



Determination of SOD in black ginger extract and its effect on the liver of rats with type 2 diabetes

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Abstract

The peeling process of fresh ginger during factory production causes not only waste but also pollution to the environment. Now, by fermenting whole pieces of fresh ginger into black ginger, people are able to further enhance the nutritional value of ginger while lowering the burden on the environment. In this experiment, fresh ginger was fermented into black ginger, and superoxide dismutase (SOD) crudely extracted from both fresh ginger and black ginger were gavaged separately in type 2 diabetic rats to compare the SOD activity and effect in rat liver during the treatment of type 2 diabetes. It was found that the SOD activity of extracts from fermented black ginger was much higher than that of extracts from fresh ginger. In addition, the activity of SOD in the liver of rats gavaged with black ginger extract is higher than that in the liver of rats gavaged with fresh ginger extract, which shows that black ginger extract has certain protective effects on the liver.

Keywords: black ginger; SOD activity; type 2 diabetes; liver.

Practical Application: Reducing the waste caused by ginger peel and benefiting the environment, enhancing SOD activity in fermented black ginger extract, delivering a protective effect on the liver.

1 Introduction

China is one of the world's major ginger growers as well as exporters. However, China's exported goods mainly consist of fresh ginger and dried ginger traded at low prices, which results in a huge waste of raw material resources. Under such circumstances, it is of great social and economic significance to develop deep-processed products with high added value based on the main active components of ginger (Wang, 2015). The peeling process of ginger during factory production results in large amounts of wasted ginger peel and creates a huge burden to the environment. However, the ginger peel contains rich nutrient substances. Through the extraction and proper use of these active components, the value of ginger could be fully exploited (Chen, 2013) and the otherwise wasted ginger peel could be turned into treasure. This contributes to the achievement of goals set by the UN Environment Assembly by reducing the waste of resources and leading a green and low-carbon lifestyle.

Pharmacologically, ginger has many beneficial effects including antipyretic, analgesic, anti-inflammatory, sedative, hypnotic, anticonvulsant, cardiostimulatory, increasing myocardial contractility, anti-hypoxia, anti-ulcer, antiemetic, increasing gastric acid secretion, hepatoprotective and cholagogic, anti-platelet aggregation, antioxidant, anti-pathogenic microorganism, etc (Cui et al., 2011). Gingerol is a kind of phenolic substance in ginger and 6-gingerol is the main functional components, which has been reported to have significant anti-inflammatory,

anti-oxidant, anti-apoptotic effects, and to regulate blood sugar and blood lipids (Li et al., 2017; Choi et al., 2018; Rahmani et al., 2014). It has also been reported that the duration of 6-Gingerol compounds available in the colon was up to 12 h, which may favor their antioxidant potential and healthy effects (Majdoub et al., 2019). Moreover, 6-gingerol could prevent DSS-induced chronic UC via anti-inflammatory and antioxidative mechanisms and preservation of the Wnt /p-catenin signaling pathway (Ajayi et al., 2018).

SOD, also known as orgotein, is an active substance derived from the living body that can eliminate harmful substances generated from the metabolic process of living organisms (Liu, 2016). As one of the important enzymes associated with biological oxidation, SOD is known as "the first line of defense in the antioxidant system of living organisms". SOD can effectively prevent the damage of oxygen free radicals in the body, avoid the damage of ionizing radiation on the skin, eliminate superoxide anion free radical generated by biochemical metabolism in the body, delay the aging of the skin, and has spot-removing and anti-wrinkle effects (Zhang et al., 2017). In the medical field, SOD is mainly used for the treatment of myocardial ischemia, ischemia-reperfusion syndrome, inflammation, cancer, and other diseases caused by the damage of superoxide anion such as some autoimmune diseases (Feng et al., 2021). In addition, it protected the brains of rats from high-fructose diet induced oxidative stress by altering serum levels of SOD (Binmowyna et al.,

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2021). With the enhancement of people's standard of living, diabetes has become a very common chronic disease in China with its incidence rising year by year. At present, diabetes has become a public concern that poses major threats to the health of Chinese people.

Diabetes is a group of chronic and metabolic diseases, which can cause a series of illnesses that bring huge negative impact on the physical conditions of the patients, seriously compromise their quality of life, and impose a heavy burden on the patients themselves, their families, and the society at large. On all accounts, available data shows that the prevention and treatment of diabetes demand immediate attention. Therefore, it is imperative to carry out scientific research into the right medicines for treating the disease or slowing its progression. There are two general categories of diabetes: type 1 diabetes and type 2 diabetes. Type 1 diabetes has obvious clinical symptoms, including excessive drinking, frequent urination, and overeating, while type 2 diabetes generally has no typical symptoms, so most patients are unaware of their illness and only learn about their situation by accident during a physical check-up. In addition, with the increased prevalence of type 2 diabetes, the age at onset is getting younger and younger today. Diabetes mellitus is a group of physiological dysfunctions associated with hyperglycemia-mediated oxidative stress and apoptosis in pancreatic β -cells. As we learn more about the pathophysiology of diabetes mellitus, loss of functional β -cells mass has been considered as the key mechanism leading to the diabetes mellitus (Guthrie & Guthrie, 2004). Hyperglycemia-mediated oxidative stress and apoptosis in pancreatic β -cells have been found to play major role in the development of diabetes mellitus (Lim et al., 2018). Therefore, preventing the oxidative stress and apoptosis in pancreatic β -cells may contribute to the prevention and treatment of diabetes mellitus (Qiao et al., 2021). There are many western medicines available for treating diabetes at the present stage. However, despite their treatment effect, these medicines can cause different degrees of damage to the liver and other organs of patients with significant toxic and side effects. Many plants antioxidant and anti-atherosclerotic effects have already been described and were indicated to be mainly due to its high levels of phenolic compounds (Morais et al., 2020; Yoshime et al., 2019). This not only greatly reduces the toxic side effects of drugs but also conforms to the current green health concept of treating diseases.

This experiment used fresh ginger as the raw material. Under particular temperature and humidity conditions, the fresh ginger was fermented into black ginger products. In this fermentation process, the tissues in fresh ginger were damaged and the substances went through a series of chemical reactions. After the fermentation process, changes occurred in both the composition and functions of fresh ginger, resulting in the generation of a richer amount of nutrient substances and stronger physiological activity (Zong et al., 2019). Following this, the SOD components in fresh ginger and black ginger were extracted respectively and compared against each other. By gavaging the extracted SOD substances in diabetic rats, this experiment compared the SOD activity of extracts from fresh and black ginger and examined their respective effect

on the liver. Although there are already some studies on the chemical composition and pharmacological effects of ginger, few studies have been done on the effects and components of fermented black ginger, resulting in the lack of knowledge about the efficacy of black ginger. Under such circumstances, this research is of great importance in leveraging the rich ginger resources in China and strengthening the development of black ginger products.

2 Materials and methods

2.1 Materials and reagents

The fresh ginger was purchased in the farmers' market of Heping Village, Arding Street, Kundulun District, Baotou City.

Experimental reagents

The reagents used in the experiment are of analytical grade purity. Coomassie brilliant blue G-250 (CAS No. 6104-58-1) and disodium EDTA (CAS No. 6381-92-6) were purchased from Beijing Solarbio; Tris (CAS No. 77-86-1) was purchased from Shandong Suihua Biological Technology Co., Ltd.; anhydrous ethanol (CAS No. 64-17-5) was purchased from Tianjin Concord Technology Co., Ltd.; ammonium sulfate (CAS No.: 7783-20-2), potassium dihydrogen phosphate (CAS No.: 7778-77-0), pyrogalllic acid (CAS number: 87-66-1), sodium carbonate (CAS No.: 497-19-8), sodium chloride, (CAS No.: 7647-14-5), pepsin (CAS No.: 9001-75-6), pancreatin (CAS No.: 8049-47-6), alloxan (CAS No.: 2244-11-3) were purchased from Shanghai Yuanye Biological Technology Co., Ltd.

Experimental equipment

High-speed refrigerated centrifuge (Eppendorf Centrifuge 5417R, Eppendorf China Co., Ltd.), ultra-violet and visible spectrophotometer (UV-1600, Shanghai Mapada Instruments Co., Ltd), pH testing pen (AZ8692, AZ Technology Co., Ltd.), food processor (JYL-C012, Hangzhou Joyoung Life Electric Co., Ltd.), vortex mixer (GL-88B, Haimen Kylin-Bell Lab Instruments Co., Ltd.), medical refrigerator (BCD-240SDPN, Qingdao Haier Co., Ltd.), constant temperature & humidity fermentation chamber (BPS-100CL, Shanghai Yiheng Scientific Instrument Co., Ltd.), disposable sterilized syringe (5mL, Lianhua Medical Supplies Co., Ltd.), ice maker (XB70, GRANT), Sinocare blood glucose meter (GA-3 model, Sinocare Inc.).

2.2 Experimental animals

- (1) Six-week-old male SD rats of specific pathogen-free (SPF) grade weighing 200-240 g were provided for the experiment. The rats were purchased from SPF (Beijing) Biotechnology Co., Ltd. (tested by Suzhou Xishan Biotechnology Inc.), which can be used for safety experiments and other experiments related to nutrition and growth as well as endocrine and toxicology. According to the habits of SD rats that those kept in the same cage tend to be more docile and easier to

catch, while those kept alone tend to be timid and easily frightened, the rats for the experiment were housed three per cage.

- (2) Modeling of type 2 diabetic rats. The rats were randomly divided into 3 groups, with 10 rats in the control group and 20 rats in the experimental group. The rats in the experimental group were fasted with free access to water for 12 hours and were injected intraperitoneally with 2% alloxan at a dose of 200 mg/kg. After the injection was completed, fasting blood glucose was measured in the experimental group of rats through tail-tip blood collection after 72 hours. Rats with blood glucose concentrations less than 7.0 mmol/L were considered as rats with failed modeling and needed to be re-modeled. Rats with blood glucose concentrations greater than 7.0 mmol/L were considered as hyperglycemic rats with successful modeling, which were then randomly divided into the fresh ginger group and the black ginger group (Li & Ning, 2020), with 10 rats in each group.
- (3) Animal treatment. The rats in the control group and the rats in the experimental group were placed in the same dark and quiet environment. Rats in the control group were gavaged with normal saline. Hyperglycaemic rats with blood glucose concentrations greater than 7.0 mmol/L were gavaged with ginger juice and black ginger juice respectively at a dose of 400 mg/kg/d using a syringe with a gavage needle. After two weeks of gavage, the rats were executed and the livers, kidneys, and pancreas were removed, weighed and the blood was washed from the surface of the organs with normal saline and then dried with filter paper. The measurement solution was prepared according to the tissue ratio (liver tissue homogenate: saline = 1:9) and immediately centrifuged at 4 °C for 15 minutes at 5000 rpm. The supernatant from the centrifugation was used for measurement (Ning et al., 2015).

2.3 Preparation of black ginger

Due to the high quality requirement on the fresh ginger to be fermented into black ginger, the ginger purchased for the experiment was carefully selected: the fresh ginger must be in whole pieces and free from defects, with no signs of spoilage, mould, or decay due to microbial growth. The cleaned fresh ginger was then put into a constant temperature and humidity chamber at 68 °C and 95% humidity and fermented for 30 days to obtain black ginger (Ning et al., 2016).

2.4 Extraction of SOD components in ginger

- (1) Full dissolution of SOD. Wash the fresh ginger first with plenty of water and then distilled water, and drain on filter paper. Weigh and take about 200 g of black ginger and fresh ginger, and put them separately into the food processor until they are well homogenized. Next, filter the homogenate with four layers of gauze to remove crude

fibres and other components. The resulting filtrate was the ginger extract. Then add 5-fold volume of phosphate buffer (0.05 mol/L) to the ginger extract and get a mixture of about 25 mL. Continue to grind the mixture for 20 min, let stand for 1 h to fully dissolve the SOD into the buffer, then centrifuge at 3500 r/min for 20 min, and take the supernatant (Ning et al., 2015).

- (2) Removal of heteroprotein. Add 0.25 volume of chloroform-ethanol mixture into the supernatant and stir for 20 min, then centrifuge at 3500 r/min for 20 min, and the supernatant obtained was the crude enzyme solution (Liao et al., 2011);
- (3) Precipitation separation of SOD. Add an equal volume of cold acetone (4 °C) to the crude enzyme solution, stir for 20min, centrifuge at 3500 r/min for 20 min to obtain SOD precipitate. Then dissolve the SOD precipitate in phosphate buffer (first add 2 mL and then 3 mL), perform heat treatment at 55-60 °C for 20 min, and then centrifuge to obtain SOD enzyme solution (Yu et al., 2012).

2.5 Determination of SOD in ginger

- (1) Determination of SOD activity. The enzyme activity was determined with the auto-oxidation of pyrogallol method in GB/T5009.171-2003 (Ning Yue bao et al.,2015). The amount of SOD enzyme required to inhibit the auto-oxidation rate of pyrogallol by 50% at 25 °C with 1 mL SOD enzyme solution per minute is an enzyme activity unit (Lei et al., 2012).
- (2) Determination of the auto-oxidation rate of pyrogallol. At 25 °C, add 2.35 mL of Tris-hydrochloric acid buffer, 2.00 mL of phosphate buffer, and 0.15 mL of pyrogallol solution into the test tube in order, then add the pyrogallol solution, and gently shake up the mixture in the test tube. Pour the mixture into the prepared cuvette, set the wavelength of the spectrophotometer at 325 nm, and measure the absorbance value. Then subtract the absorbance value measured 1 min later from the absorbance value measured at the beginning, and get the auto-oxidation rate of pyrogallol $\Delta A_{325}(\text{min}^{-1})$ (Zhao, 2001).
- (3) Determination of the inhibition rate of SOD to pyrogallol auto-oxidation. To determine the inhibition rate of SOD enzyme to pyrogallol auto-oxidation, add the sample solutions specified above according to the determination of the auto-oxidation rate of pyrogallol described to set the inhibition rate of pyrogallol auto-oxidation at about $1/2\Delta A_{325}(\text{min}^{-1})$, i.e., $\Delta A_{325}(\text{min}^{-1})$. The following Table 1 shows the order of sample addition for SOD activity determination.

Pyrogallol rapidly auto-oxidizes under alkaline conditions, releasing O₂- and producing a coloured intermediate

product. After the reaction starts, the mixture first turns yellow-green and then yellow after a few minutes, with the linear time maintained at 3~4 min. In the presence of SOD, the auto-oxidation reaction is inhibited. Determination of the inhibition rate of SOD from black and fresh ginger to pyrogallol auto-oxidation: according to the determination method of the auto-oxidation rate of pyrogallol as specified in GB/T5009.171-2003. The reagents and instruments used in this method are relatively common and easily accessible. Due to its advantages of fast and simple operation, good repeatability, and high sensitivity, it is currently the most widely used test method (Wan et al., 2010).

(4) SOD activity calculation (Equation 1) Table 2 shows the calculation criterion of SOD activity.

$$\text{SOD Activity (U/g)} = \frac{\Delta A_{325} - \Delta A'_{325}}{\Delta A_{325} 50\%} \times 100\% \times 4.5 \times \frac{D}{V} \times \frac{V_1}{m} \quad (1)$$

2.6 Data analysis

All data results were expressed as mean+standard deviation, and t-test was performed for comparison between groups. Group difference was considered significant when $P < 0.05$ and highly significant when $P < 0.01$. All data were processed using SPSS 20.0 statistical software.

3 Results and discussion

3.1 Changes of blood glucose levels in rats

Table 3 shows the changes of blood glucose in rats during modeling:

3.2 Determination of SOD activity in crude extracts from ginger

According to Table 4, which shows the determination results of SOD activity in extract, the SOD activity of non-fermented fresh ginger was 20.42 U/g, while the SOD activity of fermented black ginger was 312.73 U/g. It can be calculated that the SOD activity of fermented black ginger was 15.32 times higher than that of fresh ginger before the experiment.

3.3 Determination of SOD activity in rat liver by extract

According to Table 5, which shows the determination results of SOD activity in rat liver by extract, the SOD activity in the liver of rats in the fresh ginger group was 312.73 ± 2.08 U/g, while the SOD activity in the liver of rats in the black ginger group was 566.64 ± 5.37 U/g. By comparing the results, it was found that the SOD activity in the liver of rats gavaged with black ginger extract was higher than that of rats in the fresh ginger group.

3.4 Changes of biochemical indices in rat liver

Table 6 shows the changes of biochemical indices in rat liver and compares the changes observed in the fresh ginger group, black ginger group, and the control group. The result indicates that the hepatoprotective effect of black ginger crude extract in rats is higher than that of fresh ginger extract.

Table 1 . Sample addition for SOD activity determination.

Reagent solution	Fresh ginger	Black ginger	Sequence
Tris-hydrochloric acid buffer/mL	2.35	2.35	1
Phosphate buffer/mL	1	1	2
SOD enzyme solution/mL	1	1	3
Pyrogallol solution/mL	0.15	0.15	4

Table 2. Calculation criterion of SOD activity.

In formula	Meaning	Unit
ΔA_{325}	Auto-oxidation rate of pyrogallol	
$\Delta A'_{325}$	Inhibition rate of SOD enzyme solution to pyrogallol auto-oxidation	
4.5	Total volume of reaction mixture	mL
V	Volume of enzyme solution added	mL
V1	Total volume of sample solution	mL
D	Dilution multiple of enzyme solution	
M	Total sample volume	g

Table 3. Changes of blood glucose levels in rats.

Time	Control group	Fresh ginger group	Black ginger group
Before modeling	6.4 ± 2.2	6.4 ± 2.2	6.4 ± 2.2
After modeling	5.8 ± 1.6	15.7 ± 1.1	16.1 ± 0.8

Table 4. Determination results of SOD activity in extract.

Sample	SOD Activity (U/g)
Fresh ginger	20.42
Black ginger	312.73

Table 5. Determination results of SOD activity in rat liver by extract.

Group	SOD Activity (U/g)
Fresh ginger group	385.73 ± 2.08
Black ginger group	566.64 ± 5.37

Table 6. Biochemical indices in mice.

Biochemical indicators	Unit	Control group	Fresh ginger group	Black ginger
Alanine aminotransferase U/L	72.32 ± 2.3	78.45 ± 2.1	72.33 ± 1.9	
Total protein	q/L	55.78 ± 1.23	67.3 ± 5.1	50.40 ± 1.4
Albumin	q/L	12.68 ± 0	22.47 ± 2.33	17.52 ± 6.1
Globulin	q/L	25.4 ± 1.1	32.1 ± 1.2	29.2 ± 4.5
Albumin/Globulin		0.5 ± 0.6	0.7 ± .13	2.01 ± 1.3
Total bilirubin	umol/L	2.62 ± 1.2	1.8 ± 3.7	2.2 ± 1.2
Direct bilirubin	umol/L	1.12 ± 1.3	1.3 ± 2.1	1.2 ± 0.4
Indirect bilirubin	umol/L	2.46 ± 0.54	1.23 ± 2.9	2.32 ± 0.9

4 Conclusion

In this experiment, the researchers fermented fresh ginger into black ginger, extracted active SOD substances from fresh ginger and black ginger respectively, constructed the model of type 2 diabetic rats, and gavaged SOD substances from fresh ginger and black ginger in rats respectively. The results showed that the SOD activity of extracts from fermented black ginger was 15.32 times higher than that of non-fermented fresh ginger. By comparing the SOD activity of extracts from both fresh and black ginger in the liver of type 2 diabetic rats, it was found that the SOD activity of extracts from black ginger in rat liver was higher than the SOD activity of extracts from fresh ginger, which indicated that black ginger can significantly enhance the SOD activity in rat liver and has a protective effect on the liver of type 2 diabetic rats. During the fermentation process, the complex sulfides in the fresh ginger were degraded, while the irritating odour first turned stronger, then gradually faded away until it finally disappeared. The proteins in black ginger were broken down into peptides, while the content of free amino acid and phenolic compounds continued to rise. The increased amount of active SOD substances in black ginger resulted in higher nutritional value and greater antioxidant effect as compared with fresh ginger. Given the lack of black ginger products available at the current stage, the research and development of black ginger products hold promising prospects, aiming to provide a certain theoretical basis for the future research and development of ginger health food and drugs with the function of regulating and lowering blood glucose.

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