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Antioxidant and Hypoglycemic Activity of Polysaccharide from Tea

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Abstract - Tea polysaccharide had high antioxidant activity and it could be used to cure diabetes. Antioxidant activity of tea polysaccharide (TPS) from three kinds of tea (green tea, oolong tea and black tea) were compared, the result indicated that oolong tea polysaccharide (OTPS) had the highest antioxidant activity. In order to explicate the mechanism of antioxidant and hypoglycemic activity, the streptozotocin (STZ)-induced diabetes mice model (DM) was established. The influence of OTPS on blood-glucose, content of MDA and NO, and activities of GSH-PX, SOD, NOS in serum, kidney and liver were investigated. The result showed that after four weeks injection of OTPS to DM mice, the blood-glucose of three treatment group reduced by 14.5%, 21.5% and 33.3%, respectively, comparing to the model control. The reduction effect of OTPS increased with the rise of dose. The activity of SOD and GSH-PX elevated significantly, while the activity of NOS decreased. The content of MDA and NO reduced significantly. The above results imply that antioxidant activity was enhanced. Comparing to XKW treatment, the effect of a dose of 300mg/(kg bw) OTPS was much better. The research showed that the OTPS had a significant effect on reducing blood glucose, and could enhance the antioxidant activity of DM mice.

Key words - Polysaccharide, Tea, Antioxidant, Activity, Diabetes, Blood glucose

Introduction

Tea is one of the most popular beverages in the world, especially in Asia and Pacific area. It is not only a beverage, but also an important part of the life. Tea consumption benefits health, since there are many kinds of functional component in tea extract, such as tea polyphenol, tea polysaccharide, etc. In recent years, polysaccharide is getting more and more attention of scientists from different countries. Some experimental have proved that polysaccharide are effective antioxidants and has pharmacological activities, such as reducing blood glucose, anti-cancer, anti-tumor and immunomodulatory function. Tea has traditionally been used to cure diabetes in East Asia, especially in China and Japan (Chen and Xie, 2001). In recent years, scientists have made a great advance in Pharmacological and chemical studies. Tea polysaccharide was one of the main components related to the hypoglycemic activity, it also have immunological, anti-radiation, anti-blood coagulation, anti-cancer, and anti-HIV activities (Isiguki, Takakuwa and Takeo, 1992; Wang *et al.*, 2000; Wang and Wang, 1992; Zhou, Ding, Wang and Xie, 1997).

Diabetes is one of the main diseases that threatened the health of human beings. As the development of society, the living level was elevated. The ratio of diabetes increased rapidly due to the changing of lifestyle. The number of diabetic was more than 100 million according to the orresponding authoE-mail address: nidj@mail.hazu.edu.cnstatistical data from WHO. Diabetes is a kind of chronic and lifelong disease, which can't be cured radically. At present, the main method of curing diabetes is still medication. Because of the side-effect of synthesized medicament, scientists are trying hard to find new natural medicine to cure this disease. Since 1987, Japanese scientist presented that TPS was the pharmacological component of tea in curing diabetes, Japanese and Chinese scholars have been studying on its bioactivity, physicochemical property and glycosyl component. (TPS has been reported to have hypoglycemic, antilipemic, antioxidant, and anti-radiation activity. The hypoglycemic activity was the focus of TPS activity studies.) Japanese scientist researched the TPS extracted with cold water, warm water and boiling water, the results showed that the hypoglycemic activity of the TPS from cold water was better. The hypoglycemic activity decreased when the temperature rose. TPS separated from green tea and chromatograph with synthetic resin HP_2MG had been reported by Tadakazu Takeo to be effective in hypoglycemic activity. The effects of green tea TPS on the high blood glucose of alloxan-induced mice model and streptozotion-induced mice model were studied. It showed that TPS could reduce the blood glucose significantly. TPS from green tea with different solvent was applied to assay the effect of reducing blood glucose, and the TPS that precipitated with acetone was the best. The blood

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glucose of DM mice was reduced by neutral polysaccharide (NTPS) and acidic polysaccharide (ATPS) with molecular mass less than 3×10^4, which were separated from green tea with D315 macroporous resin. Green tea TPS could prevent the alloxan-induced high blood glucose and improve the glucose tolerance.

Free radicals and active oxygen are important molecules, which can induce oxidant damage. The role of free radicals and active oxygen is becoming increasingly recognized in pathogenesis of many human diseases, including cancer, aging, and atherosclerosis (Perry, 2000). Until but now the studies on antioxidant activity of TPS have mainly focused on the assay of activity, the mechanism of activity has not yet been well explicated. The antioxidation mechanism of TPS requires further research, and the correlation between antioxidant activity and hypoglycemic activity of TPS is remain obscure, which need more experiment to clarify.

Most of above the reference of the TPS pharmacological activities were based on green tea. There was little report about other kinds of tea. TPS from three representative kinds of tea in China, including green tea, black tea and Oolong tea were applied to DM mice. The result showed that Oolong tea and black tea were better in hyperglycemic activity than green tea. TPS were extracted from green tea, Oolong tea and black tea made of the same kind of tea-leaves. TPS from oolong tea (OTPS) have the highest hyperglycemic and antioxidant activity, the TPS activity from black tea was higher than that from green tea. It indicated that the TPS activity of different kinds of tea was not the same. We mainly took Oolong tea as material in this paper for further study, after OTPS preparation, the antioxidant activity and the blood glucose of DM rats were investigated.

Materials and Methods

Materials

Tea: Three kinds of tea (green tea, oolong tea and black tea) were collected from three provinces of China (Hubei, Fujian and Yunnan), respectively.

Wistar rat (200±20g): clean grade, male, were obtained from Laboratory animal center of Shanghai Medical University.

STZ, blood glucose reagent kit was purchased from Sigma Chemical Co. Eliminated-thirst pill (XKW) was purchased from Guangzhou the first Chinese Medicine factory.

SOD, GSH-PX, MDA, NOS, NO reagent kits were purchased from Biological Engineering Institute (Nanjing, China).

All other chemicals used were of analytical purity.

Preparation of TPS

TPS was prepared as described by Ni De jiang (2003).

Assay for the scavenging effect on hydroxyl radicals

The deoxyribose method for determining the rate of reaction of hydroxyl radical with antioxidant was performed, as described by Barry H, John MC, Okezie IA (1987). Reaction mixture in a final volume of 1.0ml contained deoxyribose (60mM), KH_2PO_4–KOH buffer (pH 7.4, 20mM), $FeCl_3$ (100μM), EDTA (100μM), H_2O_2 (1mM) and ascorbate acid (100μM). Solutions of $FeCl_3$ and ascorbate acid were prepared immediately before use. After incubation at 37℃ for 1 h, the color was developed by adding 1ml of 1% thiobarbituric acid (TBA) (w/v) and 1ml of 25% (v/v) HCl, which was then heated in a boiling water bath for 15 min. The absorbance of the resulting solution was measured at 532nm.

Assay for the scavenging effect on superoxide radicals

Xanthine-oxidation method was performed as described by Beauchamp C, Fridovich (1971).

Establishment of DM rat model

The rats were acclimatized to the laboratory condition for 3 days, and then fasted for 24 hours. STZ at a dose of 58mg/(kg.bw) was injected into the rats at the left abdominal cavity. STZ was newly prepared in 0.1 mol/L citrate buffer, pH 4.5, cooled by ice. The urine of mice was detected three days after injection. The content of mice blood glucose was determined by blood glucose meter. If the glucose in urine was electropositive and the value of BG was above 17.6mmol/ L, then the mouse was a DM model.

Experimental design

One normal mice group was set up, DM mice were divided into five groups: diabetic mice model, eliminated-thirst pill (XKW) treated group and three level doses of OTPS treated group. While the DM mice group were injected the same volume of physiological saline solution, the XKW treated mice were injected eliminated-thirst pills at a dose of 1.56-3.13 pills/(kg.bw). OTPS treated rats were divided into low dose group, middle dose group and high dose group, the doses per day were 50mg/(kg.bw), 100mg/(kg.bw) , and 200mg/(kg. bw), respectively. The experiment continued for 30 days.

Blood glucose assay

Blood glucose reagent kit

Table 1. Scavenging rate of -OH and O^{2-}- by TPS from three kinds of tea

	Fujian province			*Hubei province*			**Yunnan province**		
	Green tea	Oolong tea	Black tea	Green tea	Oolong tea	Black tea	Green tea	Oolong tea	Black tea
Scavenging rate of -OH(%)	51.69	56.72	31.75	59.02	59.35	29.22	26.37	30.34	16.43
Scavenging rate of O^{2-}(%)	8.45	9.41	5.38	7.92	8.80	7.49	7.40	8.65	6.30

Antioxidant activity assay

SOD activity: xanthine oxidase assay;

GSH-PX: enzymatic method;

MDA content: TBA colorimetry;

NO: nitric acid reductase method;

NOS: colorimetry.

Statistical treatment of data

All the data was analyzed by SPSS (10.0) software, and were expressed as means ± standard, the Student's test was introduced with the average of two specimen.

Results and Discussion

Scavenging effect on hydroxyl radical and superoxide radical *in vitro*

As shown in Table 1, TPS from all three kinds of tea have significant scavenging effect on hydroxyl radical and superoxide. There were some differences between the cultivar and producing area of the tea. The effect of TPS extracted from semi-fermented Oolong tea and non-fermented green tea was better than that of fermented black tea. Oolong tea, especially from Hubei province, have the highest scavenging rate of -OH and O^{2-}, which 59.35% and 8.80%, respectively. The black tea had the lowest scavenging ability was. The scavenging ability of TPS from Yunnan province was lower than the two others.

Effect on the blood glucose of DM mice

The effect of OTPS on the blood glucose of STZ-induced DM rats was shown in Table 2. The BG values of 50mg/(kg.bw) OTPS treated group, 100mg/(kg.bw) treated group, and 200mg/(kg.bw) treated group were 20.97±2.64mMol/L, 21.08±4.41mMol/L and 17.64±5.61mMol/L and the blood glucose concentration decreased by 14.5%, 21.5% and 33.5%, respectively, compared to the DM rat model group. The OTPS reduced the blood glucose of hyperglycemic mice significantly. There was a dose effect relationship. Anti-thirst pill (XKW) containing glyburide was combination of Chinese and western medicines, of which the blood glucose reduction ratio was 24.2%. It is more effective than the doses of 50mg/(kg.bw) and 100mg/(kg.bw) OTPS treatment, but less effective than a dose of 200mg/(kg.bw) OTPS treatment.

Table 2. Effect of 0TPS on BG level of diabetic mice

Group	Dose(mg/kg.bw)	N	**BG(mmol/L)**
Control		8	5.07±0.75
DM		8	26.86±1.63
DM+XKW		8	20.36±4.63a
DM+OTPS	50	8	22.97±2.64a
	100	8	21.08±4.41b
	200	8	17.64±5.61a

Note: compared with DM, a: $p<0.01$, b: $p<0.05$.

Effect of OTPS on SOD, GSH-PX, NOS activity and MDA, NO content in serum of mice

Compared to the normal rats group, the activity of serum SOD, GSH-PX of DM rats decreased markedly, as shown in Table 3. The GSH-PX and SOD activity could be enhanced by XKW and OTPS. The content of MAD was a reflection of the internal lipid peroxidation extent. The analysis of serum MDA content showed that the content in DM mice was 10.88 ±1.34nmol/ml, that was much higher than that in normal rats group with the MDA concentration of 4.98 ± 0.41nmol/ml. After injection of XKW or OTPS, the content of MDA decreased. The OTPS was more effective than XKW, and there was a dose effect relationship.

NO, which was produced from L-arginine catalyzed by NOS, play physiological and pharmacological dual roles in the body. It was very important in the programmed cell death of thymocyte cell and macrophage and immune system signal transduction. The exception of NO had a close relationship with diabetes. From Table 4, the serum NO content and NOS activity of STZ-induced DM mice were 72.24 ±10.99 umol/ L and 23.83±1.75 U/ml, respectively, which was markedly higher than that of normal mice at a level of 18.07± 5.48 umol/ L and 10.75±0.80 U/ml. It indicated that antioxidant activity increased after XKW and OTPS treatment. To our surprise, the DM mice of the dose of 200mg/(kg.bw) treated group could recover to the normal level completely. It suggested that the enhancement of immunity was of beneficial to the recovery .

Table 3. Effect of TPS from Oolong tea on GSH-PX, SOD activity and MDA content in serum

Group	Dose(mg/kg.bw)	N	GSH-PX(NU/ml)	SOD(NU/ml)	**MDA(nmol/ml)**
Control		8	174.34±2.68	355.66±11.82	4.98±0.41
DM		8	156.11±8.06	238.82±18.36	10.88±1.34
DM+XKW		8	165.97±3.27e	292.44±29.62a	9.48±1.47
DM+OTPS	50	8	166.08±3.33a	315.28±14.51a	8.29±1.47a
	100	8	163.81±2.24e	295.09±15.72ab	8.75±0.93a
	200	8	171.76±5.46cf	338.61±23.51acd	6.92±0.74ad

Note: Compared with DM, a: $p<0.01$, e: $p<0.05$; compared with OTPS50, b: $p<0.01$; c: $p<0.05$; compared with OTPS100, d: $p<0.01$, f: $p<0.05$.

Table 4. Effect of TPS from Oolong tea on NOS activity and NO content in serum

Group	Dose(mg/kg.bw)	N	NO(μmol/ml)	**NOS(U/ml)**
Control		8	18.07±5.48	10.75±0.80
DM		8	72.42±10.99	23.83±1.75
DM+XKW		8	34.78±13.11ae	12.02±0.95a
DM+OTPS	50	8	45.52±17.74b	12.32±1.83a
	100	8	59.65±19.16	12.34±1.71a
	200	8	18.07±6.75acd	10.70±0.89a

Note: Compared with DM, a: $p<0.01$, b: $p<0.05$; compared with OTPS50, c: $p<0.01$; compared with OTPS100, d: $p<0.01$, e: $p<0.05$.

Table 5. Effect of TPS from Oolong tea on GSH-PX, SOD activity and MDA content in kidney

Group	Dose(mg/kg.bw)	N	GSH-PX(NU/mgprot)	SOD(NU/mgprot)	**MDA(nmol/mgprot)**
Control		8	20.50±0.93	540.85±76.62	2.62±0.66
DM		8	15.49±0.94	477.83±18.09	4.21±0.51
DM+XKW		8	18.63±1.30a	557.33±33.17a	3.43±0.55e
DM+OTPS	50	8	21.33±0.47ad	531.58±34.59a	2.99±0.3a
	100	8	19.92±0.85ac	647.38±29.58acd	3.38±0.45c
	200	8	22.27±1.44abd	570.47±31.45ab	2.63±0.49abf

Note: Compared with DM, a: $p<0.01$, e: $p<0.05$; compared with XKW, d: $p<0.01$, f: $p<0.05$; compared with OTPS50, c: $p<0.01$; compared with OTPS100, b: $p<0.01$.

Table 6. Effect of TPS from Oolong tea on NOS activity and NO content in kidney

Group	Dose(mg/kg.bw)	N	NO(μmol/ml)	**NOS(U/ml)**
Control		8	0.84±0.35	0.44±0.11
DM		8	1.29±0.33	0.89±0.071
DM+XKW		8	0.95±0.33	0.52±0.092ac
DM+OTPS	50	8	0.91±0.37	0.32±0.068a
	100	8	0.81±0.24a	0.49±0.087ac
	200	8	1.00±0.29b	0.71±0.086acd

Note: Compared with DM, a: $p<0.01$; compared with OTPS50, c: $p<0.01$; compared with OTPS100, b: $p<0.05$, d: $p<0.01$.

Effect of OTPS on SOD, GSH-PX, NOS activity and MDA, NO content in kidney of mice

As shown in Table 5, the kidney MDA content of DM mice was 4.21±0.51nmol/mgprot, it was higher than that of normal mice group which had a content of 2.62±0.66nmol/mgprot. After injection of XKW or OTPS, the content of MDA decreased, the OTPS was more effective than XKW, but the dose effect relationship was not remarkable. The GSH-PX and SOD activity of treat group mice were all enhanced by the

Table 7. Effect of TPS from Oolong tea on GSH-PX, SOD activity and MDA content in liver

Group	Dose(mg/kg.bw)	N	GSH-PX(NU/mgprot)	SOD(NU/mgprot)	**MDA(nmol/mgprot)**
Control		8	116.70±4.53	355.14±14.34	1.10±0.18
DM		8	78.30±7.58	302.59±18.06	2.55±0.49
DM+XKW		8	91.54±8.13a	321.71±8.12a	1.45±0.35a
DM+OTPS	50	8	93.60±5.99a	325.79±10.62a	1.24±0.13a
	100	8	139.15±5.26abd	377.44±14.55abd	1.69±0.15ab
	200	8	100.29±4.23acef	349.02±11.26abcd	1.20±0.18ac

Note: Compared with DM, a: $p<0.01$; compared with XKW, d: $p<0.01$, e: $p<0.05$; compared with OTPS50, b: $p<0.01$, f: $p<0.05$; compared with OTPS100, c: $p<0.01$.

Table 8. effect of TPS from Oolong tea on NOS activity and NO content in liver

Group	Dose(mg/kg.bw)	N	NO(μmol/ml)	**NOS(U/ml)**
Control		8	0.99±0.23	0.91±0.12
DM		8	1.76±0.34	1.37±0.13
DM+XKW		8	1.36±0.39be	1.04±0.15ad
DM+OTPS	50	8	1.45±0.29b	1.06±0.11a
	100	8	1.03±0.26ac	1.06±0.12a
	200	8	1.49±0.12bd	0.75±0.15adf

Note: Compared with DM, a: $p<0.01$; compared with OTPS50, c: $p<0.01$; compared with OTPS100, b: $p<0.05$, d: $p<0.01$.

TPS, they were markedly higher than that of DM mice at a level of 15.49±0.94 NU/mgprot and 477.83±18.09NU/mgprot.

As shown in Table 6, both the kidney NO content and NOS activity of STZ-induced DM mice increased with the value of 1.29±0.33 umol/ L and 0.89±0.071U/ml, respectively, that was markedly higher than that of normal mice at a level of 0.84±0.35umol/ L and 0.44±0.11U/ml. The result indicated that antioxidant activity increased after XKW and OTPS treatment, NO content in kidney and NOS activity decreased markedly, especially that the DM mice treated with 100mg/(kg.bw) OTPS could reached normal level.

Effect of OTPS on SOD, GSH-PX, NOS activity and MDA, NO content in liver of mice

After injection of XKW or OTPS, as shown in Table 7, the content of MDA in liver decreased, the content of normal mice group was 1.10±0.18nmol/mgprot, lower than that of DM mice, which had a content of 2.55±0.49nmol/mgprot. OTPS was more effective than XKW at 50 and 100mg/(kg.bw), but the dose effect relationship was not remarkable. The GSH-PX and SOD activity of treated group mice were all enhanced by the TPS, markedly higher than that of DM mice with a level of 15.49±0.94 NU/mgprot and 477.83±18.09NU/mgprot.

As shown in Table 8, both the kidney NO content and NOS activity of STZ-induced DM mice increased, they were 1.76±0.34 umol/ L and 1.37±0.13U/ml, respectively, and markedly higher than that of normal mice at a level of 0.99±0.23umol/L and 0.91±0.12U/ml. The result indicated that antioxidant activity increased after XKW and OTPS treatment, NO content in kidney and NOS activity decreased, which the dose of 100mg/(kg.bw) was the most effective.

Results

Polysaccharides from tea had high antioxidant activity. TPS antioxidant activities were different between the cultivar and producing area of the tea. The effect of TPS extracted from semi-fermented Oolong tea and non-fermented green tea was better than that of fermented black tea and OTPS have the highest antioxidant activity. TPS have remarkable effective on curing diabetes, and its antioxidant activity was related to the mechanism of hypoglycemic activity tightly.

Discussion

Free radicals were produced by organic molecule through the homolysis of radical-mediated chain reaction, with atoms, radicals, molecules or ions. Generally, there was a dynamic balance between production, usage and scavenging of the free radicals, and it was mainly related to a variety of antioxidant defensive system, including

enzyme and non-enzyme antioxidant. The antioxidant enzymes were mainly SOD, GSH-PX, CAT and other hemoglobin peroxidase. The main function of SOD is scavenging O_2^- and turning it into H_2O_2, the latter can be turned into water with the help of CAT and GSH-PX. GSH-PX can enhance the reaction between H_2O_2 and reductive GSH, where the reaction product were water and oxidation-type glutathione (GSSG). If the activity of antioxidant in organism decreased, the overabundance free radicals would attack the multi-unsaturated fatty acid on the biomembrane, leading to lipid peroxidation. Then the anti-damage ability and stability of biomembrane would decrease and the flow ability and integrity of the biomembrane were damaged, which would destroy the function and structure of biomembrane, thereby, lead to a series of diseases. The study indicated that the antioxidant activity of STZ-induced mice decreased, MDA content increased, and the antioxidant ability of organism decreased, the activity of SOD and GSH-PX in serum of DM mice decreased obviously, while the MDA content increased, as shown in the present experiment. After OTPS treatment, the activity of SOD and GSH-PX increased, and the MDA content decreased markedly. It suggested that OTPS could enhance the antioxidant function of DM mice organism, and inhibit the lipid peroxidantion.

NO is a new kind of endocellular messenger molecule, participating in the physiological and pharmacological modulation of cardiovascular, nerve and immune system. It could relax the vessel, reduce the blood pressure, and prevent the reproduction of smooth muscle cell and thrombocyte paste. But excess NO leads to cell toxicity, the nitration of cell nucleus and destruction of the DNA structure, activity reduction of some key enzymes related to the programmed cell death and DNA reproduction. NO plays an important role in development of diabetes and nephrosis, engaging in the high filtration, blood vessel permeability enhancement and acceleration of glomerulus hardening. In the present experiment, the increase of NOS activity and NO content in serum of DM mice might aggravate the occurrence of diabetes complication as well as engage in the damage mechanism of pancreatic islet β-cell. After the treatment with OTPS, the NOS activity and NO content were decreased. Organism tissues were protected.

Diabetes is a kind of common endocrine metabolic disease, which has a characteristic that eating and drinking a lot, excreting a lot while losing weight, it always accompanying the functional diminishing of organs like kidney, liver, spleen etc. In the other morphologic experiment, the liver cell swelling and renal glomerulus enlargement was a clue of diabetes and nephrosis. In the experiment, the weight of liver and kidney of DM mice and the ratio of organ / weight increased. After OTPS treatment, the DM mice grow well and regained weight gradually, moreover, the hepatocyte and glomerulus enlargement were controlled. The immunohistochemical and the programmed death of cell also were studied in the research, the result indicated that in the liver the expression strength of bcl-2 that prevented the cell death increased markedly, and the expression strength of bax gene that enhanced the cell death faded decreased, the cell number of death was reduced (in other report). From above, OTPS was effective on curing diabetes.

There were several main mechanisms for polysaccharides to act on the glucose level; to decrease the content of MDA, NO; or to influence the activities of SOD, GSH-PX, NOS. STZ was a chemical that can cause oxidative damage. Therefore, the activity of antioxidant (in serum, kidney and liver) decreases after the injection of STZ. It was found that TPS could decrease blood glucose and increase the antioxidant activity in a similar manner, which suggests that antioxidant activity of TPS is likely to be one of the mechanisms of hypoglycemic activity.

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