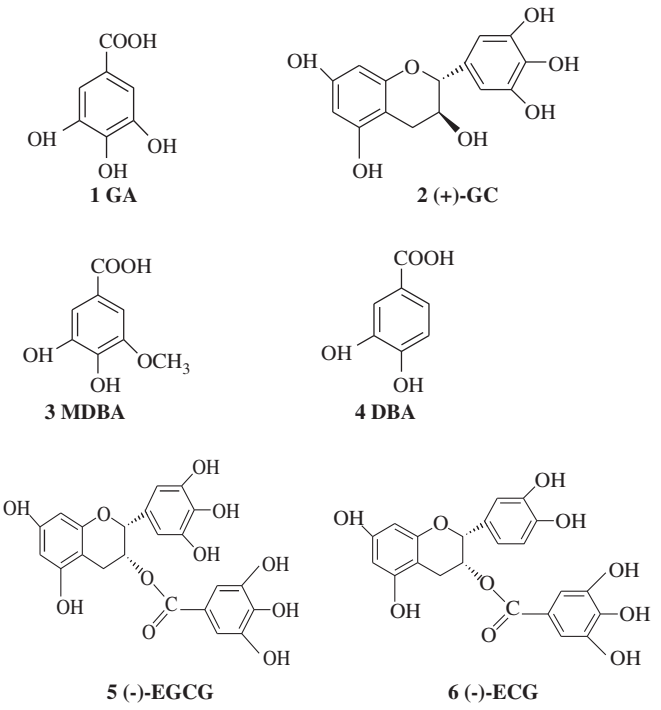

Fig. 2. Chemical structures of isolated compounds identified by Fu Zhuan Tea fractionation. 1) GA = gallic acid, 2) (+)-GC = (+)-gallocatecin, 3) MDBA = 3-methoxy-4,5-dihydroxy-benzoic acid, 4) DBA = 3,4-dihyddroxy-benzoic acid, 5) (−) EGCG = (−)-epigallocatecingallate, and 6) (−)ECG = (−)-epicatechingallate.

3.2. Fu Zhuan Tea fractionation and chemical analysis

Toxicity studies conducted in mice and rats support the safety index for consuming Fu Zhuan tea extracts (Xiao, 2007b), however characterization of tea constituents are still in their infancy (Zhu, 2006; Huang, 2007). Fig. 1 shows the series of tea fractionation steps prior to the isolation of purified compounds. Six compounds isolated from bioactive fractions of Fu Zhuan Tea were identified as 1) gallic acid (GA), 2) (+)-gallocatechingallate (GC), 3) 3-methoxy-4,5-dihydroxy-benzoic acid (MDBA) 4) 3,4-dihydroxy-benzoic acid (DBA), 5) (−)-epigallocatechingallate (EGCG), and 6) (−)-epicatechingallate (ECG) (Fig. 2). NMR chemical shifts were compared between isolated compounds and commercially available standards for confirmation of identity (data not shown). All six compounds have been previously isolated in varying quantities from preparations of *C. sinensis*. Tea catechins have been widely studied for their antioxidant capabilities (Intra & Kuo, 2007; Maatta-Riihinen, Kahkonen, Torronen, & Heinonen, 2005; Torres, Lozano, & Maher, 2005) and MDBA, also called 4-O-methylgallic acid, has been used as a biomarker for tea consumption (Hodgson et al., 2000) as well as having anti-inflammatory properties (Na et al., 2006). In animal models, extracts enriched in tea catechins have been shown to inhibit cholesterol absorption and lower plasma cholesterol (T Murase, A Nagasawa, J Suzuki, T Hase & I Tokimitsu, 2002; Yang, Wang, & Chen, 2001; Crespy & Williamson, 2004), but few studies have examined these chemicals individually. The most abundant tea catechin, EGCG, has been shown to increase LDL receptor binding activity in cultured human liver cells by increasing the conversion of sterol regulatory element binding protein-1 (SREBP-1) to its active form, resulting in lower cellular cholesterol concentrations (Bursill & Roach, 2006). These compounds have also been shown to suppress differentiation of 3 T3-L1 pre-adipocytes (Kim et al., 2010). Thus, further assessment of bioactivity related to lipid metabolism was applied herein related to transcription factor activation in liver cells.

3.3. Cytotoxicity of isolated compounds

Isolated tea components did not significantly reduce viability of liver cells at any of the doses examined (Fig. 3). These data suggest a high safety profile for Fu Zhuan Tea and are consistent with prior doses applied to Ames test and in toxicity studies following dietary intake(Xiao, 2007b). The doses that did not show adverse effects on liver cell toxicity were used for evaluation of transcriptional activation. A nonsignificant reduction in viability (less the 80%) was detected for GC, MDBA and DBA at the higher doses. These findings supported the use of 30 μg/mL to assess bioactivity, whereas up to 50 μg/mL was applied for GA, ECGC and EGC.

3.4. Dose dependent regulation of FXR and PPARδ transcriptional activation

To our knowledge there are no studies reporting the effects of these isolated tea compounds on transcriptional regulators of lipid metabolism in liver cells, and that studies to examine these mechanisms with tea extracts are limited. Testing of Fu Zhuan tea compounds GA, EGCG, ECG showed that GA demonstrated the lowest activation potential for FXR and PPARδ at all the concentrations tested when compared to ECG and EGCG (10, 30, 50 μg/ml—Table 2). The highest doses of ECG and EGCG (50 μg/mL) had an activation potential of 3.22 and 6.00 respectively for FXR (Table 2). While all three compounds were active on PPARδ, EGCG showed greatest magnitude of activation at 2.67 ± 0.21, and that of ECG was 2.04 ± 0.25. This report first demonstrated that specific tea constituents ECG and EGCG can regulate the activation of liver FXR and PPARδ. These findings support a recent report for Fu Zhuan tea extracts to activate FXR (KunBo, 2009), however a specific compound contained in the bioactive fractions was not previously described. Given that the FXR is a key regulator of bile acids, lipids and glucose (Chen et al., 2011; Trauner et al., 2010; Cariou et al., 2006) and that PPARδ has been considered a therapeutic target for diabetes and obesity through

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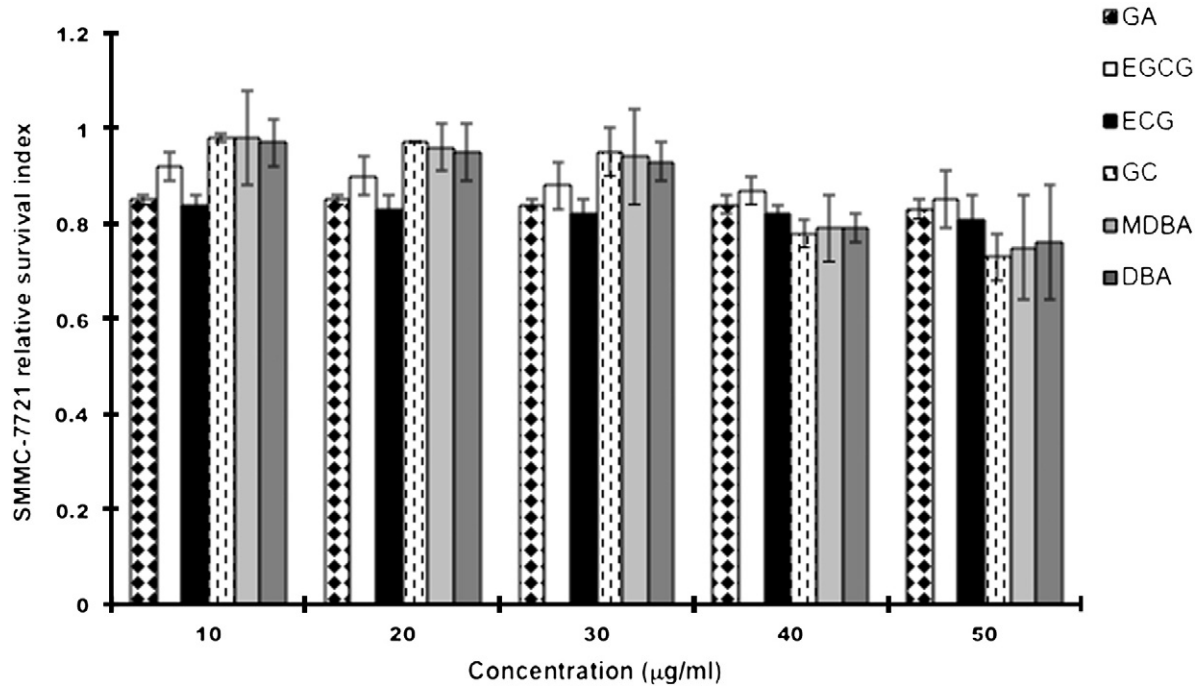

Fig. 3. Cytotoxicity testing of isolated tea compounds on SMMC-7721 cells. Dose-dependent effects of six tea compounds [GA = gallic acid, GC = gallocatecin, MDBA = 3-methoxy-4,5-dihydroxy-benzoic acid, DBA = 3,4-dihydroxy-benzoic acid, EGCG = (−)-epigallocatecingallate, and ECG = (−)-epicatechingallate] were examined on liver cells by MTS assay. No statistically significant cytotoxic effects of tea components were demonstrated at 10–30 μg/ml for all compounds tested. GC, MDBA, and DBA exhibited a nonsignificant 20% cell growth inhibition at the 40 and 50 μg/ml dose. Data is presented as mean ± SE.

enhancement of fatty acid oxidation (Cao et al., 2010; Ye et al., 2011; Yu et al., 2010), these findings suggest that future studies should be designed to examine the *in vivo* liver activation of FXR and PPARδ in mice consuming Fu Zhuan Tea.

3.5. Screening GC, MDBA and DBA bioactivity for liver transcription factor activation

A 30 μg/ml dose was determined from the liver cell viability assay for comparative bioactivity of the isolated tea components GC, MDBA and DBA (Fig. 2). Reporter gene assays for LXR were added to this high throughput screen of tea compounds based on the role of this nuclear receptor in cholesterol transport and metabolism, inflammation and glucose metabolism (Michael, Schkeryantz, & Burris, 2005). Natural compounds, including those from tea have also been screened for PPARγ agonism (Furuyashiki et al., 2004; Salam et al., 2008), and thus this model was also included herein to compare levels of bioactivity. The results in Table 3 showed that (+)-GC demonstrated the greatest magnitude of transcription factor regulation for PPARδ, PPARγ, FXR (activating) and LXR when compared to MDBA and DBA. The potential for these tea compounds to activate multiple nuclear receptor regulators of lipid catabolism in liver cells suggests promising novel mechanisms of action to explore for Fu Zhuan Tea investigations in vivo.

4. Conclusions

Some of the major tea producing countries in the world are China, Sri Lanka, Kenya, India, Indonesia, Japan, and Vietnam. Fu Zhuan Tea is one kind of post-fermented dark green tea with specific microbes of *E. cristatum* that contributes towards the special characteristics. Fu Zhuan Tea is produced in China and is a requisite drink for the herdsman of China. The herdsman cannot digest their traditional high fat food intake without drinking Fu Zhuan Tea.

Table 2
FXR and PPARδ activation by GA, EGCG and ECG.

Monomers	FXR activation[a]			PPARδ[a]		
	10.00 μg/mL	30.00 μg/mL	50.00 μg/mL	10.00 μg/mL	30.00 μg/mL	50.00 μg/mL
GA[b]	1.20 ± 0.13**	1.57 ± 0.12**	1.77 ± 0.14**	1.30 ± 0.02**	1.50 ± 0.11**	1.67 ± 0.12**
EGCG[c]	1.78 ± 0.32**	2.81 ± 0.35**	6.00 ± 0.45**	1.21 ± 0.02**	1.94 ± 0.11**	2.67 ± 0.21**
ECG[d]	1.21 ± 0.0**	2.10 ± 0.1**	3.22 ± 0.0**	1.02 ± 0.00**	1.54 ± 0.10**	2.04 ± 0.25**
2-Bro[e]	ND	ND	ND	2.99 ± 0.29	ND	ND
CDCA[f]	7.24 ± 1.71	ND	ND	ND	ND	ND

[a]Data shown as mean ± SE.
[b]Gallic acid.
[c](−)-epigallocatecingallate.
[d](−)-epicatechingallate.
[e]2-bromohexadecanoic acid (positive control for PPARδ activation).
[f]Chenodeoxycholic acid (positive control for FXR activation).
ND: not detected.
** $p<0.01$ when compared with that of the positive control.

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Table 3
GC, MDBA and DBA effects on PPARs, FXR, and LXR transcriptional activation.

Models[a]	FXR restraining	FXR activating	PPARδ	PPARγ	LXR
GC[b]	7.28 ± 0.22**	1.39 ± 1.01**	1.25 ± 0.39**	1.62 ± 0.16**	1.34 ± 0.12**
MDBA[c]	6.19 ± 0.14*	1.16 ± 1.32*	1.02 ± 0.02*	1.73 ± 0.16*	1.40 ± 0.07*
DBA[d]	3.64 ± 0.05**	0.83 ± 0.18**	0.77 ± 0.02**	0.89 ± 0.09**	0.98 ± 0.09**
22-(R)-HC[e]	ND	ND	ND	ND	6.96 ± 0.09**
Ros[f]	ND	ND	ND	4.36 ± 0.11	ND
2-Bro[g]	–	–	2.25 ± 0.25	–	–
CDCA[h]	6.44 ± 0.35	7.36 ± 0.82	–	–	–

[a]30.00 μg/mL.
[b](+)-gallocatecin.
[c]3-methoxy-4,5-dihydroxy-benzoic acid.
[d]3,4-dihyddroxy-benzoic acid.
[e]22-(R)-hydroxycholesterol, positive control of LXR activation.
[f]Rosiglitazone, positive control of PPARγ activation.
[g]2-Bromohexadecanoic acid, positive control of PPARδ activation.
[h]Chenodeoxycholic acid, positive control of FXR activation.
* $p<0.05$ when compared with that of the positive control.
** $p<0.01$ when compared with that of the positive control.

Furthermore, the herdsmen who eat high fat foods and drink Fu Zhuan Tea do not suffer from hyperlipidemia. The compelling observational findings in US volunteers of improved blood lipid parameters reported herein are consistent with these traditional uses. The decreased HbA1c levels following Fu Zhuan Tea consumption for 120 days is a novel finding and provides strong rationale for continued chemical characterization and assessment of bioactive components that may influence production of advanced glycation end products. The effects of Fu Zhuan Tea on insulin sensitivity and glucose regulation are also highly relevant to novel therapeutic applications for type II diabetes.

Fu Zhuan Tea is different from other post-fermented tea as a result of the *E. cristatum* exposure during flowering and influence on processing. While the differences in both chemical content and bioactivity may be due to Fu Zhuan Tea processing (i.e., fermentation) and not to botanical varieties, further biomedical research is warranted to substantiate these observed medicinal properties. Based on reports from the bioactivity of Fu Zhuan Tea extracts, six compounds were isolated and showed differential levels of transcription factor regulation in liver cells. These data suggest a role for Fu Zhuan Tea compounds to regulate lipid catabolism. Tea and drug comparison study designs will be required to identify unique health promoting and chronic disease fighting properties across the different types of Chinese brick, post fermented teas. Future Fu Zhuan Tea/ PHatea® investigations may assess bioavailability of selected tea compounds, establish bioactivity of liver transcriptional activation in vivo, and identify pharmacodynamics mechanisms of synergy with current drug treatments, such as statins and metformin. PHatea® has only recently become available for consumption in the US and clinical research studies for the chronic disease fighting properties in humans are still under development. Epidemiological studies in various populations will also be necessary to inform the utility of PHatea® for control and management of metabolic syndromes, hyperlipidemia, and alterations in glucose homeostasis/metabolism that may be seen in obesity, pre-diabetes and non-insulin dependent type II diabetics.

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