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Extraction of proanthocyanidins from grape seeds and their protective effect on spinal cord injury in rats

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Abstract

Proanthocyanidins were extracted and purified from grape seeds, and the protective effect and mechanism of proanthocyanidins on spinal cord injury were explored. After extraction optimization and purification, the proanthocyanidins with purity of 92.53% were obtained. The rats were divided into control, model and treatment groups. The spinal cord injury model was established in model and treatment groups. Then, the treatment group was treated with 40 mg/kg proanthocyanidins. On the 1st, 3rd and 7th day after modeling, compared with model group, in model group the Basso-Beattie-Bresnahan scores were significantly decreased ($p < 0.05$). On the 7th day after modeling, compared with model group, in treatment group the spinal cord tissue superoxide dismutaseand glutathione peroxidase levels were significantly increased ($p < 0.05$), the malondialdehyde level was significantly decreased ($p < 0.05$), the necrosis factor α and interleukin 1βlevels were significantly decreased ($p < 0.05$), and the interleukin 10 level was significantly increased ($p < 0.05$). In conclusion, proanthocyanidins have protective effect on spinal cord injury in rats. The mechanisms may be related to its reducing oxidative stress and inflammation in spinal cord tissue.

Keywords: proanthocyanidins; spinal cord injury; rats; oxidative stress; inflammation.

Practical Application: Proanthocyanidins are applied to protect against spinal cord injury in rats.

1 Introduction

Spinal cord injury is often caused by the spine burst or displacement due to different ways of external force. It is one of the most common severe diseases in clinic. In recent years, with the development of industry, construction, sports and transportation, the incidence of spinal cord injury is increasing (Miyakoshi et al., 2021). Spinal cord injury can be divided into two stages including primary injury and secondary injury. Studies have shown that the secondary spinal cord injury may lead to the further neurodegenerative diseases (Austin & Fehlings, 2008; López-Serrano et al., 2016). It has been found that the oxidative stress and inflammation play an important role in spinal cord injury (Zhaohui & Shuihua, 2020; Guan & Wang, 2021). Therefore, how to reduce the oxidative stress and inflammation is the focus of clinical treatment of spinal cord injury.

Proanthocyanidins are a class of active phenolic compounds mainly existing in grape seeds and grape skins. In recent years, scholars have done a lot of research on the physical and chemical properties and biological characteristics of proanthocyanidins. They find that, proanthocyanidins have the strong effect in resisting oxidative stress (Chen L et al., 2020), reducing apoptosis (Mantena et al., 2006), regulating immunity (Williams et al., 2016), and preventing cancer (Chen et al., 2014). In addition, they have the characteristics of high efficiency, low toxicity and high bioavailability (Choy et al., 2013). It is found that proanthocyanidins can enhance the stability of the nervous system (Moreira et al., 2010). However, whether proanthocyanidins have protective effect on spinal cord injury has not been reported. In this study, proanthocyanidins were extracted from grape seeds, and the extraction technologies were investigated. Then, the rat model of spinal cord injury was established, and the protective effect and mechanism of proanthocyanidins on spinal cord injury were explored.

2 Materials and methods

2.1 Extraction of proanthocyanidins from grape seeds

A 500 g of petroleum ether-degreased grape seed powder was added to the extraction kettle, and then the ethanol solution was added. The extraction was performed under different ethanol concentration, solid-liquid ratio, extraction temperature and extraction time. Finally, the extraction solution was filtered. The filtrate was concentrated by rotary evaporation, followed by freeze-drying. The crude proanthocyanidins product was obtained. After weighing, the content of proanthocyanidins in product was determined. The extraction yield was calculated.

2.2 Purification of crude proanthocyanidins product

The crude proanthocyanidins product was dissolved with water to a concentration of 50 mg/ml, and then was added to the pretreated AB-8 macroporous adsorption resin column. The gradient elution was performed using 20-80% ethanol solution. The target elution solution was collected, followed by concentration under

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reduced pressure and freeze-drying. The products were loaded to the Sephadex LH-20 gel chromatography column, followed by gradient elution using 20-100% ethanol solution. The target elution solution was collected. After concentration under reduced pressure and freeze-drying, the final proanthocyanidins product was obtained. After weighing, the content of proanthocyanidins in final product was determined.

2.3 Establishment of spinal cord injury model

Thirty SPF-grade male SD rats (250-300 g) were divided into control, model and treatment groups, with 10 rats in each group. In model and treatment groups, the rats were anesthetized by intraperitoneal injection of 10% chloral hydrate, and were fixed in prone position. A 3 cm longitudinal incision was made in the posterior midline of back to expose the paravertebral muscles. The T10 lamina, spinous process and transverse process were exposed by blunt dissection of muscles. The spinal canal was expanded to expose the spinal cord, keeping the dura mater intact. The T10 spinal cord was struck with a 60 g·cm impactor (10 g metal rod falling from 6 cm height). After staying for 3 min, the metal rod was removed. The success criteria for establishment of SCI model were as follows: the dura mater presented purple; there were swelling, edema and hemorrhage in spinal cord; the rats presented spasmodic shaking of both lower extremities and spasmodic swing of tail. In control group, only T10 spinal cord was exposed, without striking, the other surgical operations were the same with other two groups. During the modeling, one and two rats died in model and treatment groups, respectively. After surgery, penicillin (80000 U/day) was injected intramuscularly for three days. The bladder massage was performed to assist the urination.

2.4 Treatment methods

Immediately after modeling, the treatment group was intraperitoneally injected with 40 mg/kg proanthocyanidins. The control and model groups were synchronously intraperitoneally injected with equal volume of normal saline. The treatment was performed once a day, for seven days.

2.5 Basso-Beattie-Bresnahan (BBB) behavioral scoring

On the 1st, 3rd and 7th day after modeling, the hind limb motor function of rats in each group was evaluated using BBB scores by double-blind method (Basso et al., 1995). The score of 0 point and 21 points represented total paralysis and normal motor function, respectively.

2.6 Biochemical index detection

On the 7th day after modeling, the rats were sacrificed under anesthesia. The injured spinal cord tissue was obtained and the homogenate was made. After centrifuging at 3000 r/min and -10 °C for 20 min, the supernatant was obtained. The protein content was determined by Coomassie brilliant blue method. The oxidative stress indexes including superoxide dismutase (SOD) activity and glutathione peroxidase (GSH-Px) activities and malondialdehyde (MDA) content were measured using the corresponding kits. The inflammatory factors including necrosis factor α (TNF-α), interleukin (IL)-1β and IL-10 were detected using enzyme-linked immunosorbent assays.

2.7 Statistical analysis

The statistical analysis was performed using SPSS 18.0 software. Data were presented as mean ± SD. The comparison of data among three groups was conducted using single-factor analysis of variance test with SNK-q test. A p-value of < 0.05 (2-tailed) was considered as statistically significant.

3 Results

3.1 Extraction and purification results

The optimization experiments showed that, the optimal extraction conditions of proanthocyanidins from grape seeds were as follows: ethanol solution concentration, 60%; solid-liquid ratio, 1:4 (g: ml), extraction time, 90 min; extraction temperature, 75 °C. Under these conditions, the yield of proanthocyanidins was 10.2%. After purification using AB-8 macroporous resin column and Sephadex LH-20 gel chromatography column, the content of proanthocyanidins in the final product was 92.53%.

3.2 General condition of rats

After striking of spinal cord, the rats in control group presented normal condition. In model group, the rats presented typical spasmodic shaking of both lower extremities and spasmodic swing of tail. After recovery from anesthesia, the both lower extremities suffered from flaccid paralysis, and the urine retention and urinary incontinence occurred. From three days after modeling, partial rats began to have a small amount of activities, less food intake, and severe urinary retention. Since then, the diet and activities gradually increased. In treatment group, the symptoms were basically the same with model, but the degree was obviously reduced. After bladder massage, all rats were able to urinate autonomously.

3.3 Effect of proanthocyanidins on BBB scores of rats with spinal cord injury

From the 1st day to the 7th day after modeling, the BBB scores of in three groups gradually increased. At each time point, the BBB scores in model and treatment groups were significantly lower than those in control group, respectively ($p < 0.05$). Compared with model group, the BBB scores in treatment group at each time point were significantly decreased ($p < 0.05$) (Figure 1).

3.4 Effect of proanthocyanidins on spinal cord tissue SOD, GSH-Px and MDA levels in rats with spinal cord injury

As shown in Figure 2, on the 7th day after modeling, compared with control group, in model and treatment groups the spinal cord tissue SOD and GSH-Px levels were significantly decreased, respectively ($p < 0.05$), and the MDA level was significantly increased, respectively ($p < 0.05$). Compared with model group, in treatment group the SOD and GSH-Px levels

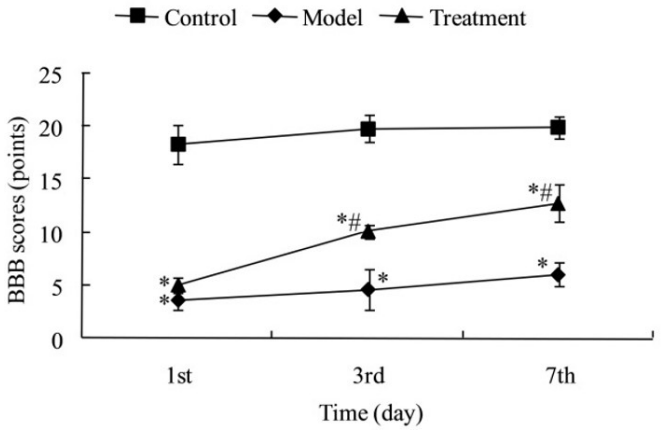

Figure 1. Comparison of BBB scores among three groups. Control: n = 10; model: n = 9; treatment: n = 8; 1st day: F = 364.691, p < 0.001; 3rd day: F = 313.741, p < 0.001; 7th day: F = 255.884, p < 0.001; *p < 0.05 vs. control group; #p < 0.05 vs. model group; BBB: Basso-Beattie-Bresnahan.

were significantly increased, respectively (p < 0.05), and the MDA level was significantly decreased (p < 0.05).

3.5 Effect of proanthocyanidins on spinal cord tissue TNF-α, IL-1β and IL-10 levels in rats with spinal cord injury

On the 7th day after modeling, the spinal cord tissue TNF-α and IL-1β levels in model and treatment groups were significantly higher than those in control group, respectively (p < 0.05), and the IL-10 level in model and treatment groups was significantly lower than that in control group (p < 0.05). Compared with model group, in treatment group the TNF-α and IL-1β levels were significantly decreased, respectively (p < 0.05), and the IL-10 level was significantly increased (p < 0.05) (Figure 3).

4 Discussion

Proanthocyanidins are internationally recognized as one of the most effective natural antioxidants. Studies have found

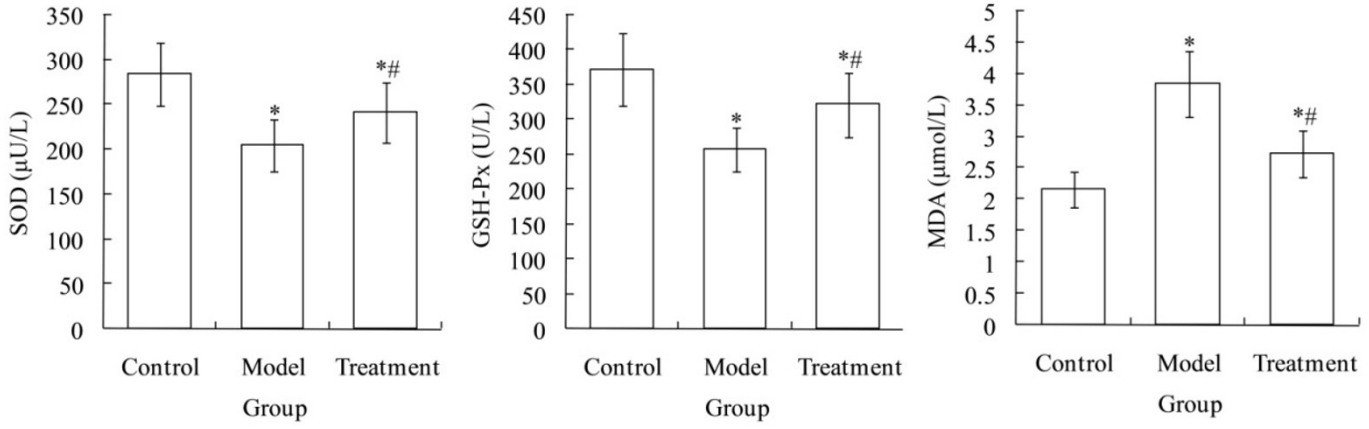

Figure 2. Comparison of spinal cord tissue SOD, GSH-Px and MDA levels among three groups. Control: n = 10; model: n = 9; treatment: n = 8; SOD: F = 14.083, p < 0.001; GSH-Px: F = 41.980, p < 0.001; MDA: F = 15.573, p < 0.001; *p < 0.05 vs. control group; #p < 0.05 vs. model group; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase; MDA: malondialdehyde.

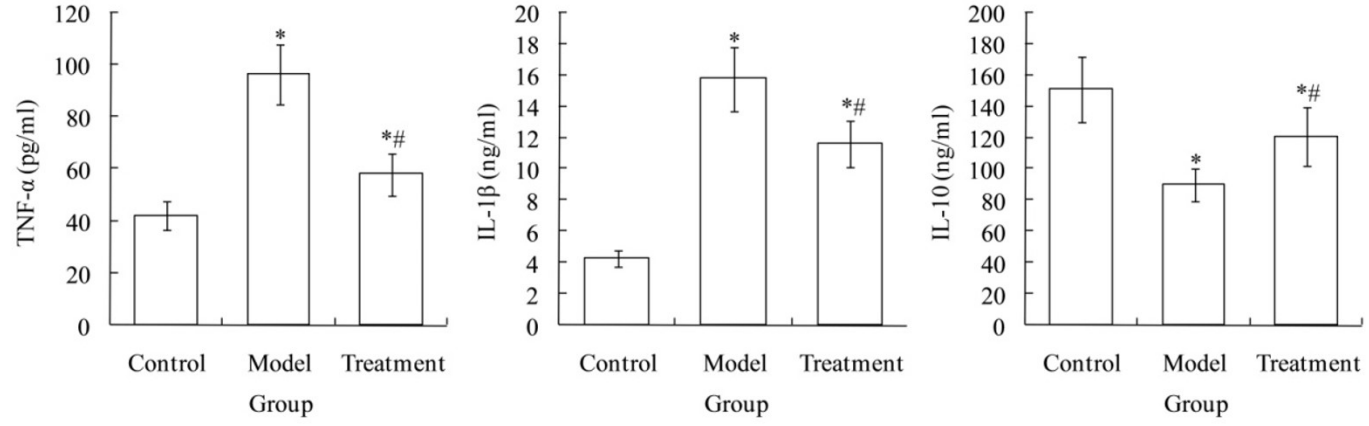

Figure 3. Comparison of spinal cord tissue TNF-α, IL-1β and IL-10 levels among three groups. Control: n = 10; model: n = 9; treatment: n = 8; TNF-α: F = 94.820, p < 0.001; IL-1β: F = 151.406, p < 0.001; IL-10: F = 29.541, p < 0.001; *p < 0.05 vs. control group; #p < 0.05 vs. model group; TNF-α, necrosis factor α; IL-1β, interleukin 1β; IL-10, interleukin 10.

that proanthocyanidins from grape seed has many biological functions, such as anti-oxidation, anti-inflammation, anti-tumor, anti-radiation, anti-obesity, reducing blood lipid and protecting cardiovascular system (Rauf et al., 2019). They are widely used in the fields of food, pharmacy and medical care. In this study, proanthocyanidins were extracted and purified from grape seeds, and the content of proanthocyanidins in the final product was 92.53%. Then, the rat model of spinal cord injury was established, and the protective effect of proanthocyanidins on spinal cord injury was explored. Results showed that, compared with model group, in treatment group, the spinal cord injury symptoms were obviously relieved, and the BBB scores were significantly decreased. This indicates that, the proanthocyanidins treatment has the protective effect on spinal cord injury in rats.

Studies have confirmed that the excess of free radicals and lipid peroxidation are the important reasons for the secondary injury of spinal cord injury. After spinal cord injury, a large number of free radicals cannot be eliminated, resulting in continuous lipid peroxidation to generate more free radicals, affecting the respiratory function of nerve cells, and eventually causing the nerve cell death (Lucas et al., 2002; Ishii et al., 2018). SOD is an enzyme that catalyzes the disproportionation of superoxide anion. It can protect cells against the damage of oxygen free radicals (Kalra et al., 1988). GSH-Px is an important enzyme which reduces toxic peroxides to non-toxic hydroxyl compounds, and promote the decomposition of H_2O_2, so as to protect the structure and function of cell membrane from the interference and damage of oxides (Kawai et al., 1988). MDA is the final product of lipid peroxidation of polyunsaturated fatty acids (Vagnozzi et al., 1999). SOD, GSH-Px and MDA are important indicators of oxidative stress. In this study, on the 7th day after modeling, compared with model group, in treatment group the spinal cord tissue SOD and GSH-Px levels were significantly increased, and the MDA level was significantly decreased. This suggests that proanthocyanidins can alleviate the spinal cord injury in rats by reducing the oxidative stress.

Inflammation is closely related to the spinal cord injury. The interaction of various pro-inflammatory and anti-inflammatory factors directly affects the prognosis of spinal cord injury (Hu et al., 2015). TNF-α and IL-1β are the pro-inflammatory factors, which are produced by activated macrophages and participate in the recruitment of inflammatory cells. TNF-α can reflect the severity of spinal cord injury. It can significantly decrease the expression of Caspase-3 and caspase-8 by inhibiting the TNF-α receptor, thus alleviating the apoptosis of neurons and oligodendrocytes (Chen et al., 2011). IL-1β is expressed in the early stage of spinal cord injury. It can stimulate the expression of TNF-α after spinal cord injury and activate the microglia/macrophage system, thus aggravating the damage of spinal cord and slow down the recovery of motor neuron function (Sato et al., 2012). IL-10 is a negative regulator of immune response. It can inhibit TNF-α after spinal cord injury, and plays the role of neuroprotection and promotion of injury repair (Bethea et al., 1999). In the present study, on the 7th day after modeling, compared with model group, in treatment group the spinal cord tissue TNF-α and IL-1β levels were significantly decreased, and the IL-10 level was significantly increased. This indicates that, proanthocyanidins can reduce the inflammatory response, which may be related to its protective effect on spinal cord injury in rats.

5 Conclusion

In conclusion, proanthocyanidins are extracted from grape seeds. The optimal extraction conditions were as follows: ethanol solution concentration, 60%; solid-liquid ratio, 1:4 (g: ml), extraction time, 90 min; extraction temperature, 75 °C. Under these conditions, the yield of proanthocyanidins was 10.2%. After purification, the content of proanthocyanidins in the final product is 92.53%. Proanthocyanidins have protective effect on spinal cord injury in rats. The mechanisms may be related to its reducing oxidative stress and inflammation in spinal cord tissue. However, as there are many factors involving the spinal cord injury, the action mechanisms of proanthocyanidins still need to be further clarified.

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