Comparative Study of the Anti-Obesity Effects of Green, Black and Oolong Tea Polysaccharides in 3T3-L1 Preadipocytes

LI Juan[1], LIU Rui[1,2], WU Tao[1], GUO Yu[1], ZHANG Min[1,2,*]

(1. Key Laboratory of Food Nutrition and Safety, Ministry of Education, Tianjin University of Science and Technology, Tianjin 300457, China;
2. Tianjin Food Safety and Low Carbon Manufacturing Collaborative Innovation Center, Tianjin 300457, China)

Abstract: This study aimed to evaluate the anti-obesity effects of green tea polysaccharides (GTP), black tea polysaccharides (BTP) and oolong tea polysaccharides (OTP) in 3T3-L1 preadipocytes. The monosaccharide compositions of GTP, BTP and OTP were analyzed using gas chromatography, and the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide (MTT) assay was used to determine the effects of three tea polysaccharides (TPs) on the proliferation of 3T3-L1 preadipocytes. The cell cycle was determined by flow cytometry. The preadipocytes were differentiated into adipocytes using the traditional "cocktail" method and the differentiation rate was measured based on absorbance. The triglyceride (TG) content was measured to analyze lipid accumulation in adipocytes using the phosphoric acid oxidase peroxidase (GPO-PAP) method. The expression of transcription factors related to adipocyte differentiation was analyzed by real-time polymerase chain reaction (RT-PCR). The inhibition of the proliferation and differentiation of 3T3-L1 preadipocytes by three TPs were significantly different ($P < 0.05$). With the addition of 100 μg/mL TPs, the differentiation rates were (62.00 ± 6.61)% (GTP), (82.95 ± 4.25)% (BTP) and (97.24 ± 5.80)% (OTP). In addition, GTP, BTP and OTP could regulate the cell cycle, and the proportions of G0/G1 phase cells after 5 days were (68.52 ± 2.28)%, (67.11 ± 1.68)% and (59.69 ± 1.35)%, respectively. Further investigation of the total RNA extracted from the differentiated preadipocytes revealed that the addition of TPs (100 μg/mL) could regulate the expression of related cytokines. In this study, the inhibitory effect of GTP was more significant than those of BTP and OTP. Up-regulation of of adiponectin (ADP) expression activated the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway, which regulates the expression of related adipocyte factors, and ultimately leading to inhibition of TG synthesis and 3T3-L1 preadipocyte differentiation.

Key words: tea polysaccharides (TPs); anti-obesity activity; 3T3-L1 preadipocytes; differentiation; gene expression

不同茶多糖对3T3-L1前脂肪细胞分化的抑制作用比较

李 娟[1]，刘 锐[1,2]，吴 涛[1]，郭 郁[1]，张 民[1,2,*]

（1.天津科技大学 食品营养与安全教育部重点实验室，天津 300457；
2.天津食品安全低碳制造协同创新中心，天津 300457）

摘 要：本实验利用小鼠3T3-L1前脂肪细胞对绿茶多糖（green tea polysaccharides，GTP）、红茶多糖（black tea polysaccharides，BTP）和乌龙茶多糖（oolong tea polysaccharides，OTP）的减肥作用进行评价。使用气相色谱对GTP、BTP、OTP的单糖组成进行分析。采用噻唑蓝染色法测定3 种茶多糖（tea polysaccharides，TPs）对3T3-L1前脂肪细胞增殖活力的影响。使用流式细胞仪检测3 种TPs对3T3-L1前脂肪细胞细胞周期的影响。采用传统"鸡尾酒"法诱导3T3-L1细胞分化成脂后，测吸光度并计算其分化率。采用甘油磷酸氧化酶-过氧化物酶（glycerol phosphate oxidase-peroxidase，GPO-PAP）法测定细胞中甘油三酯（triglyceride，TG）含量的变化。采用实时荧光定量聚合酶链式反应（real-time polymerase chain reaction，RT-PCR）技术测定与脂质代谢相关基因的表达量。结果显示3 种TPs均能显著抑制3T3-L1前脂肪细胞的增殖与分化（$P<0.05$）。添加100 μg/mL的TPs能使细胞分

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作者简介：李娟（1990—），女，硕士研究生，研究方向为植物多糖活性及分子营养学。E-mail：1097130466@qq.com

*通信作者：张民（1972—），男，教授，博士，研究方向为食品科学。E-mail：zm0102@tust.edu.cn

化率显著下降至（62.00±6.61）%（GTP）、（82.95±4.25）%（BTP）、（97.24±5.80）%（OTP）。另外，在细胞培养至第5天检测表明，这3 种TPs显著促进G0/G1期细胞数量的累积，细胞比例分别为（68.52±2.28）%（GTP）、（67.11±1.68）%（BTP）、（59.69±1.35）%（OTP）。RT-PCR分析结果显示TPs可以调控相关脂肪细胞因子的表达。在本研究中，在3T3-L1前脂肪细胞中GTP的减肥作用强于BTP和OTP。TPs的加入上调脂联素的表达从而激活腺苷酸活化蛋白激酶信号通路调控相关脂肪细胞因子的表达，并最终抑制TG的合成与3T3-L1前脂肪细胞的分化。

关键词：茶多糖；减肥作用；3T3-L1前脂肪细胞；分化；基因表达

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Obesity is a risk factor for arteriosclerosis, diabetes, and hyperlipidemia and is a major public health problem in many countries[1-3]. Adipose tissue plays an important role in lipid metabolism, including triglyceride (TG) storage and fatty acid release[4-6]. Adipogenesis involves un-differentiated preadipocytes converting to differentiated adipocytes and plays a key role in fat mass growth. Controlling adipogenesis is a potential strategy for obesity prevention[7], and 3T3-L1 preadipocytes have been widely used as an *in vitro* model for analyzing adipocyte differentiation[8]. The occurrence of obesity was found to be related to the disorder of adipocytes factors and the related regulatory transcription factors[9-11].

Recently, studies have provided evidence that tea polysaccharides (TPs) are one of the main bioactive components of tea with anti-obesity activity[12]. However, the effect of the three types of TPs has rarely been compared. In this study, we explored the anti-obesity effects of green tea polysaccharides (GTP), black tea polysaccharides (BTP) and oolong tea polysaccharides (OTP) on precursor and mature adipocytes; the cell cycle; the TG content and expression of adipogenic transcription factors, such as peroxisome proliferator-activated receptor gamma (PPARγ) and sterol regulatory element-binding protein-1c (SREBP-1c)[13]. However, as important regulatory factors, the relationship between PPARγ and SREBP-1c and acetyl-CoA carboxylase (ACC), lipoprotein lipase (LPL), stearoyl-CoA desaturase enzyme 1 (SCD1) and adiponectin (ADP) remains obscure.

In this study, the anti-obesity effects of these factors on lipid metabolism and the relationship between these factors were evaluated. In addition, the results of this study can provide a reference for further exploration, development and utilization of highly efficient functional foods with health benefits for obese people.

1 Materials and Methods

1.1 Materials and reagents

3T3-L1 preadipocytes were obtained from the Chinese Basic Medical Center (resource number: 3111C0001CCC000155). GTP, BTP, OTP were prepared by the Laboratory of Food Additives and Functional Ingredients at Tianjin University of Science and Technology[14].

Dulbecco's modified Eagles medium (DMEM), 0.25 g/100 mL (in phosphate buffered saline (PBS)) trypsin, oil red O staining kits, 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-*H*-tetrazolium bromide (MTT), calf serum (CS), fetal bovine serum (FBS) Gibco Co. (USA); isobutyl methyl xanthine (IBMX), dexamethasone, bovine insulin Sigma-Aldrich Co. (USA); TG enzyme assay kit Pulilai (Beijing, China); RNA extraction kit, One-Step SYBR®PrimeScript™ Plus real-time polymerase chain reaction (RT-PCR) kit Takara Biotechnology (Da Lian) Co. Ltd. (Japan). All other chemicals were of analytical grade.

1.2 Instruments and equipments

Gas chromatograph Agilent Co. (USA); calibur flow cytometer Becton Dickinson (USA); inverted phase contrast microscope Olympus Co. (Japan); microplate reader Thermo Co. (USA); real-time polymerase chain reaction (RT-PCR) instrument Bio-Rad (USA); CO_2 incubator Shellab Co. (USA).

1.3 Methods

1.3.1 Determination of the main components of TPs

The neutral sugar, acidic polysaccharide, protein and polyphenol contents in the three types of GTP, BTP and OTP were determined by the phenol-sulfuric acid method[15], sulfuric acid-carbazole method[16], Coomassie brilliant blue method[17] and Folin-phenol method[18], respectively.

1.3.2 Determination of the sugar composition of TPs by gas chromatography

Samples of 10 mg of GTP, BTP and OTP were hydrolyzed using trifluoroacetic acid. Eight monosaccharide standards (rhamnose, arabinose, ribose, xylose, mannose, *D*-galacturonic acid, galactose and glucose), and an internal standard (inositol) and the hydrolysates of the TP samples were derivatized before analysis by gas chromatography. The percentages of the sugars were calculated according to the peak area. The formula for calculation is as formula (1):

$$P_i/\%=\frac{S_i f_i M_s}{S_s f_s M_m}\times 100 \quad (1)$$

Where, P_i is the percentage content of component *i*/%; S_i is the peak area of the measured group; S_s is the peak area of the internal standard; f_i is the weight correction factor of the measured component; f_s is a weight correction factor for an internal standard; M_m is the mass of the sample being tested/g; M_s is the internal standard mass/g.

1.3.3 3T3-L1 preadipocytes culture

The preadipocytes were cultured in DMEM containing a high glucose concentration (4 500 mg/L) supplemented with 10% CS (*V*/*V*), penicillin (100 U/mL) and streptomycin (100 U/mL) in an atmosphere of 5% CO_2 at 37 ℃.

1.3.4 Preadipocytes viability assay

The MTT assay was used to assess cell toxicity and viability[19]. 3T3-L1 preadipocytes were treated with GTP, BTP and OTP at 50, 100, 200 and 400 μg/mL, and treatment with 0 μg/mL served as the control group (NC). After 1, 3 and 5 days, a 5 mg/mL MTT solution (30 μL) was added, and the cells were incubated at 37 ℃ for 4 h. Thereafter, the supernatant was completely removed, and dimethyl sulphoxide was used to dissolve the formazan crystals. The optical density (OD) value was read at 570 nm using a microplate reader. The cell viability rate was calculated according to the formula (2).

$$\text{Cell viability rate}/\%=\frac{\text{OD}_{\text{experimental group}}}{\text{OD}_{\text{control group}}}\times 100 \quad (2)$$

1.3.5 Cell cycle measurement

3T3-L1 preadipocytes were treated with 100 μg/mL GTP, BTP, OTP and NC (0 μg/mL). After 3 and 5 days, the tested cells were harvested by trypsin digestion and adjusted to a density of 4×10^5 cells/mL. The cells were washed with cold PBS and re-suspended in 1 mL of ethanol. The treated cells were fixed with 70% ethanol and incubated overnight at 4 ℃. The fixed cells were centrifuged and re-suspended in 1 mL of a propidium iodide solution (containing 500 U/mL RNase) for 30 min at room temperature. The sample was stored in the dark at 4 ℃, and the cell cycle was analyzed using a calibur flow cytometer .

1.3.6 3T3-L1 preadipocytes differentiation

3T3-L1 preadipocytes were differentiated into adipocytes using the traditional "cocktail" method[20-21]. After cells had reached confluence, they were incubated for 2 days, and the culture medium was then changed to a differentiation-induction medium containing 10 μg/mL insulin, 1 μmol/L dexamethasone, and 5 mmol/L IBMX in DMEM with 10% FBS (*V*/*V*), which was then treated with GTP, BTP and OTP (50, 100, 200 and 400 μg/mL) every 2 days. After 2 days (Day 4), the differentiation-induction medium without dexamethasone and IBMX was replaced. After two more days (Day 6), the cells were cultured in growth medium without insulin. After 8 days of culturing, 90% of the preadipocytes had differentiated into adipocytes. The cells in these conditions were harvested for further studies.

1.3.7 Oil red O staining

Lipid accumulation in the induced 3T3-L1 preadipocytes was determined using the oil red O staining method[22]. The cells were washed twice with PBS and then fixed with 10% formalin in PBS (pH 7.4) (*V*/*V*) for 1 h. The cells were stained with oil red O solution in isopropanol for 2 h and then washed 3 times with PBS. The fat droplets in the adipocytes were stained red by oil red O dye, the oil red O dye was eluted with isopropanol, and the OD was measuring at 520 nm. The differentiation rate was calculated according to the formula (3).

$$\text{Differentiation rate}/\%=\frac{\text{OD}_{\text{control group}}-\text{OD}_{\text{experimental group}}}{\text{OD}_{\text{control group}}}\times 100 \quad (3)$$

1.3.8 Determination of TG in adipocytes

Different concentration of GTP, BTP and OTP (50, 100, 200 and 400 μg/mL) were added on day 0 of 3T3-L1 preadipocytes differentiation in the culture medium, and the culture solution was discarded on day 8, followed by washing twice with PBS. The TG levels in the adipocytes were quantified using a TG enzyme assay kit.

1.3.9 RNA extraction and RT-PCR

Gene primers were designed using Premier Primer 5 software. The following primers were used: forward

5′-CTGTCCCTGTATGCCTCTG-3′ and reverse 5′-TGATGTCACGCACGATTT-3′ for *β-actin* (reference gene); forward 5′-GACCACTCGCATTCCTTT-3′ and reverse 5′-CCACAGACTCGGCACTCA-3′ for *PPARγ*; forward 5′-CTTCTGGAGACATCGCAAAC-3′ and reverse 5′-GGTAGACAACAGCCGCATC-3′ for *SREBP-1c*; forward 5′-ACTGCCACTTCAACCACA-3′ and reverse 5′-ATCAGCGTCATCAGGAGA-3′ for *LPL*; forward 5′-CTCCTGCTTTGGTCCCTC-3′ and reverse 5′-GCCAGTGCTGCCGTCATA-3′ for *ADP*; forward 5′-TGACCTGAAAGCCGAGAA-3′ and reverse 5′-CGTTGAGCACCAGAGTGTAT-3′ for *ACC*; and forward 5′-TGACCTGAAAGCCGAGAA-3′ and reverse 5′-CGTTGAGCACCAGAGTGTAT-3′ for *SCD1*. An RNA extraction kit was used to extract the total RNA from the NC and experimental groups (100 μg/mL TPs). The RNA was used in RT-PCR experiments using a One-Step SYBR®PrimeScript™ Plus RT-PCR kit. The amplification conditions were 42 ℃ for 5 min and 95 ℃ for 10 s, followed by 40 cycles of 95 ℃ for 5 s, 55 ℃ for 30 s, and 72 ℃ for 30 s. The results were normalized using *β-actin* as a reference gene. The amount of expression was calculated using the $2^{-\Delta\Delta Ct}$ method.

1.4 Statistical analysis

Data are expressed as the $\bar{x} \pm s$. Group results were compared by analysis of variance (ANOVA), followed by Duncan's test using SPSS software. $P < 0.05$ was considered as significant difference; $P < 0.01$ was considered as extremely significant difference.

2 Results and Analyses

2.1 Main components of TPs

TPs are biologically active compounds in tea and are a type of acidic polysaccharide or acidic glycoprotein. As shown in Table 1, GTP, BTP and OTP contained a small amount of protein. The contents of neutral sugars were not significantly different ($P > 0.05$), but the uronic acid content of GTP was significantly higher than that of BTP and OTP ($P < 0.05$).

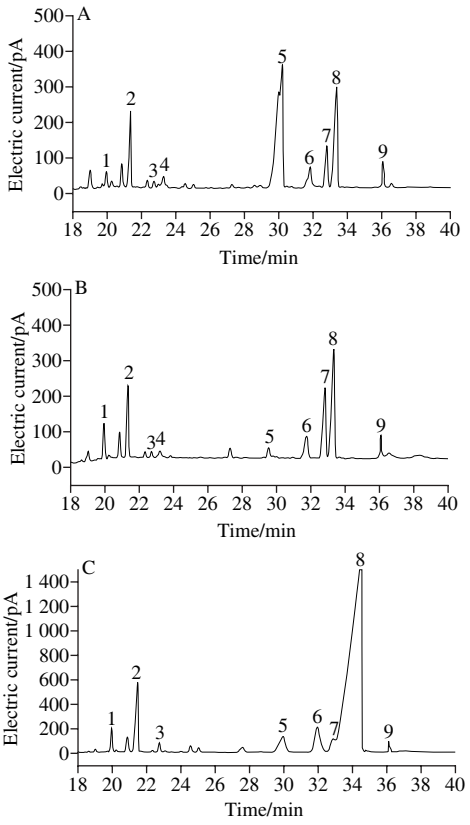
Table 1 Major compositions of GTP, BTP and OTP

%

TPs	Neutral sugar content	Uronic acid content	Protein content	Polyphenol content
GTP	34.76 ± 0.04[a]	30.93 ± 0.23[a]	19.25 ± 0.52[a]	5.64 ± 0.06[a]
BTP	38.9 ± 0.12[a]	11.92 ± 0.08[b]	17.00 ± 0.16[a]	6.20 ± 0.15[a]
OTP	40.66 ± 0.04[a]	12.89 ± 1.54[b]	16.70 ± 0.02[a]	5.82 ± 0.13[a]

Note: Different letter indicate significant difference in the same line ($P < 0.05$).

2.2 Monosaccharide compositions of TPs



A. GTP; B. BTP; C. OTP. 1–9. rhamnose, arabinose, xylose, ribose, *D*-galacturonic acid, mannose, galactose, glucose and inositol.

Fig. 1 Gas chromatograms of GTP (A), BTP (B) and OTP (C)

The monosaccharide compositions of GTP, BTP and OTP were determined by gas chromatography (Fig. 1). BTP and OTP were mainly composed of arabinose, mannose and glucose. The molar percentage of glucose was the highest in BTP and OTP at 32.65% and 52.80%, respectively. The main components of GTP were arabinose, glucose and *D*-galacturonic acid, and the molar percentage of *D*-galacturonic acid was the highest in GTP at 31.25% (Table 2).

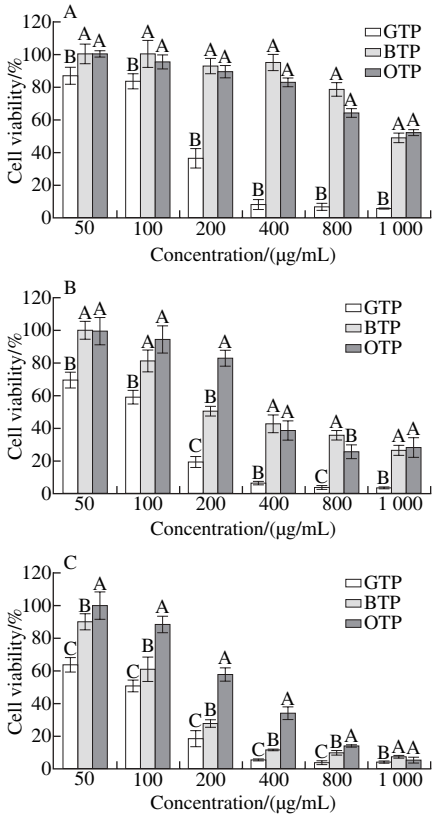
Table 2 Monosaccharide compositions of three TPs

Monosaccharide composition	GTP	BTP	OTP
Rhamnose	5.04	10.08	4.46
Arabinose	13.32	17.77	24.35
Xylose	2.74	2.03	1.65
Ribose	4.95	4.14	
D-galacturonic acid	31.25	6.57	5.10
Mannose	9.28	11.05	6.25
Galactose	9.94	15.71	5.39
Glucose	23.48	32.65	52.80

2.3 Effect of TPs on 3T3-L1 preadipocytes proliferation

The MTT assay results demonstrated that the inhibitory effect of the TPs on 3T3-L1 preadipocytes increased with

increasing treatment time and concentration. As shown in Fig. 2, after treatment of the 3T3-L1 preadipocytes with GTP, BTP and OTP, the inhibition effect of GTP was extremely significantly higher than those of BTP and OTP ($P < 0.01$). There was no significant difference between the effects of BTP with OTP on the first day ($P > 0.05$). However, as time prolonging, the samples treated with 200 μg/mL showed extremely significant difference among the three types of TPs on the third day ($P < 0.01$). Considering that the cell viability was greater than 50%, when the TPs concentration was 100 μg/mL on the fifth day, the cell viability of the GTP group was (50.71 ± 3.69)%, whereas those of the other two groups were (60.39 ± 7.50)% (BTP) and (88.5 ± 4.81)% (OTP).



A–C. 1, 3 and 5 days. Different capital letters among groups indicate extremly significant differences between TPs ($P < 0.01$).

Fig. 2 Effects of TPs on the cell viability of 3T3-L1 preadipocytes

2.4 Cell cycle detection

G0/G1, S, G2 and M are different phases of the cell cycle. Differentiation of 3T3-L1 preadipocytes into mature adipocytes is initiated by mitotic clonal expansion (MCE), a prerequisite for differentiation, in which G0/G1-growth-arrested preadipocytes are induced to differentiate[23]. Researchers believe that when the cell growth is delayed in the G0/G1 phase and prevented from entering the next phase for a long period of time, the cells will never enter the next phase and finish DNA replication. With long-term retention, the G1 phase will activate certain intracellular mechanisms and initiate the cell program death signal, ultimately inducing cell apoptosis[24-25]. To investigate whether the effect of TPs on the 3T3-L1 preadipocytes proliferation and pre-differentiation stages was associated with the cell cycle, 3T3-L1 preadipocytes were treated with the TPs for 3 and 5 days in the early stage of adipogenesis, and the cell quantities were analyzed. As shown in Table 3, the cell cycle was arrested in the G0/G1 phase in a time-dependent manner after TPs treatment. Compared with the NC, the TPs delayed the cell cycle from the S phase to the G2/M phase at 5 days, with increasing cell quantity of G0/G1 phase cells ($P < 0.05$). Notably, compared with the other groups, the proportion of G0/G1 phase cells induced by GTP was the highest on the fifth day.

Table 3 Effects of TPs on the cell cycle of 3T3-L1 preadipocytes at 3 and 5 days

Time/d	Group	Cell proportion/%			Apoptosis rate/%
		G0/G1	S	G2/M	
3	NC	55.56 ± 1.42	25.18 ± 0.64	18.60 ± 0.76	0.29 ± 0.08
	GTP	67.57 ± 1.49*	11.38 ± 1.94*	21.30 ± 1.37*	6.53 ± 0.61*
	BTP	54.71 ± 1.12	31.94 ± 1.29*	13.52 ± 1.00*	4.37 ± 0.56*
	OTP	67.67 ± 3.27*	5.85 ± 1.78*	26.32 ± 1.27*	6.37 ± 0.40*
5	NC	55.92 ± 0.63	19.88 ± 0.29	23.5 ± 0.74	0.73 ± 0.08
	GTP	68.52 ± 2.28*	17.34 ± 1.67	13.73 ± 1.00*	14.31 ± 4.95*
	BTP	67.11 ± 1.68*	17.36 ± 2.07*	17.57 ± 1.22	9.39 ± 2.22*
	OTP	59.69 ± 1.35*	21.32 ± 2.33	20.50 ± 2.63	5.05 ± 0.76*

Note: *. indicate significant difference compared with NC ($P < 0.05$).

2.5 TPs inhibit the differentiation of 3T3-L1 preadipocytes

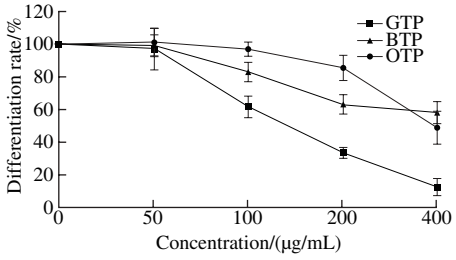
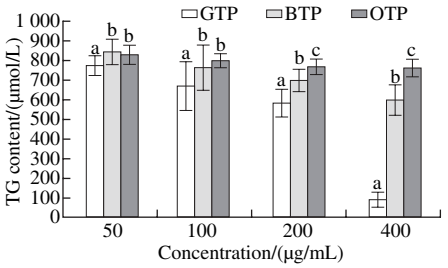


Fig. 3 Effects of TPs on the differentiation of 3T3-L1 preadipocytes

During the differentiation of 3T3-L1 preadipocytes, we added different concentrations of TPs (50, 100, 200 and 400 μg/mL). In addition, the differentiation rate of 3T3-L1 preadipocytes significantly decreased with increasing TPs concentration ($P < 0.05$), indicating a potential anti-obesity effect in 3T3-L1 preadipocytes. Fig. 3 showed significant differences in the effects of the three types of TPs on cell differentiation. The inhibition of preadipocytes differentiation by GTP was the strongest at the same concentration of TPs. At

100 μg/mL GTP, lipid accumulation was inhibited to approximately (62.00 ± 6.61)% of that in the NC cells. However, the cell differentiation rates of the BTP and OTP groups were (82.90 ± 4.25)% and (97.24 ± 5.80)%, respectively, at the same concentration.

2.6 Effect of TPs on the TG content



Different letters among groups indicate significant differences between TPs ($P < 0.05$).

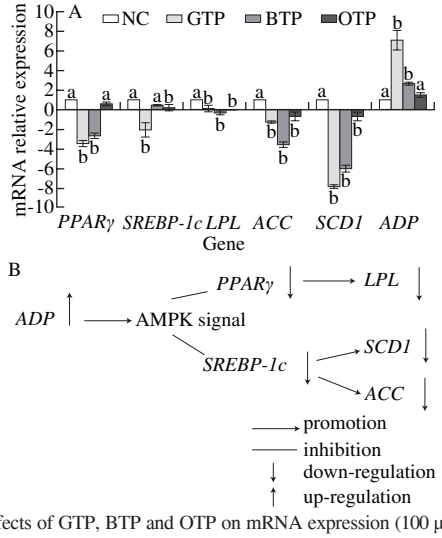
Fig. 4 Effects of TPs on the TG content

At the stage of adipocyte differentiation, a high level of TG accumulated in the adipocytes. The addition of TPs significantly decreased the amount of oil droplets and TG levels in the cells, indicating that the TPs are potent inhibitors of adipogenesis. As shown in Fig. 4, with the increase in the TPs concentration, the inhibitory effect of GTP on the TG content was significantly stronger than those of BTP and OTP ($P < 0.05$). In addition, at the same concentration, GTP also showed a stronger inhibitory effect, which was especially evident at 400 μg/mL TPs, where the TG content was (91.28 ± 14.47) μmol/L in the GTP group, but (596.50 ± 81.95) and (762.07 ± 43.84) μmol/L in BTP and OTP group. This further proved that GTP had effectively inhibited the differentiation of 3T3-L1 preadipocytes.

2.7 Effect of TPs on the expression of adipogenic genes

The RT-PCR results showed that the mRNA expression of *LPL*, *SREBP-1c*, *PPARγ* and *ACC* in the TPs groups were down-regulated compared to that in NC. In addition, the mRNA expression of *ADP* was up-regulated by the TPs ($P < 0.05$) (Fig. 5A).

Adipose tissue plays an important role in whole-body energy production[26], connects the endocrine and metabolic pathways and regulates the balance of energy[27]. An imbalance between energy intake and energy consumption will affect the development of adipose tissue. The three types of TPs inhibited the proliferation and differentiation of 3T3-L1 preadipocytes. Adipocyte differentiation is accompanied by a variety of transcription factors and the expression of specific genes for adipocyte factors[28]. *SREBP-1c* and *PPARγ* mainly regulate the expression of lipid biosynthesis-related genes and promote the synthesis of fatty acids[29]. *PPARγ* is the most adipose tissue-specific transcription factor in the *PPAR* family and plays an important role in the regulation of preadipocytes differentiation; its expression is closely related to obesity, type Ⅱ diabetes, high lipid hyperinsulinemia and insulin resistance[30-31]. The *SREBP-1c* gene encodes the key nuclear transcription factor *SREBP-1c*, which has been shown to regulate *de novo* lipogenesis and TG-rich lipoprotein metabolism experimentally. *SREBP-1c* overexpression induces insulin resistance, diabetes, nonalcohol fatty liver disease, and accelerates atherosclerosis in mice[32]. At the same time, some studies have indicated that the target gene is activated by up-regulation of the expression of *PPARγ*, resulting in the accumulation of fat[33]. These two genes (*SREBP-1c* and *PPARγ*) can activate adipocyte factor LPL[34]. ACC is a key enzyme in TG synthesis, preadipocyte differentiation and adipogenesis and plays a very important role in the regulation of fatty acid synthesis[35]. Studies have shown that the promotion of *SREBP-1c* expression could up-regulate *ACC* expression and then promote the synthesis of fatty acids and TGs[36]. *SCD1* is highly expressed in liver and adipose tissue. In studies on *SCD1* as a target for obesity intervention, *SCD1* inhibition during 3T3-L1 adipocyte differentiation decreased *de novo* fatty acid synthesis[37]. The *SCD1* global knock-out mouse has a lean phenotype, is resistant to diet-induced obesity, and exhibits increased *β* oxidation[33]. Some researchers believe that the expression of *SCD1* is also subject to the regulation of *SREBP-1c*[38].



A. Effects of GTP, BTP and OTP on mRNA expression (100 μg/mL) ($n = 4$, different letter indicate significant difference compared with NC ($P < 0.05$)); B. TP-mediated related lipid metabolism.

Fig. 5 Effects of TPs on the mRNA expression of adipogenic genes

As shown in Fig. 5, TG synthesis in 3T3-L1 adipocytes was inhibited by down-regulating the expression of *SCD1* after the addition of the TPs. ACC and SCD1 are key enzymes in the biosynthesis of fatty acids and unsaturated fatty acids, and down-regulation of their expression directly inhibited lipid synthesis. Therefore, TPs can down-regulate *PPARγ* and *SREPB-1c* such that the downstream genes (*ACC* and *SCD1*) synthesis are inhibited, thereby inhibiting TG synthesis and exerting a favorable effect on lipid metabolism disorders. *ADP* is involved in the metabolism of glucose and lipids, increasing the oxidation of fatty acids, enhancing the insulin resistance of the liver, and promoting insulin sensitivity[39]. In addition, some studies have suggested that *ADP* can activate the adenosine monophosphate-activated protein kinase (AMPK) signaling pathway by activating two receptors, ADP1 (adipor1) and ADP 2 (adipor2)[40]. Impaired *ADP* can lead to insulin resistance and obesity[41]. Fig. 5A showed that adding the TPs resulted in up-regulation of *ADP* expression compared to NC and *ADP* being upstream of the AMPK protein. This up-regulation inhibited the expression of AMPK such that a series of lipid metabolism-related genes were inhibited[42]. Thus, the TPs mediated the AMPK signaling pathway by up-regulating the expression of *ADP*, which can inhibit the expression of *PPARγ* and *SREBP-1c*[43], subsequently leading to the down-regulation of *LPL* and *ACC* expression and ultimately inhibiting the differentiation of 3T3-L1 preadipocytes and TG accumulation (Fig. 5B).

In summary, the inhibition effects on the proliferation and differentiation of 3T3-L1 preadipocytes by the three types of TPs were significantly different. The GTP group was significantly different in its regulation of the expression of all adipocyte factors ($P < 0.05$), indicating that the inhibitory effect of GTP was more significant than those of BTP and OTP. As shown in Table 1, the content of uronic acids in GTP was obviously higher than that in BTP and OTP, which was further confirmed by the monosaccharide composition (Fig. 1), revealing that GTP contained more *D*-galacturonic acids. Uronic acid, as an acidic polysaccharide, exhibited greater weight loss and lipid-lowering activity in a previous study[44], indicating that uronic acid plays a significant role in inhibiting the proliferation and differentiation of preadipocytes.

3 Conclusion

The glucose content in BTP was the highest, followed by OTP. However, the main component of GTP was galactose. The TPs, especially GTP, significantly inhibited the growth and differentiation of 3T3-L1 preadipocytes ($P < 0.05$). TPs increased the number of 3T3-L1 preadipocytes in the G0/G1 phase and had a significantly positive effect on cell apoptosis. Through RT-PCR, we found that the three types of TPs down-regulated the expression of *PPARγ*, *SREBP-1c*, *LPL*, ACC and *SCD1* and up-regulated the expression of *ADP*. Changes in the mRNA expression of these genes will affect the inhibition of 3T3-L1 preadipocytes differentiation, the accumulation of TGs and the regulation of lipid metabolism.

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