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Analyzing and comparising capacity of glucose- and uric acid-lowering effects of yellow tea with different varieties and grades in zebrafish model

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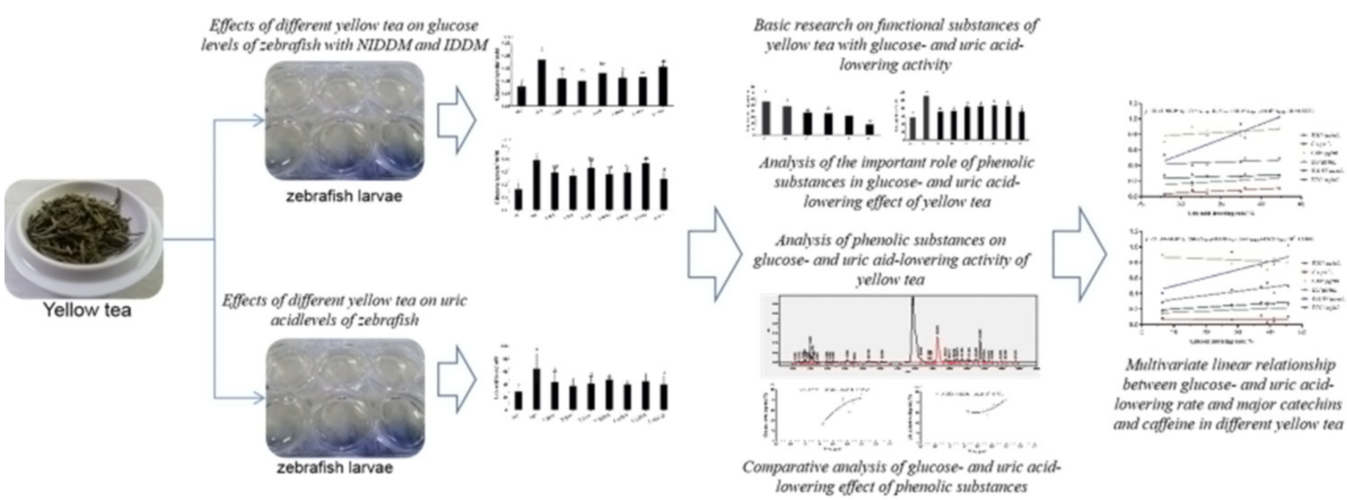
Abstract

The glucose-lowering effects (GLE) and uric acid-lowering effects (UALE) of tea have been widely reported, but the differences in quality and activity of different varieties and grades (DVG) are still unknown. Herein, yellow tea (YT) were chosen, and in this study the effects of YT with DVG on GLE and UALE were investigated and comparised in zebrafish with diabetes and hyperuricemia, and the mechanism of action were deeply researched. Results showed that there were distinct differences in GLE and UALE of YT with DVG after zebrafish evaluation. The GLE and UALE of population cultivar with all different grades were relatively better than that of special early species. The GLE of bud tea and small tea was better than that of large tea. The UALE of small tea was better than that of large tea and then better than that of bud tea. The differences in GLE and UALE may be related to the content levels of phenols because the GLE and UALE was significantly reduced when phenols directly removed from YT solution. Regression analysis revealed that the highest GLE and UALE does not require the highest or lowest content of each phenolic substance, but depend on what components.

Keywords: yellow tea; zebrafish; diabetes; hyperuricemia; glucose-lowering effects; uric acid-lowering effects.

Practical Application: Our results provided some basic information for variety and grade screening and utilizing based on their specific functional characters, and developing functional enhanced food and drinks.

Graphical Abstract



1 Introduction

With the improvement of living standards, quality of life is that each of us pursued. However, instead, many diseases arise from what people really don't know about in their daily life. For example, diabetes, cancer, tumor, gout arthritis, obesity, etc have become worldwide diseases. And most importantly, these diseases may threaten to roll back life expectancy, and bring the poverty due to illness. Studies have consistently shown us that many diseases caused by the changes of environmental factors (Skinner et al., 2010). As one of them, diabetes, characterized with hyperglycemia, and is caused by a group of metabolic disorders (Li et al., 2020), may lead to many complications, such as diabetic nephropathy, atherosclerosis, eye diseases, hypertension. According to statistics, the numbers of patients with type II diabetes increased significantly in the past two decades. The main reason behind it is

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that the diminution of physical activity, eat too much unhealthy food and get too heavy. In other words, most cases of diabetes result from the unhealthy lifestyle.

Aside from diabetes, gout is another health risk (Li et al., 2022). It is a metabolic disease of tissue damage due to long-term purine metabolic disturbance and the increase of blood uric acid. Gout arises when excess uric acid builds up in the joints. And also gout is one kinds of inflammatory arthritis, and is caused by metabolic disorder. Up to now, unfortunately, there are no proven treatments or cure for any form of diabetes or gout arthritis. Of course, they all can be well controlled by series of new drugs. However, chronic drug use can lead to adverse drug reactions, such as nausea, vomiting, diarrhea, etc. Herein, healthy, safe and effective assistant treatment measures are needed.

Studies have consistently shown us that nutrition plays a big part in many diseases (Ji et al., 2022), and a healthy life style can help prevent many metabolic diseases, such as diabetes (Chen et al., 2023; Fan et al., 2022; Zapata et al., 2022). Furthermore, some chronic diseases require special diets that should be monitored by your physician. For example, high-fiber or low glycemic index diets helps to reduce the risk of diabetes (Bai et al., 2023). Controlling of energy intake and reasonable diet, weight reduction helps to prevent gout attacks.

It is fair to say that we are looking for everything that is good for health. Because of this, tea, one amazing plants appeared in our horizons, and is famous for lots of a magical effect for anticancer, anti-tumor, anti-diabetes (Liu et al., 2022a, b), anti-gout, antioxidant (Zahidin et al., 2018), restoration circadian rhythm disruption (Hu et al., 2022), anti-obesity (Huang et al., 2013), regulation the balance of intestinal flora (Sun et al., 2021; Zhang et al., 2020), etc. Experiments on modern animals (Liu et al., 2022a, b; Zhang et al., 2020) and people (Nagao et al., 2009; Mortazavi et al., 2018) have found that tea has good ability to regulate the balance of energy metabolism. And the possible regulatory pathways of tea on glucolipid metabolism were as follows: inhibiting lipid absorption by forming complex with lipids and lipases (Xu et al., 2020), reducing glucose digestion and absorption by inhibiting amylase activity (Sun et al., 2017), inhibiting gluconeogenesis (Collins et al., 2007), enhancing glucose consumption and utilization (Lin & Lin, 2008) etc. Of course, researchers suggested that tea polyphenols (TPs) (Macena et al., 2022; Tan et al., 2022), caffeine (CAF), tea polysaccharide (Zhou et al., 2022a, 2023; Kim et al., 2022), and other functional ingredients (Rubanka et al., 2020; Wang et al., 2023) contained in tea play a key role in lowering blood glucose levels. In addition, studies suggested that TPs (Zhou et al., 2022b), tea pigment (Liu et al., 2020) and other functional components contained in tea also play an important role in lowering uric acid levels.

However, tea is one kinds of functional component complex, only use pure substance of tea for functional evaluation neither scientific nor comprehensive evaluate the effects of tea. Therefore, in view of the problem, more in-depth research are needed. Yellow tea is one kinds of special tea in China, and it is liked by tea consumers because of its unique textures and tastes, such as it has three-yellow feature of dry tea yellow, tea soup yellow and the phyllotaxis (has soaked tea dregs) yellow (Zhang et al., 2019a, 2022).

Meanwhile, there are many kinds of yellow tea existed in China (the homeland of tea), including different varieties and grades. According to the old tenderness of its fresh tea leaves, yellow tea can be divided into yellow bud tea, yellow small tea and yellow large tea. Most importantly, the sources of fresh tea leaves exist breed difference, and the breed difference will lead to different levels of biological activity (Guedes et al., 2022). Until now, we know yellow tea like other tea also has superior ability to regulate the balance of energy metabolism (Zhou et al., 2018a; Wu et al., 2022) and helps to control gout attack (Jin et al., 2016; Wu et al., 2021).

However, at present, there are only a few studies focused on the difference of glucose- and uric acid-lowering performance of different varieties and grades of yellow tea, and uncover its internal regulatory mechanism. Therefore, this study takes different varieties and grades of yellow tea (not pure substance of tea) as the object, and investigate the effects of different tea on glucose- and uric acid-lowering rate in zebrafish with type I and II diabetes and hyperuricemia. And on this basis, we deeply investigated and reveled the internal regulatory mechanism of yellow tea on glucose- and uric acid-lowering performance by combined basic research on functional substances and using data mining technology. This research provides some basic information for the theory of lowering glucose and uric acid in yellow tea and technical guidance for the development of functional drinks, and the final goal provides the health Gospel for special groups.

2 Materials and methods

2.1 Experimental materials

(1) Main instrument: Thermostatic Water Bath (Shanghai Jinghong Experimental Equipment Co., LTD.); Automatic microplate reader (Thermo scientific Varioskan lux, Thermo Scientific (China) Co., LTD.); Hand-held high speed homogenizer (MY-20, Shanghai Jingxin Industrial Development Co., LTD.); Inverted fluorescent microscope (Nikon SMZ25); Automatic intelligent biochemical (mold) incubator (Tianjin Hongnuo Instrument Co., LTD.); Precision electronic balance (AL-204, Mettler Toledo International Trading (Shanghai) Co., LTD.); High Speed Tabletop Refrigerated Centrifuge (3K1S, sigma Centrifuge Company, Germany).

(2) Main reagents: Bud-tea in yellow tea of population cultivar (BPC); Small tea in yellow tea of population cultivar (SPC); Large tea in yellow tea of population cultivar (LPC); Bud-tea in yellow tea of special early species (BSES); Small tea in yellow tea of special early species (SSES); Large tea in yellow tea of special early species (LSES). They were all purchased from Pingyang Tianrun Tea Co., LTD.) and they were all extracted by the following conditions: extraction temperature 80°C, extraction time 30 min, water/material ratio 50:1, and extracted twice.

Glucose (Sinopharm Chemical Reagent Co., LTD.); Alloxan (purity ≥98.0%, Hefei Bomei Biotechnology Co., LTD.); Polyvinylpolypyrrolidone (PVPP) (Shanghai Aladdin Biochemical Technology Co., LTD.); Acarbose (ACA, Bayer Medical Healthcare Co., LTD).

Glucose detection kit (glucose oxidase method) and uric acid detection kit were purchased from Nanjing Jiancheng Bioengineering Institute); potassium oxonate (PO), xanthine sodium salt (XSS) and allopurinol (ALLO) were purchased from Sigma Aldrich (Shanghai) Trading Co., LTD.).

(3) Experimental animals

Zebrafish (3-month-old, AB type). Reproduction of zebrafish was carried out by natural mating. According to 3:2 male and female zebrafish were selected and placed in a breeding box with double layer design in a 28.5°C incubator to lay eggs. When the female zebrafish layers her eggs, the embryos were collected for incubation, and the incubation water (60 μg/mL sea salt water) was changed every 24 hours, and the egg membrane and dead eggs were removed. When the zebrafish larvae incubated for five days were selected and chosen for experiments.

2.2 Experimental methods

Comparative study on hypoglycemic effect of different varieties and grades of yellow tea

(1) Effects of hypoglycemic effect of yellow tea based on NIDDM zebrafish

Based on previous studies (Liu et al., 2021, 2022c, 2023), healthy zebrafish were selected and randomly divided into 5 groups, with 60 fish in each group. They were incubated with 60 mL 0 mg/mL glucose, 22 mg/mL glucose, 22 mg/mL glucose and 25 μg/mL BPC, SPC, LPC, BSES, SSES and LSES for 24 hours, respectively. And they were denoted as NC1, NC2, T-BPC, T-SPC, T-LPC, T-BSES, T-SSES and T-LSES, respectively. At the end of the experiment, samples were taken for determination the glucose levels of zebrafish.

(2) Effects of hypoglycemic effect of yellow tea based on IDDM zebrafish

Based on our previous studies (Liu et al., 2021, 2022c, 2023), healthy zebrafish were selected and induced with 22 mg/mL glucose and 0.02 mmol/L alloxan for 24 h to establish a diabetic model. Then, the diabetic zebrafish were randomly divided into 8 groups, with 60 fish in each group. They were incubated with 60 mL of 22 mg/mL glucose, 22 mg/mL glucose and 25 μg/mL BPC, SPC, LPC, BSES, SSES and LSES, 22 mg/mL glucose and 25 μg/mL ACA for 24 hours, which were recorded as MC, T-BPC, T-SPC, T-LPC, T-BSES, T-SSES and T-LSES and T-ACA, respectively. Another 60 healthy zebrafish larvae were chosen and incubated with 0 mg/mL glucose for 48 h as NC control group. After the experiment samples were taken for determination the glucose levels.

Comparative study on uric acid-lowering effects of different varieties and grades of yellow tea

Referring to the modeling scheme of Zhang et al. (2019b), and based on our exploration, 250 μM XSS and 400 μM PO were selected to establish a zebrafish model of hyperuricemia. According to the above experimental protocol, healthy zebrafish were selected and randomly divided into 6 groups, with 60 fish in each group. They were incubated with 60 mL of pure water, 250 μM XS+400 μM PO and 0, 25 μg/mL yellow tea with different varieties and grades, 250 μM XS+400 μM PO+25 μg/mL of ALLO solution for 24 hours, respectively. And they were recognized as NC, MC, BPS, SPS, LPS, BSES, SSES, LSES and ALLO, respectively. Samples were taken at the end of the experiment to determine the level of uric acid.

Basic research on functional substances of yellow tea with glucose- and uric acid-lowering activity

Analysis of phenolic substances on glucose- and uric aid-lowering activity of yellow tea

TPs is a general terms for phenolic substances in tea, and is one major bioactive components of tea. In this study, the phenolic substances in SPC solution were directly removed by PVPP (one kind of adsorbing material). The adsorption efficiency of PVPP were studied through determination the levels of TPs in yellow tea solution according to National Tea Standardization Technical Committe (2018). Most importantly, the glucose- and uric acid-lowering activity of yellow tea with phenolic substances removed were investigated and evaluated through the usage of zebrafish model mentioned above.

The specific experimental process is as follows: Firstly, yellow tea solution were divided into 6 experimental groups, 30 mL 0.00, 0.33, 0.66, 1.33, 2.67 and 6.00 μg/mL PVPP were weighed and added to each group, respectively. And then the mixed solution was shaken at room temperature for 30 min and centrifuged at 3500 r/min for 20 min. Next, the supernatant of each group was absorbed and used for determination of the TPs levels and functional evaluation.

The functional evaluation procedure is as follows:

(1) Healthy zebrafish were selected and randomly divided into 8 groups, with 60 fish in each group. They were incubated with 60 mL 0 mg/mL glucose, 22 mg/mL glucose and 0 and 25 μg/mL yellow tea with different removal efficiency of TPs for 24 hours, respectively. And they were denoted as NC1, NC2, T0, T1, T2, T3, T4 and T5, respectively. At the end of the experiment, samples were taken for the determination of glucose levels of zebrafish.

(2) Healthy zebrafish were selected and randomly divided into 8 groups, with 60 fish in each group. They were incubated with 60 mL of pure water, 250 μM XS+400 μM PO and 0, 25 μg/mL yellow tea with different removal efficiency of TPs, 250 μM XS+400 μM PO+25 μg/mL of ALLO solution for 24 hours, respectively. And they were recognized as NC, MC, T0, T1, T-2, T3, T4, T5 and T6, respectively. Samples

were taken at the end of the experiment to determine the level of uric acid.

Analysis of the relationship between phenolic substances and glucose- and uric aid-lowering activity of yellow tea

Since phenolic substances play an important role in the glucose- and uric acid-lowering effect of yellow tea, the relationship between phenolic substances and the glucose- or uric aid-lowering activity of yellow tea were studied by correlation analysis.

The process of correlation analysis is as follow: Firstly, the phenolic substances in different solution of different varieties and grades of yellow tea mentioned above were detected according to National Tea Standardization Technical Committe (2018). Next, the correlation analysis was carried out between the content level of the phenolic substances and their corresponding glucose- or uric acid-lowering effects mentioned above. Most importantly, the activity of glucose- or uric acid-lowering effect were deeply analyzed to clarify the "dose-effect" relationship, and to the give an anticipatory indication for directed screening potential effector with strong glucose- and uric acid-lowering values.

2.3 Detection methods

Glucose or uric acid levels were measured as follows process: 5 fish:100 μL phosphate buffer salt solution were mixed and homogenized by a high-speed handheld homogenizer for 1 min until the tissue was fully lysed, and then centrifuged at 2500 rpm for 10 min at 4 °C. After that, 5.0 μL of the supernatant sample was taken to determine the glucose concentration by glucose detection kit (glucose oxidase method), and to determine the uric acid concentration by uric acid detection kit. The assay procedures and methods were carried out according to the steps specified in the kit instructions.

2.4 Statistical analysis

All the experimental data were preliminarily processed by Excel 2016 and plotted by Graphpad Prism 8.0. One-way ANOVA was performed by SPSS Statistics 24.0, and all data were presented as mean ± standard deviation. The significance of differences was analyzed and performed by the Duncan's analysis and multiple comparisons. $p < 0.05$ indicates significant differences were found between treatment groups.

3 Results and discussion

3.1 Analysis of glucose-lowering effect of different varieties and grades of yellow tea

Figure 1a shows that, compared with group NC2, the glucose levels in groups T-BPC, T-SPC, T-LPC, T-BSES, T-SSES and T-LSES were decreased by 45.70% ($p < 0.05$), 41.29% ($p < 0.05$), 28.10% ($p < 0.05$), 39.11% ($p < 0.05$), 37.23% ($p < 0.05$) and 15.47% ($p > 0.05$), respectively.

Compared with group MC, the glucose levels in groups T-BPC, T-SPC, T-LPC, T-BSES, T-SSES, T-LSES and T-ACA were decreased by 24.39% ($p < 0.05$), 31.54% ($p < 0.05$), 14.98% ($p > 0.05$), 27.95% ($p < 0.05$), 25.78% ($p < 0.05$), 6.35% ($p > 0.05$) and 37.33% ($p < 0.05$), respectively (Figure 1b).

The results showed that the glucose-lowering effects of different varieties and grades of yellow tea exist some difference. In the same variety but not the same grades, the optimal order of glucose-lowering effects was: small tea and bud tea were stronger than large tea; In the same grade but not the different species, the glucose-lowering effects of the population cultivar was slightly stronger than the special early species.

At present, studies on the therapeutic effects of different kinds of tea on diabetic animals model have been reported (Xu et al., 2020; Macena et al., 2022; Tan et al., 2022), but there are only a few studies focused on the effects of different varieties and grades of tea on diabetes, with particularly in yellow tea. Our findings showed that within the same tea category, the glucose-lowering effects of yellow tea varies according to its variety and grade, which may be related to the different levels of functional components contained in tea (Tan et al., 2022). Therefore, further basic researches on functional substances are needed.

3.2 Analysis of uric acid-lowering effect of different varieties and grades of yellow tea

As can be seen in Figure 2, compared with the group MC, the uric acid levels of zebrafish in groups T-BPC, T-SPC, T-LPC, T-BSES, T-SSES, T-LSES and T-ALLO were decreased by 33.25%, 42.26%, 37.43%, 27.91%, 37.97%, 31.40% and 37.99%, respectively, and with all significant differences ($p < 0.05$).

Although no significant difference in uric acid-lowering effects were found among different varieties and grades of yellow tea

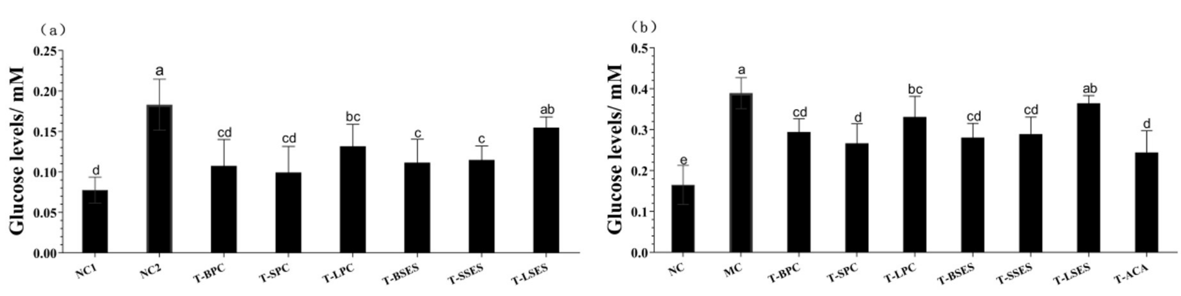


Figure 1. Effects of different varieties and grades of yellow tea on the glucose-lowering effects in zebrafish with NIDDM (a) and IDDM (b)

($p > 0.05$), there still exist some difference. In the same variety but not the same grades, the optimal order of uric acid reduction was: small tea, large tea, bud tea; In the same grade but not the same species, the bioactive action of uric acid-lowering of population cultivar was slightly stronger than that of the special early species.

3.3 Basic research on functional substances of yellow tea with glucose- and uric acid-lowering activity

Analysis of the directed removing efficiency of phenolic substance in yellow tea by PVPP

As can be seen from Figure 3a, with the increase of PVPP dose concentration, the total TPs clearance rate increased significantly, and showed an obvious linear relationship ($y = 14.82x + 0.7701$ $R^2 = 0.9989$), indicating that PVPP has a good adsorption and clearance effect on phenolic substances.

The total amount of TPs in group T5 were decreased by 89.17%. Among the main catechins, the clearance rate of PVPP on EGCG in yellow tea solution was the strongest, and the levels of EGCG was decreased by 96.15%. Moreover, the clearance rate of ECG, EGC, EC and C were 89.52%, 66.07%, 61.44% and 12.46%, respectively. In addition, PVPP had very little effect on CAF clearance (Figure 3b).

Analysis of the hypoglycemic effect of yellow tea with phenolic substances removed in various degrees

The changes in the hypoglycemic performance of yellow tea after the PVPP directed removal of phenolic substances in yellow tea solution was shown in Figure 4. Figure 4 shows that compared with the highest glucose-lowering rate of 55.93% in group T0, The glucose-lowering rate of T1, T2, T3, T4 and T5 were decreased by 8.68, 18.60, 20.37, 24.48, and 37.98 percentage points, respectively.

Current studies have shown that phenolic substance plays an important and key role in glucose-lowering effects of tea (Tan et al., 2022). Our results showed PVPP has a good clearance efficiency for TPs and its main catechin monomers EGCG, ECG, EGC and EC. Meanwhile, our findings showed that the hypoglycemic performance of yellow tea decreased significantly with the increase clearance rate of TPs and its main catechin monomer, indicating that phenolic substances plays an important and key role in the glucose-lowering effects of yellow tea.

Figure 5 shows the actual effective administration dose of TPs in different varieties and grades of yellow tea. According to Figures 1a and 5, the differences in hypoglycemic performance of different varieties and grades of yellow tea may be related to the different actual effective dose concentration of phenolic substances contained in them.

Most importantly, correlation analysis showed that there were multiple strong correlations ($R^2 \geq 0.6$) or higher levels of relationships between the glucose-lowering rate of yellow tea and the actual effective administration dose of TPs and its main catechin monomer contained in them. For example, the regression equation between the glucose-lowering rate (y, %) and the actual effective administration dose of TPs (x, μg/mL) was: $y = -538.45x^2 + 373.52x - 25.707$ ($R^2 = 0.7083$, $0.29 \leq x \leq 0.55$) (Table 1).

Analysis of the uric acid-lowering effect of yellow tea with phenolic substances removed in various degrees

Figure 6 showed that compared with group MC, the uric acid levels of zebrafish in groups T0, T1, T2, T3, T4 and T5 were decreased by 35.52%, 33.55%, 24.98%, 24.22%, 21.81% and 23.43%, respectively, and the differences were all significant ($p < 0.05$).

Among them, therapeutic effects in treatment groups T0 and T1 reached to highest levels, and with a similar levels to treatment of positive therapeutic agent-ACA (T6, 35.43%).

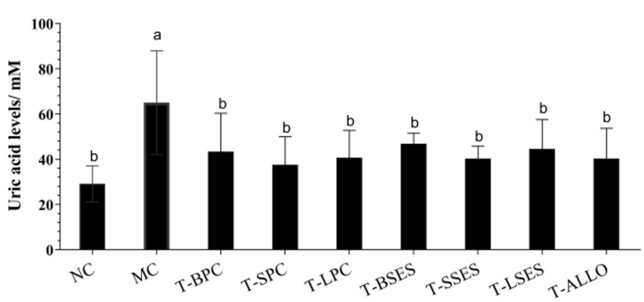


Figure 2. Analysis of uric acid-lowering effect of different varieties and grades of yellow tea

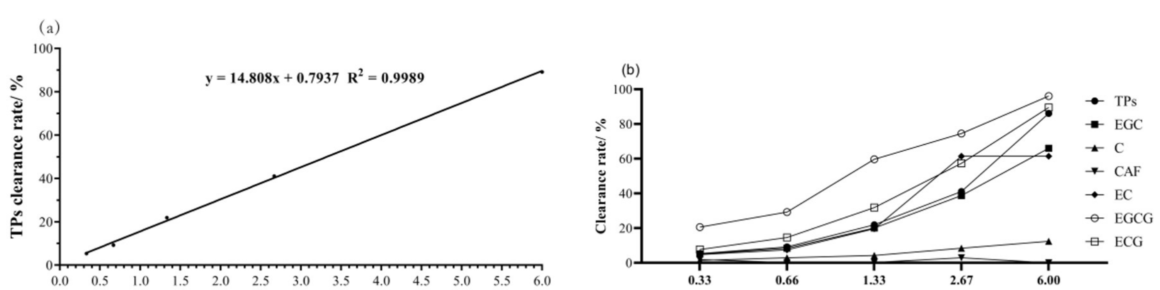


Figure 3. Effects of PVPP on TPs (a) and its major catechins (b) clearance rate of yellow tea solution. Note: a represent the dose-effect relationship between PVPP concentration (μg/mL) and TPs clearance rate (%). b represent the clearance rate of TPs and its main catechins in yellow tea of different PVPP addition groups.

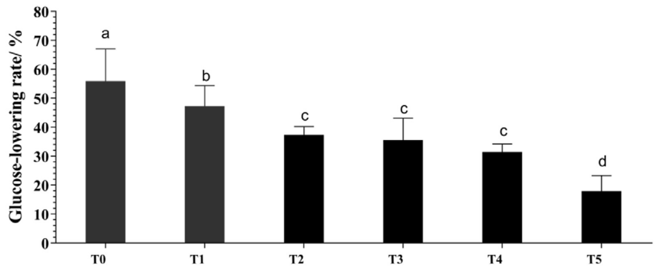


Figure 4. Changes of hypoglycemic performance of yellow tea after PVPP directed removal of phenolic substances. Note: T0, T1, T2, T3, T4 and T5 represent the reduction levels of glucose when compared the control group NC2.

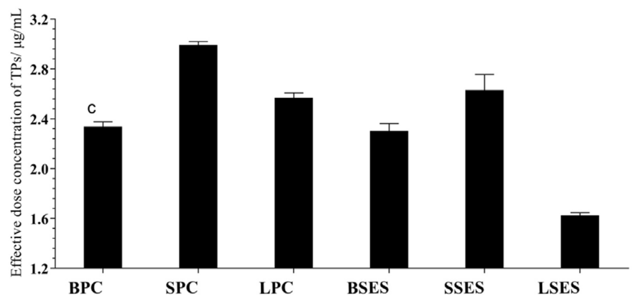


Figure 5. The actual effective administration dosage concentration of TPs in the solution of different varieties and grades of yellow tea. Note: Bud-tea in yellow tea of population cultivar (BPC); Small tea in yellow tea of population cultivar (SPC); Large tea in yellow tea of population cultivar (LPC); Bud-tea in yellow tea of special early species (BSES); Small tea in yellow tea of special early species (SSES); Large tea in yellow tea of special early species (LSES). They were all purchased from Pingyang Tianrun Tea Co., LTD.) and they were all extracted by the following conditions: extraction temperature 80 °C, extraction time 30 min, water/material ratio 50:1, and extracted twice.

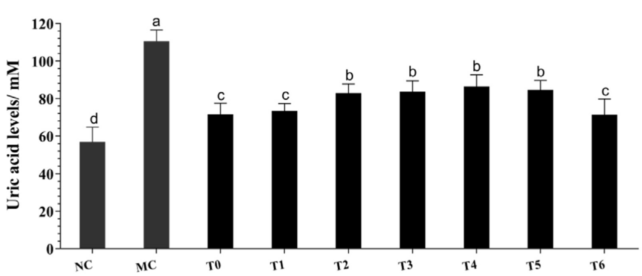


Figure 6. Effect of yellow tea after PVPP directed removal of phenolic substances on uric acid-lowering activity in zebrafish with hyperuricemia.

Except for groups T0 and T1, the therapeutic effects in other four treatment groups exhibited a relative lower levels to ACA.

According to Figure 3a and Figure 6, when the levels of TPs in yellow tea solution was reduced by 5.14%, it had little effects on the uric acid-lowering performance of yellow tea. Moreover, the uric acid-lowering performance was significantly reduced when the clearance rate of TPs in yellow tea solution was increased to 8.48% and above, suggesting that TPs played an important role in lowering uric acid.

However, results also showed that when TPs clearance rate above 8.48%, the uric acid-lowering activity of yellow tea was basically maintained at a certain levels of 21.81-24.98%, indicating that the uric acid-lowering rate of yellow tea did not exhibit a obvious dose dependent effect with the increase clearance rate of TPs. On the contrary, there may exist some other functional components in yellow tea also play an important role in the uric acid-lowering of yellow tea, and which helps to maintain the stability of uric acid-lowering performance, and further experimental studies are needed.

Tea is rich in TPs, tea polysaccharides, alkaloids, tea pigments (theaflavins) and other functional components, and it is one well-known substance for its health benefits. Studies have shown that TPs and its catechins (Jin et al., 2016; Tan & Chen, 2015) can improve hyperuricemia by reducing the production of uric acid and increasing the excretion of uric acid (Zhou et al., 2022b). Chen & Tan (2017) established a mouse model of hyperuricemia induced by PO. Their results showed that gavage of TPs (extracted from green tea) could significantly reduce the serum uric acid level, reduce the activity of xanthine oxidase in the liver, reduce the expression of URate transporter 1 (URAT1) in the kidney, but significantly increase the expression of organic anion transporter (OAT) 1 and 3. These results suggested that the uric acid-lowering effect of TPs is closely related to reducing uric acid synthesis and promoting uric acid excretion.

The uric acid-lowering properties of yellow tea solution remained stable for a certain levels after the TPs clearance rate reached to a certain level, which may be related to other active components contained in yellow tea. Except for TPs, theaflavins (Wu et al., 2021; Zhou et al., 2018b; Liu et al., 2020) also have strong uric acid-lowering activity. The content levels of theaflavins contained in yellow tea (Pingyang Yellow Soup) was 0.0494% (Ye et al., 2015). Wu et al. (2021) found that continuous gavage of theaflavins at medium and high doses (20 and 60 mg/kg) for 7 days could significantly ($p < 0.01$) reduce serum uric acid levels in mice. Zhou et al. (2018b) found that 10 mg/kg theaflaxanthins could significantly reduce serum uric acid level and increase the mRNA expression of sodium-dependent phosphate transporter NPT4, glucose facilitated transporter 9, and renal URAT1. These results suggested that the effect of theaflavins on lowering uric acid might be related to inhibiting the activity of xanthine oxidase and regulating the expression levels of anion transporter gene and protein.

Analysis of the effect of phenolic substances on the glucose- and uric acid-lowering effect of yellow tea

In view of the important role of phenolic substances in glucose- and uric acid-lowering of yellow tea, the phenolic substances was separated by HPLC according to National Tea Standardization Technical Committe (2018). Through comparative study, 35 common peaks were separated from the samples of yellow tea with different varieties and grades, which were marked as peak 1 to peak 35 in chronological order (Figure 7). Subsequently, the correlation (including exponential, linear, logarithmic, polynomial and power exponential, etc.) between each peak and the corresponding glucose- or uric

Table 1. Comparative analysis of the effect of phenolic substances on glucose- and uric acid-lowering effects in yellow tea.

Item	Correlation (Regression equation)		
	Glucose-lowering effects	Uric acid-lowering effects	Ranges of x values
TPs	$y = 111.78e^{-3.456x}$ ($R^2 = 0.7560$)	$y = 10.848x^2 - 40.878x + 68.665$ ($R^2 = 0.7931$)	$1.63 \le x \le 2.99$
	$y = -538.45x^2 + 373.52x - 25.707$ ($R^2 = 0.7083$)		
EGC	$y = -460.29x^2 + 471.6x - 81.064$ ($R^2 = 0.7046$)	/	$0.29 \le x \le 0.54$
	$y = 115.07x^{1.5158}$ ($R^2 = 0.7444$)		
C	$y = 10181x^2 - 1476.2x + 78.603$ ($R^2 = 0.4784$)	$y = 64.267x^{0.2247}$ ($R^2 = 0.6068$)	$0.06 \le x \le 0.11$
CAF	/	$y = -711.37x^2 + 1177.7x - 449.43$ ($R^2 = 0.5230$)	$0.71 \le x \le 0.90$
EC	$y = 2836.1x^2 - 920.2x + 94.263$ ($R^2 = 0.7356$)	/	$0.08 \le x \le 0.26$
EGCG	$y = -90.913x^2 + 156.91x - 27.6$ ($R^2 = 0.4716$)	$y = 18.848x + 21.129$ ($R^2 = 0.7368$)	$0.41 \le x \le 1.03$
	$y = 42.681x^{0.7668}$ ($R^2 = 0.4592$)	$y = 28.223x^2 - 21.288x + 34.075$ ($R^2 = 0.7895$)	
ECG	$y = 2.3807e^{10.2x}$ ($R^2 = 0.9005$)	/	$0.18 \le x \le 0.29$
	$y = 487.66x^2 + 41.52x - 8.8098$ ($R^2 = 0.8153$)		
TC	$y = -26.38x^2 + 106.22x - 66.941$ ($R^2 = 0.6098$)	$y = 19.845x^2 - 52.815x + 65.169$ ($R^2 = 0.8438$)	$1.09 \le x \le 2.14$
	$y = 16.458x^{1.336}$ ($R^2 = 0.6338$)		
TEC	$y = -68.724x^2 + 157.04x - 49.839$ ($R^2 = 0.5626$)	$y = 33.925x^2 - 47.227x + 46.536$ ($R^2 = 0.8220$)	$0.59 \le x \le 1.30$
	$y = 33.881x^{1.0216}$ ($R^2 = 0.5552$)		
TNEC	/	/	
CAF/TP	$y = 111.78e^{-3.456x}$ ($R^2 = 0.7560$)	/	$0.29 \le x \le 0.56$
	$y = -538.45x^2 + 373.52x - 25.707$ ($R^2 = 0.7083$)		
CAF/C	$y = 0.275x^2 - 8.1578x + 84.918$ ($R^2 = 0.4185$)	$y = 44.511e^{-0.018x}$ ($R^2 = 0.6083$)	$7.15 \le x \le 23.23$
		$y = -0.6087x + 43.541$ ($R^2 = 0.5960$)	
CAF/EC	$y = 1.7064x^2 - 23.58x + 97.406$ ($R^2 = 0.7686$)	/	$3.03 \le x \le 10.75$
CAF/EGCG	$y = 71.325e^{-0.631x}$ ($R^2 = 0.6668$)	$y = 17.239x^2 - 59.794x + 78.866$ ($R^2 = 0.7700$)	$0.84 \le x \le 2.23$
	$y = -23.838x^2 + 58.09x + 4.417$ ($R^2 = 0.7377$)		
CAF/ECG	$y = 131.3e^{-0.421x}$ ($R^2 = 0.7505$)	$y = -8.1692x^2 + 62.124x - 76.505$ ($R^2 = 0.5436$)	$2.20 \le x \le 4.93$
	$y = -6.8567x^2 + 42.394x - 26.843$ ($R^2 = 0.7100$)		
CAF/EGC	$y = 89.53e^{-0.514x}$ ($R^2 = 0.6765$)	$y = -9.98x^2 + 44.839x - 11.147$ ($R^2 = 0.4713$)	$1.31 \le x \le 3.16$
	$y = -11.23x^2 + 38.911x + 5.2128$ ($R^2 = 0.6919$)		
CAF/TEC	$y = -1045.8x^2 + 1562.2x - 534.19$ ($R^2 = 0.7539$)	$y = 55.451x^2 - 132.85x + 104.58$ ($R^2 = 0.7395$)	$0.29 \le x \le 0.55$
CAF/TNEC	$y = -37733x^2 + 47743x - 15049$ ($R^2 = 0.8966$)	$y = -49.447x^2 + 129.11x - 42.984$ ($R^2 = 0.5051$)	$0.88 \le x \le 1.80$

Notes: EGCG, epigallocatechin gallate; EC, epicatechin gallate; ECG, epicatechin gallate; EGC, epigallocatechin catechin; C, catechin; CAF, caffeine; TC, total catechins, TC = EGC + C + EC + EGCG + ECG; TEC, total ester catechins, TEC = ECG + EGCG; TNEC, total non-ester catechins, TNEC = C + EGC + EC.

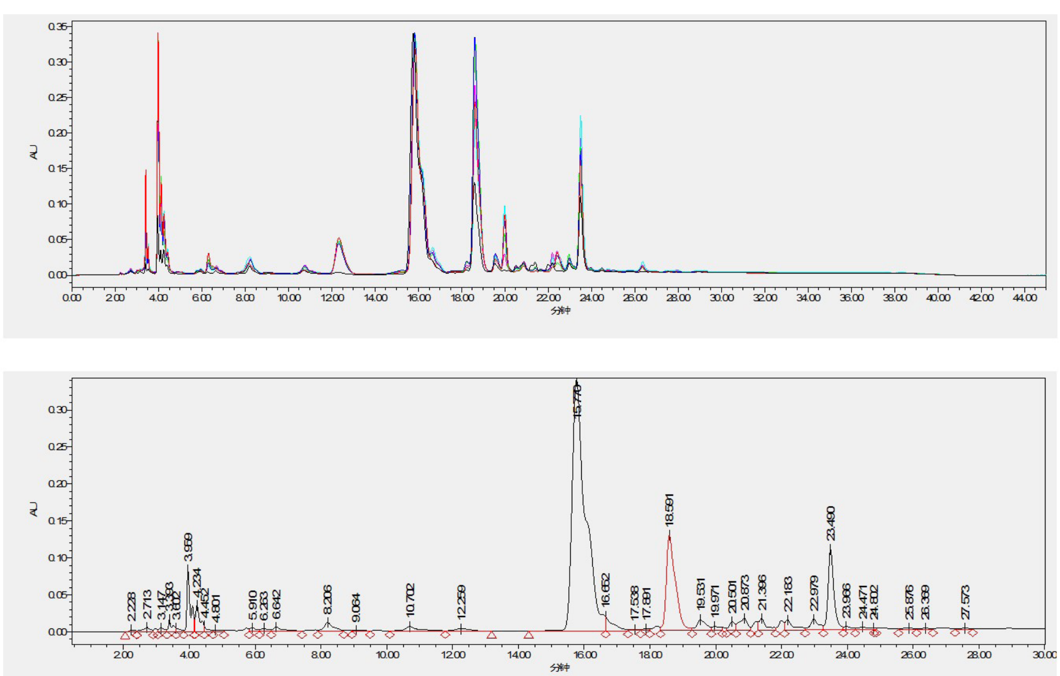


Figure 7. Analysis of phenolic substances in different varieties and grades of yellow tea solution based on liquid chromatography.

acid- lowering rate of the sample was analyzed, and the results were showed in Table 2.

In term of lowering glucose, according to R^2 values, peak 1, peak 4, peak 5, peak 6, peak 7, peak 8, peak 9, peak 13, peak 16, peak 23, peak 26, peak 29, peak 33, peak 34 reached the level of extremely strong correlation ($0.8 < R^2 \leq 1.0$); Peak 10, peak 18, and peak 24 reached the levels of strong correlation ($0.6 < R^2 \leq 0.8$) (Table 2).

Due to phenolic substances is one kind of mixture, the "Dimension Reduction" principal component analysis method in SPSS statistics 24.0 was used to carry out the dimension reduction principal component analysis, and to find out the weight of multi-factor influence. The results are shown in Table 2 and Figure 8. From the perspective of the influence weight coefficient of a single factor, the influence weight coefficient of each peak ranged from 0.22% to 5.95%, and the influence weight above 3.0% accounted for a total of 42.85% (Figure 8).

According to the above studies, the influence weight coefficients of ECG (peak 29), EGC (peak 13), EGCG (peak 21), C (peak 15), EC (peak 18) and CAF (peak 17) were: 5.05%, 3.36%, 2.88%, 1.11%, 0.59% and 0.22%, respectively, accounting for 13.21% of the weight coefficient in total (Table 2). Among them, the correlation coefficient between glucose-lowering rate and ECG (peak 29), EGC (peak 13) and EC (peak 18) reached the level of strong correlation ($R^2 > 0.6$), which showed power exponent, power exponent and polynomial (binomial) correlations according to the highest R^2 value level, respectively (Table 2).

The synergistic or antagonistic effects between hypoglycemic performance and phenolic substances were simply evaluated, and the results were shown in Table 1 and Figure 9. In terms of glucose-lowering effects, the results showed that within the observed actual administration dose range, in addition to CAF and C, the relationship between glucose-lowering rate and EGC, EGCG, ECG, TPs, TEC, CAF/ECG, CAF/EGC, and CAF/EGCG all showed a parabolic shape (binomial) with an opening downward type (Table 1, Figure 9) according to the common characteristics of the highest R^2 values. At the aspect of uric acid-lowering effects, the results showed that within the observed

Table 2. Analysis of the role of phenolic substances in glucose- and uric acid-lowering effects in different varieties and grades of yellow tea.

Item	IWCN */ %	Correlation (Regression equation)	
		Glucose-lowering effects	Uric acid-lowering effects
P 1	1.54	$y = -0.062x^2 + 4.4788x - 27.926$ ($R^2 = 0.8214$)	$y = -0.0285x^2 + 2.2832x - 0.5215$ ($R^2 = 0.6788$)
P 2	2.88	/	$y = -0.0029x^2 + 1.5943x - 176.59$ ($R^2 = 0.5640$)
P 3	0.98	/	/
P 4	5.01	$y = 0.014x^{1.1343}$ ($R^2 = 0.8860$)	/
P 5	5.08	$y = 0.0021x^{1.5993}$ (R2 = 0.8548)	/
P 6	4.52	$y = 3E\text{-}05x^{1.6817}$ ($R^2 = 0.8151$)	/
P 7	5.63	$y = -0.0001x^2 + 0.2965x - 165.95$ ($R^2 = 0.8707$)	/
P 8	5.95	$y = 0.0299x^{1.1458}$ ($R^2 = 0.9264$)	/
P 9	2.98	$y = 0.0063x^2 - 0.995x + 45.81$ ($R^2 = 0.8082$)	/
P 10	3.04	$y = -0.0104x^2 + 4.7854x - 495.28$ ($R^2 = 0.7848$)	$y = 0.0015x^2 - 0.5513x + 85.155$ ($R^2 = 0.4672$)
P 11	3.64	$y = -0.0002x^2 + 0.1902x - 11.376$ ($R^2 = 0.5045$)	$y = -9E\text{-}05x^2 + 0.0888x + 16.547$ ($R^2 = 0.5602$)
P 12	2.90	$y = 0.0005x^2 - 0.347x + 77.896$ ($R^2 = 0.5550$)	/
P 13 (EGC)	3.36	$y = 1E\text{-}07x^{2.818}$ ($R^2 = 0.8190$)	/
P 14	2.92	$y = -0.0062x^2 + 0.4846x + 33.624$ ($R^2 = 0.4790$)	/
P 15 (C)	1.11	$y = 0.0002x^2 - 0.2406x + 80.747$ ($R^2 = 0.4674$)	$y = 10.06x^{0.2043}$ ($R^2 = 0.6108$)
P 16	4.83	$y = 0.0926x^{0.7489}$ ($R^2 = 0.9419$)	
P 17 (CAF)	0.22	/	$y = -1E\text{-}06x^2 + 0.0448x - 447.72$ ($R^2 = 0.5230$)
P 18(EC)	0.59	$y = 7E\text{-}05x^2 - 0.1536x + 99.768$ ($R^2 = 0.7061$)	/
P 19	1.64	/	$y = 4.1311x^{0.437}$ ($R^2 = 0.9158$)
P 20	1.43	/	$y = 0.0059x^2 - 1.0844x + 82.753$ ($R^2 = 0.4955$)
P 21 (EGCG)	2.88	$y = -6E\text{-}07x^2 + 0.0154x - 58.408$ ($R^2 = 0.5718$)	$y = 1E\text{-}07x^2 - 0.0014x + 34.022$ ($R^2 = 7895$)
P 22	3.25	/	$y = -8E\text{-}05x^2 + 0.1672x - 48.574$ ($R^2 = 0.8011$)
P 23	3.49	$y = 0.152x^{0.741}$ ($R^2 = 0.8789$)	/
P 24	2.93	$y = -0.0007x^2 + 0.1823x + 29.955$ ($R^2 = 0.6201$)	/
P 25	1.72	/	$y = -0.0002x^2 + 0.2668x - 36.418$ ($R^2 = 0.5749$)
P 26	4.83	$y = 56.83e^{-0.003x}$ ($R^2 = 0.9165$)	$y = -8E\text{-}05x^2 + 0.0621x + 29.002$ ($R^2 = 0.4728$)
P 27	0.45	/	/
P 28	1.17	/	$y = 21.891e^{0.0007x}$ ($R^2 = 0.7363$)
P 29 (ECG)	5.05	$y = 2E\text{-}15x^{4.4037}$ ($R^2 = 0.9656$)	/
P 30	0.27	$y = 0.0063x^2 - 1.8049x + 144.93$ ($R^2 = 0.5858$)	$y = 0.0004x^2 - 0.0464x + 31.434$ ($R^2 = 0.6160$)
P 31	2.10	/	$y = -0.0009x^2 + 0.3216x + 10.56$ ($R^2 = 0.5442$)
P 32	5.36	$y = -0.0418x^2 + 5.301x - 126.42$ ($R^2 = 0.4359$)	$y = -0.0177x^2 + 2.2415x - 32.2$ ($R^2 = 0.5723$)
P 33	1.39	$y = -0.0879x^2 + 6.6326x - 37.59$ ($R^2 = 0.9359$)	/
P 34	4.57	$y = 0.0067x^{1.5448}$ ($R^2 = 0.9482$)	$y = -0.0007x^2 + 0.2827x + 11.078$ ($R^2 = 0.6604$)
P 35	0.29	/	/

Note: P, peak; * Influence weight coefficient after normalization (IWCN); EGCG, epigallocatechin gallate; EC, epicatechin gallate; ECG, epicatechin gallate; EGC, epigallocatechin catechin; C, catechin; CAF, caffeine. The "x" value in the table is calculated by the measured peak area instead, and the "y" value represents the glucose-lowering rate (%), or uric acid-lowering rate (%). R^2 represent the strength of co-relationship with "x" and "y"; when $0.0<R^2\leq0.2$, indicates very weak correlation or no correlation; $0.2 < R^2 \leq 0.4$, indicates weak correlation; $0.4 < R^2 \leq 0.6$, indicates moderate correlation; $0.6 < R^2 \leq 0.8$, indicates strong correlation; $0.8 < R^2 \leq 1.0$, indicates markedly strong correlation; The above final equation of correlation listed in table according to the highest level of R^2 value, which does not mean that there is only one equation of correlation.

actual administration dose range, the relationship between uric acid-lowering rate and EGCG, TPs, TEC and CAF/EGCG all showed a parabolic shape (binomial) with an opening upward type (Table 1, Figure 9) according to the common characteristics of its highest R^2 values.

Combined with the typical characteristics of parabola, the glucose-lowering effects of yellow tea does not require the highest or the lowest levels of phenolic substances contained in it, but there exist a relative optimal value, at which the glucose- and uric acid-lowering effect will be significantly enhanced. In addition, there may exist interaction effect between phenolic substances, which is worthy of further attention and research. This study lays a foundation for future research on multi-component interaction analysis.

In view of the important components that may play an important role in the glucose- and uric acid-lowering effect of yellow tea, the results of Pearson correlation analysis and multiple linear regression analysis are shown in Table 3-4 and Figure 10-11.

The multiple regression equation of glucose-lowering rate and C, CAF, EC, EGCG and ECG was obtained after removing of EGC, and equation was: $y = -231.99-340.63x_C + 206.32x_{CAF} + 118.10x_{EC}-16.04x_{EGCG} + 426.25x_{ECG}$ ($R^2 = 1.0000$) (Figure 10). Like the glucose-lowering effects, the multiple regression equation of uric acid-lowering rate and C, CAF, EC, EGCG and ECG was obtained after removing of EGC, and equation was: $y = 14.51+ 66.16x_C + 7.73x_{CAF} + 4.93x_{EC} + 16.55x_{EGCG}-14.69x_{ECG}$ ($R^2 = 1.0000$) (Figure 11).

Through regression analysis we find that the combined effects of C, CAF, EC, EGCG and ECG had a significant effect on the glucose- and uric acid-lowering effects, which worthy of further attention and research. Comprehensive research presents that phenolic substances plays an important and key role in glucose- and uric acid-lowering effects and its content levels in is closely related to the strength of biologic activity. Due to tea has many other functional components, and its key roles need to be further studied. Most importantly, whether our findings are a common phenomenon, further experimental studies based on multi-category, multi-variety and multi-batch are still needed.

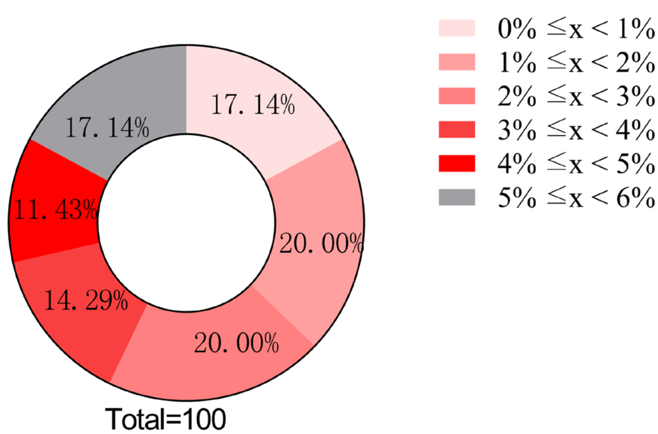


Figure 8. Sectional statistics of influence weight coefficients after normalization.

Table 3. Pearson correlation between glucose-lowering rate and main catechins and caffeine of different varieties and grades of yellow tea.

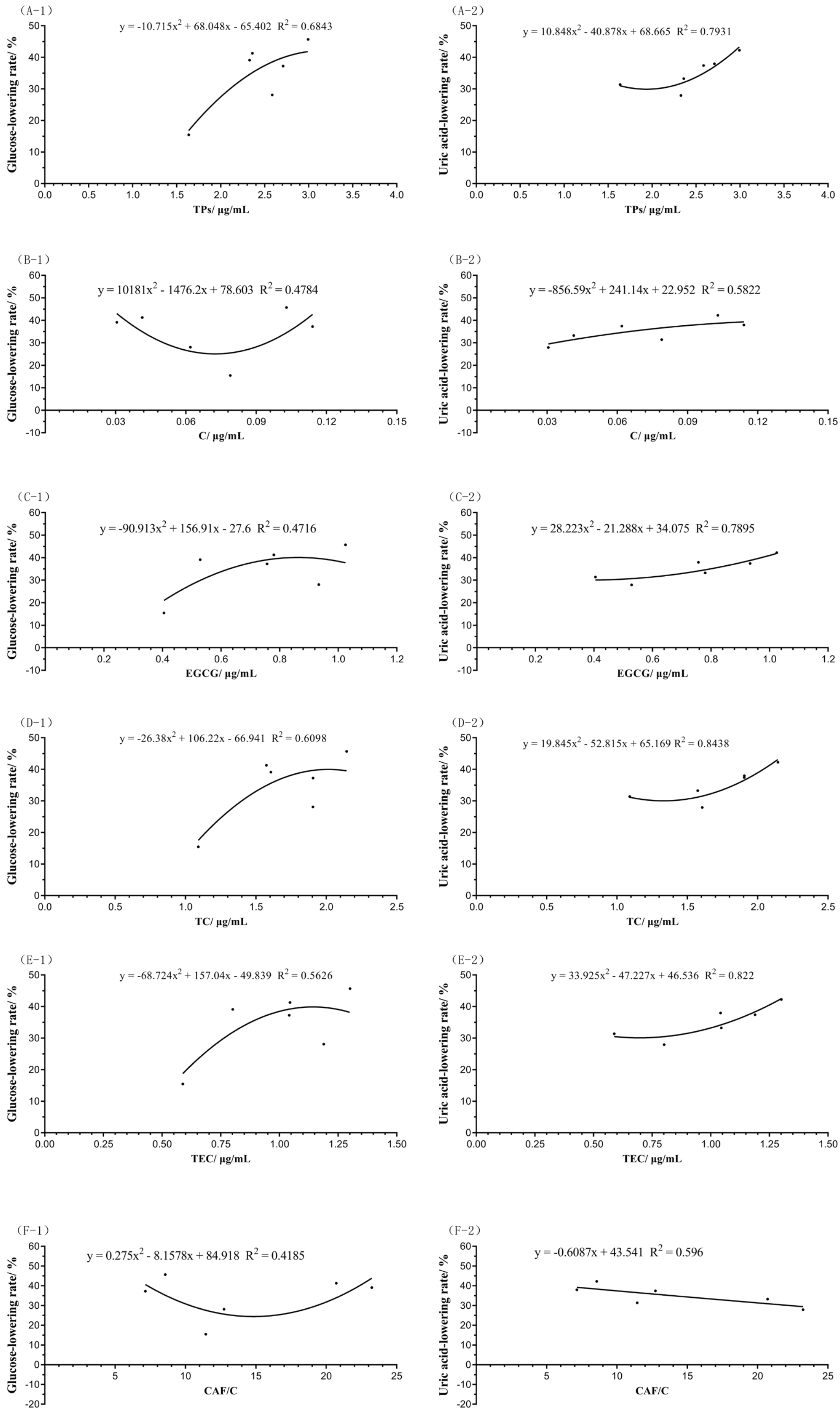
	GLR	EGC	C	CAF	EC	EGCG	ECG
GLR	1.0000	0.7850	-0.0030	-0.2490	0.3030	0.5930	0.9020
EGC	0.7850	1.0000	-0.0410	-0.7110	0.7050	0.4330	0.9020
C	-0.0030	-0.0410	1.0000	0.5170	0.4860	0.3260	0.0570
CAF	-0.2490	-0.7110	0.5170	1.0000	-0.4210	0.1000	-0.4800
EC	0.3030	0.7050	0.4860	-0.4210	1.0000	0.3050	0.4910
EGCG	0.5930	0.4330	0.3260	0.1000	0.3050	1.0000	0.6290
ECG	0.9020	0.9020	0.0570	-0.4800	0.4910	0.6290	1.0000

Note: GLR represent the glucose-lowering rate.

Table 4. Pearson correlation between uric acid-lowering rate and main catechins and caffeine of different varieties and grades of yellow tea.

	UALR	EGC	C	CAF	EC	EGCG	ECG
UALR	1.0000	0.1860	0.7530	0.4240	0.4080	0.8580	0.3750
EGC	0.1860	1.0000	-0.0410	-0.7110	0.7050	0.4330	0.9020
C	0.7530	-0.0410	1.0000	0.5170	0.4860	0.3260	0.0570
CAF	0.4240	-0.7110	0.5170	1.0000	-0.4210	0.1000	-0.4800
EC	0.4080	0.7050	0.4860	-0.4210	1.0000	0.3050	0.4910
EGCG	0.8580	0.4330	0.3260	0.1000	0.3050	1.0000	0.6290
ECG	0.3750	0.9020	0.0570	-0.4800	0.4910	0.6290	1.0000

Note: UALR represent the uric acid-lowering rate.


(A-1)
y = -10.715x2 + 68.048x - 65.402 R2 = 0.6843
Glucose-lowering rate/ %
TPs/ μg/mL
(A-2)
y = 10.848x2 - 40.878x + 68.665 R2 = 0.7931
Uric acid-lowering rate/ %
TPs/ μg/mL
(B-1)
y = 10181x2 - 1476.2x + 78.603 R2 = 0.4784
Glucose-lowering rate/ %
C/ μg/mL
(B-2)
y = -856.59x2 + 241.14x + 22.952 R2 = 0.5822
Uric acid-lowering rate/ %
C/ μg/mL
(C-1)
y = -90.913x2 + 156.91x - 27.6 R2 = 0.4716
Glucose-lowering rate/ %
EGCG/ μg/mL
(C-2)
y = 28.223x2 - 21.288x + 34.075 R2 = 0.7895
Uric acid-lowering rate/ %
EGCG/ μg/mL
(D-1)
y = -26.38x2 + 106.22x - 66.941 R2 = 0.6098
Glucose-lowering rate/ %
TC/ μg/mL
(D-2)
y = 19.845x2 - 52.815x + 65.169 R2 = 0.8438
Uric acid-lowering rate/ %
TC/ μg/mL
(E-1)
y = -68.724x2 + 157.04x - 49.839 R2 = 0.5626
Glucose-lowering rate/ %
TEC/ μg/mL
(E-2)
y = 33.925x2 - 47.227x + 46.536 R2 = 0.822
Uric acid-lowering rate/ %
TEC/ μg/mL
(F-1)
y = 0.275x2 - 8.1578x + 84.918 R2 = 0.4185
Glucose-lowering rate/ %
CAF/C
(F-2)
y = -0.6087x + 43.541 R2 = 0.596
Uric acid-lowering rate/ %
CAF/C

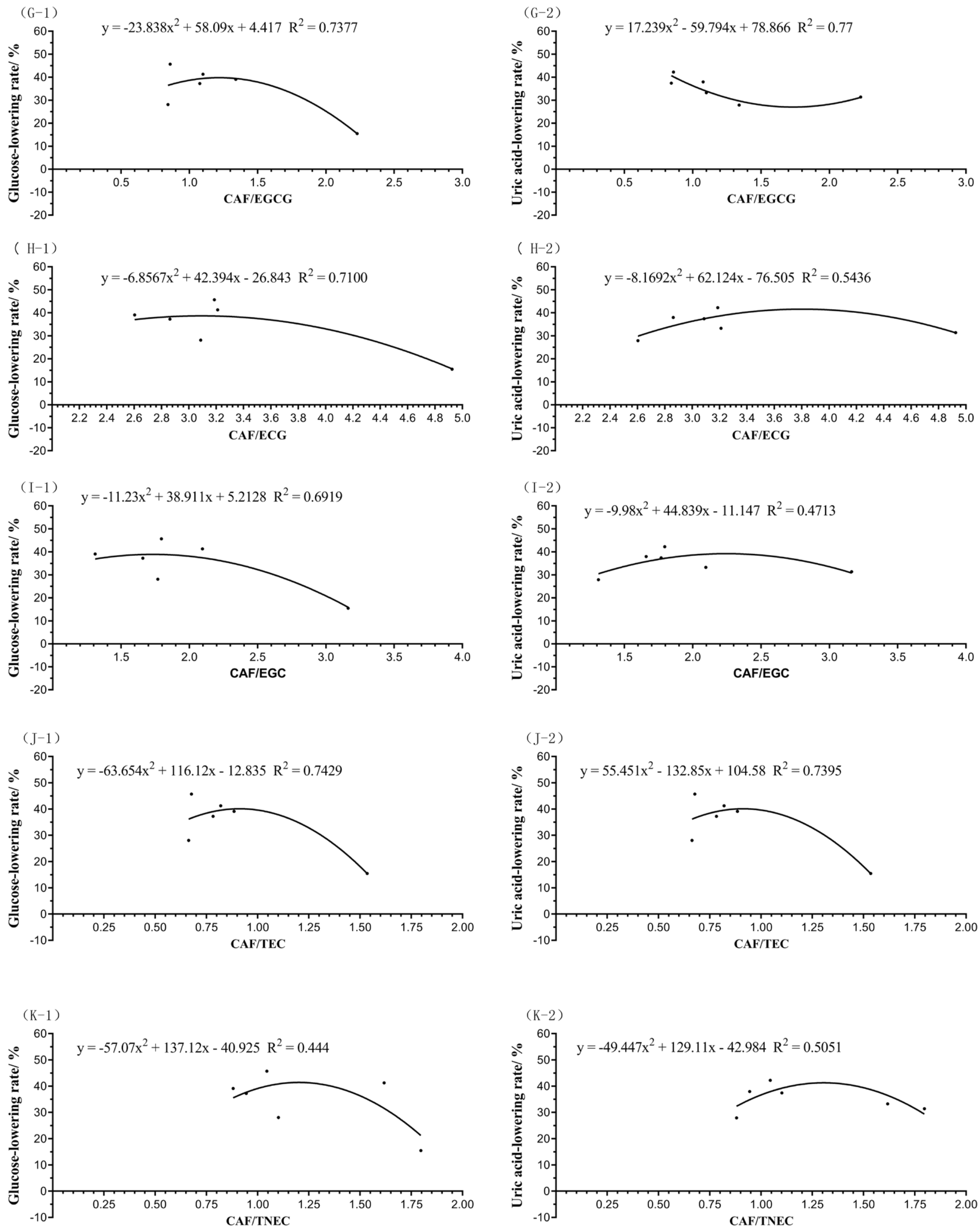


Figure 9. Comparative analysis of glucose- and uric acid-lowering effect of phenolic substances. Notes: EGCG, epigallocatechin gallate; EC, epicatechin gallate; ECG, epicatechin gallate; EGC, epigallocatechin catechin; C, catechin; CAF, caffeine; TC, total catechins, TC = EGC + C + EC + EGCG + ECG; TEC, total ester catechins, TEC = ECG + EGCG; TNEC, total non-ester catechins, TNEC = C + EGC + EC. A1-K1 shows the relationship between the glucose-lowering effects of single or combination components of TPs, C, EGCG, TC, TEC, CAF/C, CAF/EGCG, CAF/ECG, CAF/EGC, CAF/TEC and CAF/TNEC in yellow tea, respectively; A2-K2 shows the relationship between the uric acid-lowering effects of single or combination components of TPs, C, EGCG, TC, TEC, CAF/C, CAF/EGCG, CAF/ECG, CAF/EGC, CAF/TEC and CAF/TNEC in yellow tea, respectively.

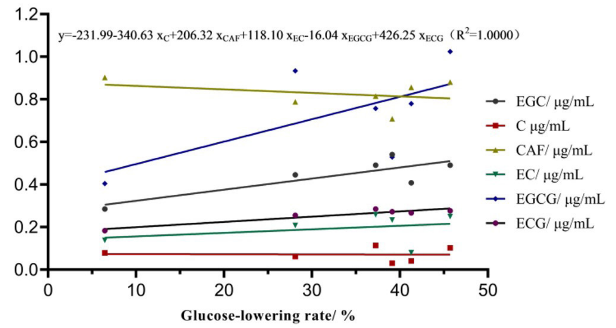


Figure 10. Multivariate linear relationship between glucose-lowering rate and major catechins and caffeine in different varieties and grades of yellow tea.

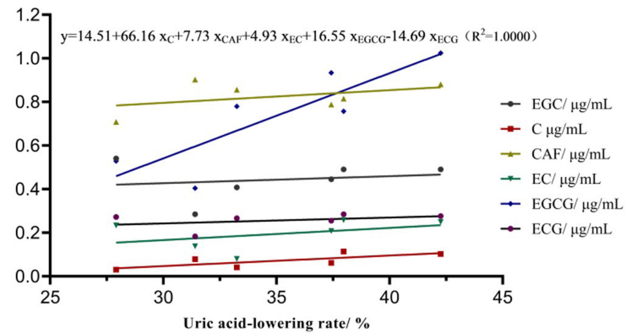


Figure 11. Multivariate linear relationship between uric acid-lowering rate and major catechins and caffeine in different varieties and grades of yellow tea.

4 Conclusions

Zebrafish model were chosen for evaluation and comparison the effects of yellow tea with different varieties and grades on glucose- and uric acid-lowering effects. Results showed that there were distinct differences in glucose- and uric acid-lowering effects of yellow tea with different varieties and grades after zebrafish evaluation. The glucose- and uric acid-lowering effects of population cultivar with all different grades were relatively better than the special early species. The glucose-lowering effects of bud tea and small tea was better than that of large tea. The uric acid-lowering effects of small tea is better than that of large tea and then better than that of bud tea.

Most importantly, the biological activity of glucose-and uric acid-lowering effects was significantly decreased by the targeted removal of phenolic substances in yellow tea solution by phenolic adsorbents-PVPP. Combined with the basic research of functional substances, the differences in glucose- and uric acid-lowering effects was closely related to the content levels of functional components in yellow tea solution (not the yellow tea), especially with the content levels of TPs. Further studies indicated that there were multiple regression relationships between the rate of glucose-lowering effects, uric acid-lowering effects and the content of functional phenolic substances in yellow tea through combined with the basic research of functional substances and correlation analysis, among which catechin and CAF had a significant correlation. However, results also revealed that the highest biological activity of yellow tea does not require the highest or the lowest content levels of each component the better, but depend on what is it. In order to reach the highest biological activity, the content levels of each functional component had a relative optimal value, which provides basic information for exploring the internal mechanism of yellow tea in lowering of glucose and uric acid levels.

At the same time, the research revealed that the glucose- and uric acid-lowering effects of yellow tea has a good multiple linear regression relationship with C, CAF, EC, EGCG and ECG, which is worthy of attention and research.

However, unlike the glucose-lowering effects, after a certain level of targeted removal of phenolic substances, the uric acid-lowering effects of yellow tea solution did not further decrease with the increase of the removal rate of TPs. This phenomenon was probably related with other functional components which plays key role in reducing uric acid of yellow tea. Since yellow tea contains a large number of active substances, in addition to TPs and its main catechins, there are also many unknown components with potential values of glucose- or uric acid-lowering effects. Our research team will continue to carry out in-depth research and discussion by making full use of the high-throughput and efficient technical advantages of zebrafish experimental technology.

Our findings showed that the physiological activity of yellow tea varied from its species and grades, the importance of specific functor element of different physiological activities were different, and the action of physiological activities of yellow tea owed to the result of multi-component interaction. Therefore, our results provided some basic information for variety and grade screening and utilizing based on their specific functional characters, and developing functional enhanced food and drinks.

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