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Original Article

Antioxidant, Stimulating Telomerase and Anxiolytic Activities of Polysaccharide Isolated from *Sargentodoxa cuneata*

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ABSTRACT

Background: The classic anxiolytics have adverse effects in the treating anxiety disorders. **Aim:** To identify the anxiolytic polysaccharide from *Sargentodoxa cuneata.* **Materials and Methods:** The water-soluble Polysaccharide Fraction (PSC) was purified from *S. cuneata*, as well as its subfractions PSCA-1 and PSCB-1. The antioxidant effect of PSC against rotenone induced PC12 cell excitotoxicity, stimulating telomerase effect in HEK293T cell and anxiolytic effect on zebrafish were evaluated. **Results:** The pretreatment of PC12 with PSC obviously increased cell survival and SOD level. PSC also down-regulated the LDH release, ROS level and cell apoptosis rate. PSC promoted the 293T cell proliferation, associated with the mechanism of stimulating telomerase. Additionally, PSC alleviated the anxiety level in zebrafish induced by mCPP. **Conclusion:** These findings suggested that the PSC could possess the anxiolytic potential via antioxidant and telomerase activation.

Keywords: *Sargentodoxa cuneata*, Polysaccharide, Antioxidant, Telomerase, Anxiety.

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INTRODUCTION

Anxiety disorder is the most common mental disease in the world, which often lead to memory and learning impairment, endocrine disorders and social withdrawal of patients. At present, the common anxiolytics used clinically include benzodiazepines, Selective 5-HT Reuptake Inhibitors (SSRIs), and 5-HT and Norepinephrine Reuptake Inhibitors (SNRIs). These medicine have shown some efficacy in the treating anxiety disorders, yet a long-term use of these medications easily lead to apparent side effects such as dizziness, headache, gastrointestinal dysfunction, drug resistance and rebound. Therefore, it is very necessary to develop more efficient and safe alternatives based on the mechanisms including but not limited to the neurotransmitter reuptake.[1,2]

The causes of anxiety disorders are not completely clear, however, emerging research involved in oxidative stress in their development.[3,4] A clinical study revealed that there was a significantly increased levels of lipid peroxidation in patients with severe anxiety,[3] while an oxidative stress regulator guanidinoacetate, alleviated anxiety-like behavior by enhancing GPX4 activity. Conversely, the deletion of the GPX4 gene in dopaminergic neurons increased anxiety behaviors.[4] A phenolic compound rosmarinic acid is effective against several neuropsychiatric disorders, including anxiety and depression, through its powerful antioxidant and anti-inflammatory properties.[5]

The accumulation of toxic oxidised proteins in the brain, which can cause severe neuronal damage, has actually been proposed as a potential factor in the onset and progression of anxiety and other several psychiatric disorders. Thus, oxidative stress could be key to the molecular mechanisms involved in emotional disorders, and may also be the alternative target of treating the anxiety. Considering the regulation of antioxidant defences through the Nrf2 pathway, this factor has emerged as a promising approach for neuroprotection, Nrf2 clearly has vital functions in various physiological and pathological stresses, and it has been implicated as a causative factor in anxiety disorder. Though lots of evidence indicates that oxidative stress is the cause of anxiety, the relationship between oxidative stress and anxiety is not unidirectional cause and effect link, while it is complex.[6]

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And there was an association between telomere, telomerase and mental risk. Accelerated telomere shortening and decreased telomerase activity has also been reported in response to anxiety, depression.[7,8] Patients with major depressive disorder and anxiety appear to have shorter leukocyte telomeres compared to controls.[9] A comprehensive analysis of UK Biobank data supported this viewpoint, and suggested the telomere length as a predictive tool for identifying individuals at risk of suffering from anxiety. Furthermore, this analysis found that telomere shortening occurred prior to the onset of anxiety disorders.[10]

TERT-deficient mice displayed significantly higher anxiety-like behaviors. Consistently, deficiency of telomerase resulted in increased anxiety-like behavior in aged transgenic mice. This meant that the telomerase play an important role in the mood disorders.[7,11]

Exploration of new drugs for anxiety treatment is a major concern worldwide. Medicinal plants, such as passionflower, valerian and kava, are being used as a potential option for treating anxiety disorders.[12] Lots of research has been conducted with cell or animal models to screen phytochemicals like alkaloids, flavonoids, lignans, terpenes and saponins possess anxiolytic effects.[13]

In the last few years, our lab focused on the bioactive constituents from the anti-inflammatory herb known as *S. cuneata*.[14,15] *S. cuneata*, a plant endemic to China, is used in traditional Chinese medicine or ethnic medicine (called as *Hongteng*) for treatment of the arthritis, acute appendicitis, amenorrhea and painful menstruation.[16] The *S. cuneata* extracts have been investigated to exhibit anti-inflammatory, immune regulating and antioxidant effects.[15,17,18] However, no attention has been paid to the neuroprotective and anxiolytic activities of *S. cuneata*. Therefore, this investigation was carried out to study the anxiolytic effects and related mechanism of PSC isolated from *S. cuneata* for the first time, to evaluate its potential as a therapeutic agent for mental disorders. The results of this study could provide a scientific basis for finding anxiolytic constituents from herbs and further understanding the related mechanism and clinic effects as regulating mental agents.

MATERIALS AND METHODS

Ethics approval and consent to participate

Adult wild-type zebrafish (AB strains, 6 months-old) were purchased from Shanghai Fish Bio Co. Ltd., (Shanghai, China). All procedures were approved by Bozhou University ethical committee and performed strictly according to the guidelines set for the usage of animals by this committee.

Materials and reagents

The purchased plant pieces from Bozhou Medicinal Herb Market, China, were authenticated as the stems of *Sargentodoxa cuneata* (Sargentodoxaceae, previously attributed to Lardizabalaceae) by Prof. Zhen Ouyang from School of Pharmacy, Jiangsu University, where a voucher specimen (HT1012) has been deposited. High-glucose DMEM, FBS, trypsin-EDTA and PBS were obtained from Gibco Industries Inc. (Grand Island, NY, USA). MTT, rotenone were purchased from Sigma-Aldrich Company (Missouri, USA). SOD, LDH and DCFH-DA ROS assay kit were purchased from Beyotime Institute of Biotechnology (Nanjing, China). TRAPeze® telomerase detection kit was from Millipore (Boston, USA). PC12 cell line was obtained from School of Medicine, Jiangsu University, China, and HEK293T cell line was from Jarstfer's lab in Eshelman School of Pharmacy, UNC Chapel Hill, USA. Sephadex G-100 was purchased from GE Healthcare (Waukesha, WI, USA). mCPP was obtained from Shanghai Titan Technology (Shanghai, China). Zebrafish gABA ELISA Kit was from ELK Biotechnology (Wuhan, China). All other chemicals and reagents used were of analytical grade.

Preparation of *S. cuneata* polysaccharides

According to the procedures described in our previously published article,[15] PSC and its subfractions PSCA-1 and PSCB-1 were prepared. Simply, *S. cuneata* stem was extracted with distilled water, followed by ethanol precipitation. The precipitate was purified by HP-20 macroporous resin column, deproteinized and dialysis to obtain the PSC. The PSC was subjected to a DEAE-cellulose column and Sephadex-G100, to obtain two polysaccharide subfractions PSCA-1 and PSCB-1. They were stored at -20°C until used.

Content of polysaccharides

The phenol-sulfuric acid method is used to determine the content of polysaccharides, and mixture was pipetted into a 96-well plate with three replicates, 200 µL each well. The absorbance was detected with a microplate reader (Molecular Devices, USA) at wavelength of 492 nm.

FT-IR spectral analysis

One mg of PSCA-1 and PSCB-1 was separately ground with KBr and pressed into a pellet. The FT-IR spectra were analyzed on a Perkin-Elmer spectrometer from 4000 to 500 cm^{-1}.

PC12 cell culture and viability assay

According to the method previously described.[15] PC12 cells were incubated in DMEM media containing 10% (v/v) FBS, 100 U/mL penicillin and 100 µg/mL streptomycin, at pH 7.2-7.4, 5% CO_2 and 37°C. Cells in the logarithmic growth phase were chosen for further experimental procedure.

Cell viability was determined using MTT assay. Briefly, PC12 cells at the density of 1×10^5 cells/mL were seeded into 96-well plate, and incubated for 24 hr. After washed with PBS, the cells were incubated with PSC, PCPA-1 and PCPB-1 (0.1, 1, 10 and 100 µg/

mL, respectively) for an additional 24 hr. After treatment, each well was added a total of 20 µL of MTT solution (5 mg/mL) and the plates were incubated at 37°C for more 3 hr. After carefully sucking up the media, DMSO (100 µL/well) was added to dissolve the resulted formazan into solution. The absorbance at 570 nm was measured with a microplate reader.

Effect of PSC, PSCA-1 and PSCB-1 on the PC12 cells damaged by rotenone

The MTT colorimetric assay was used to test the neuroprotection of polysaccharides on PC12 cells against the damage by rotenone. Briefly, PC12 cells were seeded in 96-well plates containing 2×10^4 cells/well and randomly assigned to an experimental and a control condition with or without 10 µM rotenone. The cells were pre-treated with PSC, PCPA-1 and PCPB-1 (0, 1, 10 and 100 µg/mL, respectively) for 5 hr and exposed to rotenone (10 µM) for more 24 hr at 5% CO_2 and 37°C. After treatment, 20 µL of MTT reagent was added to each well and the plates were incubated for an additional 3 hr. After sucking up the media, 100 µL of DMSO was added to dissolve the resulted formazan into purple solution. The absorbance at 570 nm was measured on a microplate reader.

LDH release determination in rotenone-induced PC12 cells

LDH activity was determined according to the manufacturer's instruction in the assay kit. In brief, after incubation with PSC and rotenone for 24 hr, PC12 cells were lysed with 1×lysis solution for 60 min at 37°C. The supernatant was incubated for 30 min in the dark at room temperature, with substrate in buffer contained in the LDH activity assay kit. The absorbance at 490 nm was measured using a microplate reader. Data represents the percentage of LDH releases relative to vehicle control.

Inhibition of PSC on ROS level in rotenone-induced PC12 cells

After pre-treatment with PSC and rotenone for 24 hr, the media was removed and PC12 cells were washed twice with PBS and incubated with 10 µM of DCFH-DA dissolved in FBS-free medium at 37°C for 20 min in the dark, then washed three times with PBS. The fluorescence of DCFH-DA was analyzed with a microplate reader at an excitation wavelength of 488 nm and emission wavelength of 525 nm. Intracellular ROS production was expressed as DCFH fluorescence intensity in each 1×10^4 cells.

Effect of PSC on SOD level in rotenone-induced PC12 cells

After pre-treatment with PSC and rotenone for 24 hr, the media was removed and the cells were washed twice with PBS. Cells were gently scraped using a cell scraper and collected after centrifugation. Cells were re-suspended in 500 µL of PBS, dissociated by cell lysis buffer and centrifuged at 13800 g for 6 min. The SOD activity of supernatant was assessed using assay kit on a microplate reader according to the specified manufacturer's instructions.

293T cell culture and viability assay

The 293T cells (6×10^5 cells/mL) were cultured in DMEM media for 24 hr in 96-well plates and treated with various concentrations (0, 0.1, 1, 10 and 100 µg/mL) of PSC. After 48 hr treatment, 20 µL of MTT reagent was added to each well and the plates were incubated at 37°C for more 3 hr. The media was removed carefully and 100 µL of DMSO was added to dissolve the resulted formazan into purple solution. The absorbance at 570 nm was measured on a microplate reader (HTS 7000 Plus, Perkin Elmer, USA).

Preparation of 293T cell lysates

293T cells cultured with or without compounds were collected with trypsin and media, centrifuged to remove the supernatant, washed with cold DPBS, then lysed in 1× CHAPS lysis buffer (10 mM Tris-HCl pH 7.5, 1 mM $MgCl_2$, 1 mM EGTA, 0.1 mM benzamidine, 5 mM β-mercaptoethanol, 0.5% CHAPS, and 10% glycerol, supplemented before use with protease inhibitor cocktail III (Calbiochem)) by incubating on ice for 20 min. The lysate was centrifuged at 12000 g, 4°C for 20 min, and the supernatant was aliquot to tubes for further analysis. Protein concentration of extracts was determined using the Bradford Assay. The extracts were flash frozen and stored at -80°C for TRAP assay.

Telomerase activity assay with 293T cell lysate

Telomerase activity in 293T cell (cultured with compounds at the final concentration of 0.01, 0.1, 1.0, 10, 100 µg/mL) lysate was semi quantitatively assessed by the TRAP assay[19] using the TRAPeze telomerase detection kit (Millipore), with Green I Stain (Lonza) as fluorescent stain. For each TRAP reaction, 1000 cell equivalents (2 µL), 2 µL of 2% DMSO, or 1 µL of TSR8 (Positive control) were used. TA-65 (1 µg/mL) was the standard compound by which all other telomerase activators like PSC are measured.

Preparation of Zebrafish larvae

The adult AB strains zebrafish were housed at 26°C with a 14: 10 hr light-dark cycle under a recirculation system, feed with brine shrimp once a day.

The night (6:00 PM) before spawning, 2 male and 2 female zebrafish were placed into the spawning tank with a clapboard, stayed in a incubator at 28°C for 14 hr. The next morning (8:00 AM), removing the clapboard to allow the zebrafish to mate freely. Fertilized eggs were then collected and sterilized with methylene blue solution (100 mg/L) at 28°C for 10 min, then transferred to petri dishes with 0.5 mg/L methylene blue solution. The water was changed every 12 hr. Any dysplasia fertilized eggs (including dead eggs) and impurities were removed. The developed normally embryos were selected for the further study. Zebrafish larvae were fed diets of paramecium.

Determination of mCPP concentration

7 dpf zebrafish were incubated in 0.1 μmol/L, 0.2 μmol/L and 0.4 μmol/L mCPP solution at 28°C for 30 min, with 4 fish per group. These zebrafish behaviors were tracked and analyzed using a Zebrabox combined with Zebralab analysis system (ViewPoint, France).

Zebrafish larvae behaviour test

At 7 dpf, 45 zebrafish larvae were transferred to the 6-well plates, with 4 fish per well. The control group was administered with water, and PSC groups were intervened with 200 μg/mL, 400 μg/mL, 800 μg/mL solutions for 6 hr, respectively, while positive group was administered with 0.15 μg/mL of oxazepam 30 min prior to being induced with mCPP. All groups, except for control group, were exposed to 0.2 μmol/L mCPP for 30 min before being observed in Zebrabox. The entire recording process consisted of 20 min.

Determination of GABA

According to the procedure of Zebrafish gABA (gamma-Aminobutyric Acid) ELISA Kit (ELK Biotechnology, China). The OD values were recorded at of wavelength of 450 nm.

Statistical analyses

The data were analyzed by the software (GraphPad Prism 9), presented as the mean and Standard Deviation (SD). Significant differences were analyzed by paired Student's *t*-test. *P*-Values of less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Isolation and purification of polysaccharides

PSC was obtained from *S. cuneata* stem, furthermore purified by DEAE-52 cellulose and Sephadex G-100 gel columns to obtain two subfractions PSCA-1 and PSCB-1. PSC and its subfractions PSCA-1, PSCB-1 were free of protein and nucleic acid.

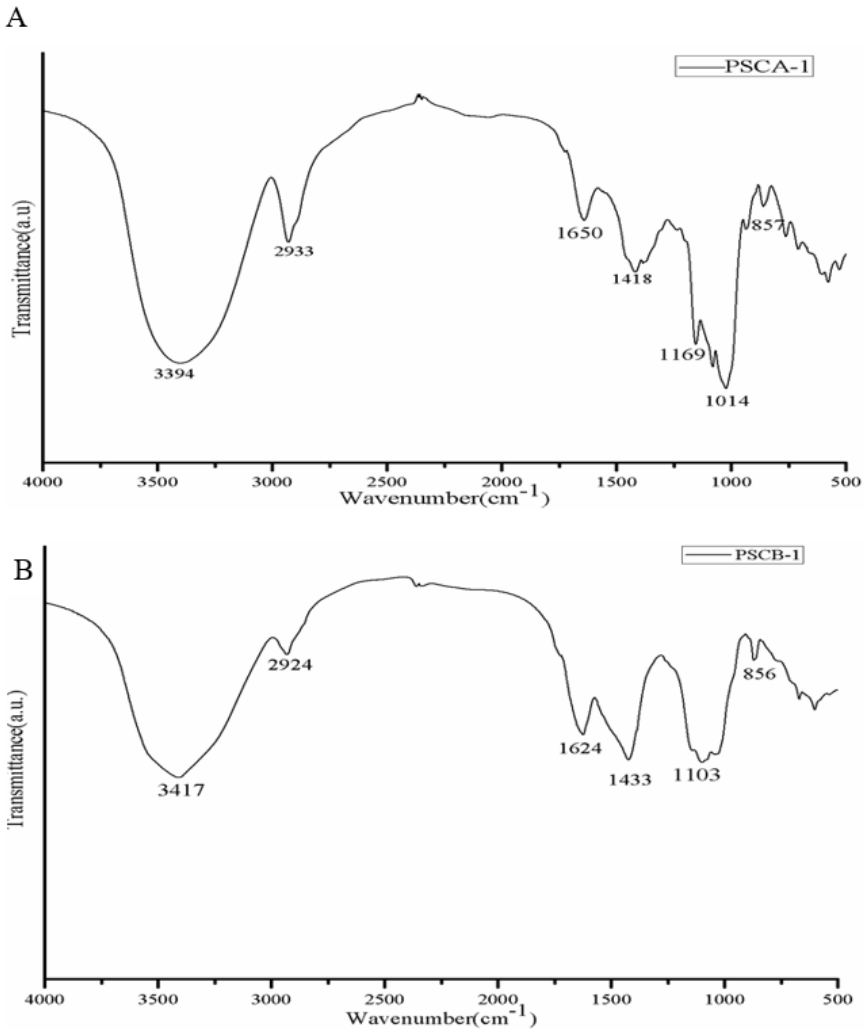

Figure 1: FT-IR spectrum (4000-500 cm^{-1}, KBr) of PSCA-1 (A) and PSCB-1 (B).

FT-IR spectral analysis

The FT-IR spectra of PSCA-1 and PSCB-1 (Figure 1) exhibited the characteristic strong and broad absorption peaks at 3394 cm^{-1} (PSCA-1) and 3417 cm^{-1} (PSCB-1) respectively, due to the stretching vibration of the OH group.[20] A weak absorption peak at 2933 cm^{-1} in PSCA-1 as well as 2924 cm^{-1} in PSCB-1 was attributed to a CH stretching vibration. The absorption peaks at 1650 cm^{-1} in PSCA-1 and at 1624 cm^{-1} in PSCB-1 correspond to the C-O stretching vibration of the carbonyl. The signals at 1418 cm^{-1} (PSCA-1), 1433 cm^{-1} (PSCB-1) was attributed to C-H bending vibration. The characteristic peaks at 1200-1000 cm^{-1} are belong to the stretching vibrations of C-O-C and C-OH in sugar moieties.[21] The occurence of peaks at 857 cm^{-1} (PSCA-1) and 856 cm^{-1} (PSCB-1) indicated the existence of α-glycosidic bonds.[22]

Effects of PSC, PSCA-1, PSCB-1 on PC12 cell viability

To ensure that PSC, PSCA-1 and PSCB-1 are not cytotoxic on the PC12 cells, the MTT method was used to determine the viability. As illustrated in Figure 2, PSC, PSCA-1 and PSCB-1 (0.1-100 μg/mL) were non-toxic to PC12 cells, even at the concentration of 200 μg/mL, the PC12 cell viability was still above 85% (data not shown in Figure 2). Thus concentration range from 1 μg/mL to 100 μg/mL was chosen for following PC12 cell experiments.

As illustrated in Figure 3, 10 μM of rotenone was obviously toxic to PC12 cells, decreased cell viability to 54.1% of control in MTT assay. PSC, PSCA-1 and PSCB-1 showed remarkable protective effect to PC12 cells. The pre-treatment with subfraction PSCA-1 enhanced respectively the cell viability to 70.6% (1 μg/mL), 94.8% (10 μg/mL), 65.2% (100 μg/mL) of control group. Similarly, PSCB-1 enhanced respectively the cell viability to 56.4% (1 μg/mL), 97.8% (10 μg/mL), 59.6% (100 μg/mL) of the control. An interesting phenomenon was found that PSC was more active than more purely subfractions PSCA-1 and PSCB-1 at the same concentrations. Indeed, PSC suppressed significantly the rotenone induced damage and cell viability increased to 82.0% (1 μg/mL), 95.3% (10 μg/mL) and 109.9% (100 μg/mL) of control group, respectively. Especially, the cell viability of 100 μg/mL group was more 9.9% than the control, which meant that PSC could reverse the damage trend induced by rotenone.

Inhibition of PSC on LDH release in rotenone-induced PC12 cells

LDH would release into the media when the cells were damaged or dead, which was used to determine the cytotoxicity of compounds. LDH leakage rate reflected the degree of cell damage or apoptosis. After exposure to 10 μM of rotenone for 24 hr, LDH release in PC12 cells increased significantly to 538.5% of control group (Figure 4). However, pre-treatment with PSC markedly decreased LDH release 24.19%, 50.2% and 66.3% compared with only rotenone group in a dose-dependent manner. Yet, they were higher than LDH level in cells with media alone of control group.

Inhibition of PSC on ROS Level in rotenone -induced PC12 cells

To investigate the effect of PSC on rotenone-induced oxidative damage in PC12 cells, the intracellular ROS level was determined by a specific fluorescent dye DCFH-DA, which leads to an enhanced fluorescent intensity, followed a generation of reactive metabolites intracellularly. As shown in Figure 5, after PC12 cells were exposed to rotenone for 24 hr, intracellular ROS level (DCF fluorescence intensity/1×10^4 cells) markedly increased to 476.1% of vehicle control group ($p<0.01$). After pretreated with PSC,

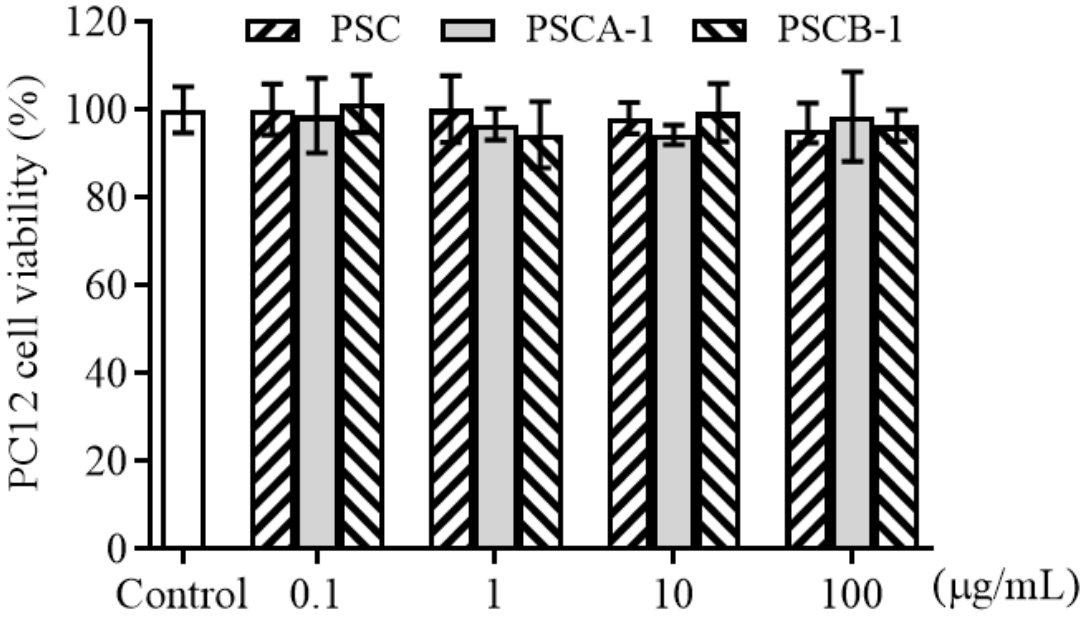

Figure 2: Effect of the PSC, PSCA-1 and PSCB-1 (0.1-100 μg/mL) on PC12 cell viability. Data are presented as mean±SD (n=3).

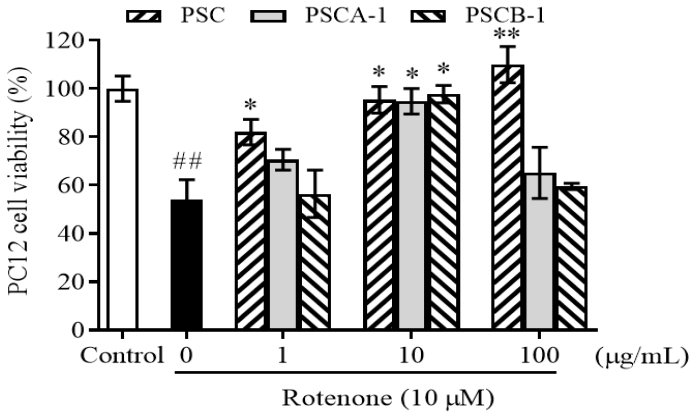

Figure 3: Protective effect of the PSC, PSCA-1 and PSCB-1 on PC12 cell damage induced by rotenone. Cells were pre-treated with PSC, PSCA-1 and PSCB-1 (1-100 μg/mL) for 5 hr and co-incubated with 10 μM rotenone for more 24 hr. Data are presented as mean±SD (n=4), ##: p<0.01, *: p<0.05, **: p<0.01 versus vehicle control.

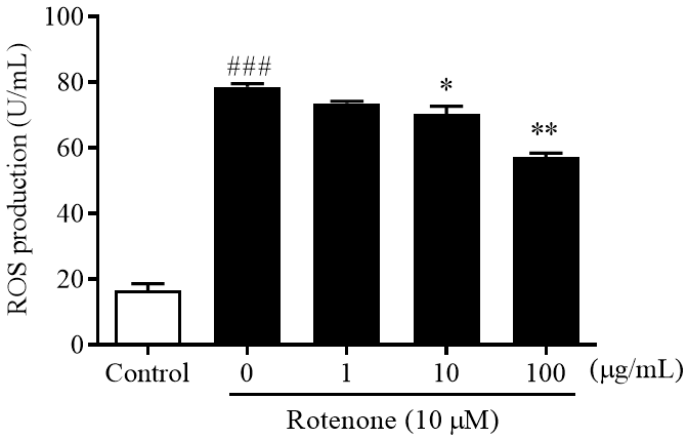

Figure 5: Effect of PSC on ROS level in rotenone damaged PC12 cells. Data are presented as mean±SD (n=4), ###: p<0.001 vs. control, *: p<0.05, **: p<0.01 vs. rotenone group.

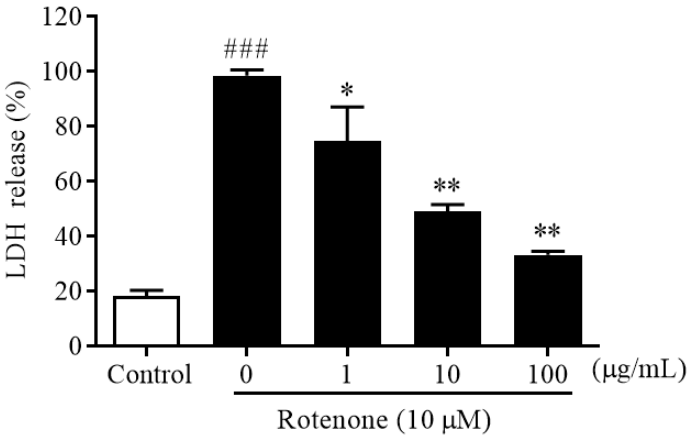

Figure 4: Effect of PSC on LDH release in PC12 cells induced by rotenone. Data are presented as mean±SD (n=4), ###: p<0.001 vs. control group, *: p<0.05, **: p<0.01 vs. only rotenone group.

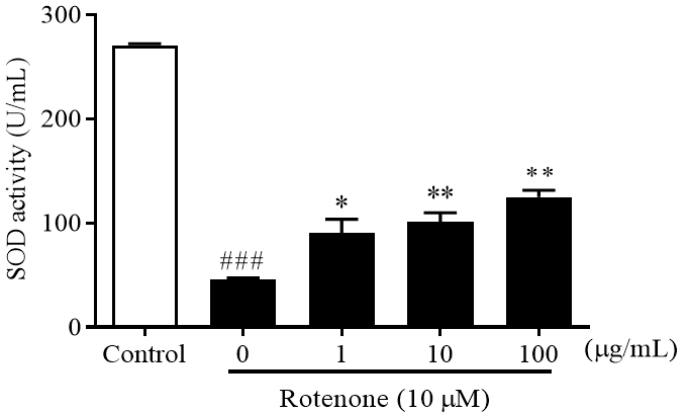

Figure 6: Effect of PSC on SOD level in rotenone induced PC12 cells. Data are presented as mean±SD (n=4), ###: p<0.001 versus control, *: p<0.05, **: p<0.01 versus rotenone group.

intracellular DCF fluorescence intensity/1×10^4 cells respectively reduced 6.5% (1 μg/mL), 10.7% (10 μg/mL) and 27.2% (100 μg/mL) compared with rotenone group. It suggested that PSC could decrease intracellular ROS level in a dose-dependent manner, which might be one of the mechanisms of protecting the rotenone-induced cells.

Effect of PSC on SOD Level in rotenone-induced PC12 cells

SOD is an important antioxidant system and can effectively protect the protein and DNA from oxidative damage. As shown in Figure 6, an obvious decrease of 83.0% of SOD activity was observed after treatment with rotenone (10 μM) for 24 hr, compared with control group (p<0.01). After pre-treatment with PSC, the SOD levels in PC12 cells markedly increased 96.2%, 120.6% and 172.1%, compared with rotenone group. Yet, all were lower than the SOD activity in the control group. The data suggested that the protection of PSC against oxidative stress could be associated with increasing SOD level.

Effect of PSC on Telomerase in 293T cells

Firstly, the effect of PSC on 293T cell proliferation was performed. The results indicated that PSC did not display cytotoxicity nor show any adverse effect on 293T cell viability at the highest treated concentration (Figure 7). Actually, PSC at the concentration of 0.1-100 μg/mL increased the proliferation of 293T cell, especially the concentration of 10 μg/mL had the most active effect, increased the cell viability 20.0% (p>0.05) compared with vehicle control group.

The subsequent TRAP assay implied that PSC could stimulate the telomerase in 293T cells. As shown in Figure 8, PSC (0.1, 1 and 10 μg/mL) stimulated the telomerase activity. Furthermore, PSC at the concentration of 1 μg/mL was the most active, it increased the telomerase activity 25.4% (p>0.05) compared with the vehicle control group (0.2% DMSO), which was more active than positive control TA-65 (20.7%). While the highest concentration of 100 μg/mL had the lowest effect on telomerase activity, only giving 2.0% increasement compared with the vehicle control.

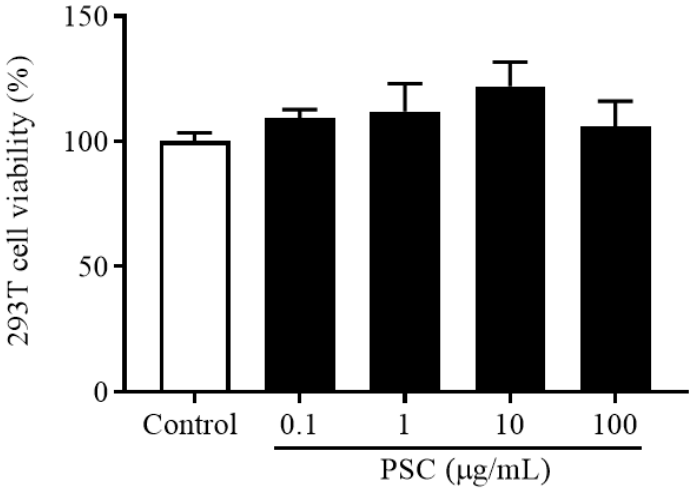

Figure 7: Effect of PSC (0.1-100 μg/mL) on 293T cell viability. Data are presented as mean±SD (*n*=3).

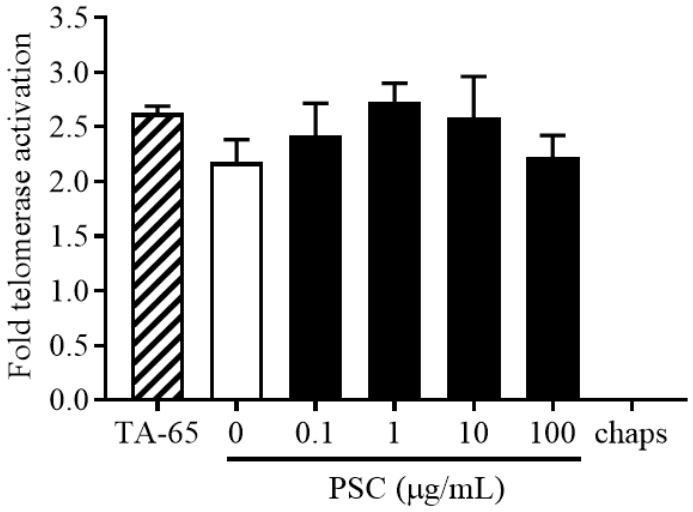

Figure 8: Stimulation of PSC on the telomerase in 293T cells. Telomerase activity quantified with ImageQuant. TSR8: positive control. TA-65: 1 μg/mL. Chaps: lysis buffer (no cell extract), negative control. Data are shown as means±SD (*n*=3).

Swimming distance of zebrafish larvae

As shown in Figure 9, the swimming distances of control group, mCPP group, positive control (0.15 μg/mL oxazepam) group, and 200 μg/mL, 400 μg/mL, 800 μg/mL PSC groups were 1626.6 mm, 2328.6 mm, 1726.1 mm, 2055.4 mm, 1691.9 mm, 1832.7 mm. mCPP increased the swimming distance 43.2% compared to control group, showed obvious the thigmotaxis (distance in the outer zone/ total distance).[23] Oxazepam could inhibited obviously the swimming distance close to the control group. PSC exhibited a dose-depend inhibition on anxiety behaviour induced by mCPP.

GABA is an amino acid that functions as the primary inhibitory neurotransmitter for the Central Nervous System (CNS). Low level of GABA may be linked to anxiety and other mood disorders. By the GABA kit assay, it could be observed that after exposure

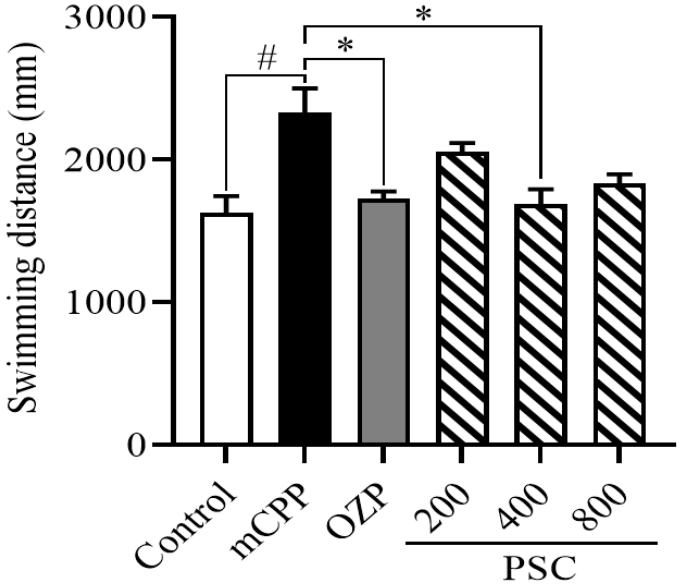

Figure 9: Effect of PSC on the swimming distance. OZP: oxazepam, #: $p<0.05$ versus control, *: $p<0.05$ versus mCPP group.

to mCPP, the GABA content in zebrafish decreases from 39.20 pg/mL to 39.01 pg/mL. While the positive control (0.15 μg/mL oxazepam) and 400 μg/mL PSC could increase the GABA content to 39.12 pg/mL and 39.16 pg/mL respectively. Of course, there is no significant difference in GABA content among all the groups, which might means that the GABA is not the key regulating role in the anti-anxiety.

Oxidative stress is one of the main contributing factors involved in cerebral biochemical impairment. The production of ROS is the main mechanism underlying this cellular redox imbalance.[24] Furthermore, by oxidative stress mechanisms, mental stress potentially impacts immune function, resulting in subsequent manifestation of psychiatric disorders.[25] Antioxidants protect biological targets against ROS, therefore, they have been considered as attractive potential therapeutic agents to counteract ROS-mediated neuronal damage.[6,24]

Short telomere length is associated various neurological and mental diseases, including Alzheimer's disease, anxiety and depression.[7] The sufficient telomerase activity will prevent telomere length gradually shorten with time and cell division. Stimulating the telomerase is thought as an efficient method to maintain the telomere length, extend the cell longevity.[26] Of course, the stimulation must be mild. If too potent, the overactive telomerase will induce an unlimited growth of cell, even increase the chances that the person will be afflicted with cancer.[27]

Rotenone is a widely used pesticide, and its exposure leads to changes of proteins and enzymes associated with dopamine synthesis and transportation in PC12 cells, contributing to the ROS formation and cell death.[28] This study proved thant PSC increased obviously rotenone-induced PC12 cell survival by increasing SOD level, down-regulating the LDH release, ROS level

and cell apoptosis rate. Furthermore, PSC possessed stimulating telomerase and anxiolytic activities.

Based on existing literature related with oxidative stress, telomerase and anxiety, there was a close connection among these three aspects. Antioxidation is an important mechanism for treating anxiety, and PSC showed good inhibitory effects on ROS in rotenone-induced PC12 cells, demonstrating excellent antioxidant and neuroprotective potential. This implied that PSC could exert its anxiolytic effects through antioxidant mechanisms. Additionally, PSC had a mild activating effect on telomerase. The activation of telomerase by PSC in this study reminded us that the anxiolytic activity of PSC may be related to telomerase activation. In fact, there is a mutually causal relationship between telomerase and anxiety, and alleviation on anxiety could be contribute to telomere maintenance and increased telomerase activity.

CONCLUSION

Given previous studies have found that PSC has good anti-inflammatory activity,[15] thus, it is certain that anti-inflammatory and antioxidant mechanisms are involved in telomerase activation and anti-anxiety effects. Therefore, further experimental verification will be conducted to reveal the causal link between treating anxiety and telomerase-activating activities of PSC, via the focus on the shared signal pathway (like Nrf2) underlying the inflammation and oxidative stress.

The polysaccharides from this plant may be applied as potential health and functional food source, to treat the psychiatric disorders.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PSC: Polysaccharide from *Sargentodoxa cuneata*; **SOD:** Superoxide dismutase; **LDH:** lactate dehydrogenase; **ROS:** Reactive oxygen species; **mCPP:** 1-(3-Chlorophenyl)piperazine; **GPX4:** Glutathione peroxidase 4; **Nrf2:** Nuclear factor erythroid 2-related factor 2; **DMEM:** Dulbeccosaccharide from *Sargentodoxa cuneata*; **SOD:** Superoxide dismutase; **LDH:** lactate dehydrogenase; **ROS:** Reactive oxygen species; **mCPP:** 1-(3-Chlorophenyl)piperazine; **GPX4:** Glutathione peroxidase 4; **Nrf2:** Nuclear factor erythroid 2-related factor 2; **DMEM:** co's PBS; **CHAPS:** 3-[(3-Cholamidopropyl)Dimethylammon io]-1-propanesulfonate; **TRAP:** Telomeric repeat amplication protocol; **TSR8:** Telomerase substrate primer extended with eight telomeric repeats AG; **dpf:** Day post fertilization; **GABA:** γ-aminobutyric acid; **FT-IR:** Fourier transform infrared spectroscopy.

SUMMARY

The polysaccharides isolated from *S. cuneata* possessed obvious anti-inflammatory and antioxidant effects, which contributed to the telomerase activation and anti-anxiety activities.

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