

Effect of pine needle extract on antioxidative ability of rats

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ABSTRACT: In order to explore the antioxidant capacity of pine needle extract, the scavenging capacity of free radicals in rat plasma was determined by pine needle proanthocyanidins extract, so as to preliminarily investigate the effect of pine needle extract on the antioxidant capacity of the body. 18 month old natural aging rats and 3 month old young rats were used as experimental animals. Randomly divided into blank control group, natural aging group, pine needle proanthocyanidins extract [1] gavage group. After 21 days of continuous administration, the eyeball was removed and the blood samples were collected. The contents of AOC, T-SOD and GSH were measured. By analyzing the different data of different groups, we can conclude the effect of pine needle extract on the antioxidant capacity of natural aging rats.

KEYWORDS: antioxidant capacity; pine needle extract; total superoxide dismutase (T-SOD); reduced glutathione (GSH).

1.Introduction

Due to the natural evolution of life and the impact of the environment, the global population age accelerated in the 21st century showing an aging trend, which will lead to Alzheimer's disease, Parkinson's syndrome and other diseases at the same time. The theory of free radical damage is the most influential one in the extensive study of aging mechanism in modern medicine. Among them, the theory of aging free radical generally holds that in the process of natural aging of organism life, it is mainly manifested in enhancing the oxidation of organism, weakening the antioxidant capacity of organism [2], reducing the activity of antioxidant enzymes such as T-SOD [3], reducing the ability of scavenging oxygen free radical [4] in vivo, which will cause irreversible damage to cells, tissues and various organs, thus causing various pathological changes And physiological metabolism disorder, accelerate the aging process of the body.

Under normal physiological and biochemical conditions, there are many defense barriers[5] and ways to eliminate free radicals. Under normal physiological conditions, the body can produce a small amount of free radicals in normal metabolism. Because there is a barrier to block the invasion of free radicals and a system to eliminate free radicals in the body, the excessive free radicals generated in the body can be eliminated in time. However, when the internal and external environment changes abnormally, a large number of generated and accumulated free radicals[6] have a strong stimulation on the body of normal free radical metabolism, affect the normal life function of the body, and endanger human life and health.

In traditional Chinese medicine, pine bark and pine needle have been used for thousands of years. According to compendium of *Materia Medica*, "pine is the length of a hundred trees, and its leaves, skin, paste, etc. produce hair, invigorate the Yang, nourish the middle, and do not starve to prolong the years; if you take it for a long time, you can firm your teeth, stop your face, and be light and not old [7]". Referring to the golden wing formula, it is also clearly recorded that the pine leaf [8] has the effect of "controlling diseases, turning white to black, and regenerating teeth [9]". Pine needle [10], as the medicinal representative part of pine tree, is non-toxic, bitter, relatively mild and easy to be compatible with other traditional Chinese medicine. Research shows that pine needles contain a lot of vitamin A, C, e and B vitamins, especially the content of vitamin C is the highest. It has been found that the pine needles of *Pinus sylvestris* contain many kinds of proteins and free amino acids. In recent years, the research results show that the pine needles contain a large number of proanthocyanidins [11] [12] which is one of the most effective free radical scavengers among the natural antioxidants [13]. Pine needles come from a wide range of sources. The planting of pine trees is less affected by climatic conditions and will not affect the supply of raw materials due to regional problems. The yield of pine needles is rich. Picking pine needles will not cause irrecoverable damage to trees.

2. Methodology

2.1 Experimental reagents and instruments

2.1.1 Experimental reagents

Drug name	Batch number	Manufacturer
T-AOC Kit	20180417	Nanjing Jiancheng Institute of Biology
GSH-Px Kit	20180417	Nanjing Jiancheng Institute of Biology
Pine needle proanthocyanidins extract	/	Self made by medical research center of Mudanjiang Medical College

Absolute ethanol	20170209	Harbin Xinda Chemical Plant
Ethyl ether	20170529	Tianjin Fuyu Fine Chemical Co., Ltd

2.1.2 Experimental instrument

Instrument	Model	Manufacturer
Ultraviolet visible spectrophotometer	TU-1901	Beijing Puxi General Instrument Co., Ltd
Small high speed crusher	WK-600A	Qingzhou FUERKANG Pharmaceutical Machinery Co., Ltd
Low speed table centrifuge	TEL-50B	Shanghai Anting Scientific Instrument Factory
Three use constant temperature water bath	HH-600	Shanghai billon Instrument Co., Ltd
Collector type constant temperature heating magnetic stirrer	90-3	Shanghai Yarong biochemical instrument factory
Vortex mixer	HQ-60	North Tongzheng Biotechnology Development Company
Electronic balance	FA1004N	Shanghai Precision Scientific Instrument Co., Ltd
Drying oven	HPX-9082 MBE	Shanghai bosun Industrial Co., Ltd. medical equipment factory
Refrigerator	BCD-224	SIEMENS

2. Experimental method

2.2.1 Animal model preparation and grouping

Twenty healthy SD rats (purchased from the Second Affiliated Hospital of Harbin Medical University), half male and half female, with a body weight of (160 ± 20) g, were normally raised in SPF animal room of Mudanjiang Medical College Medical Research Center, with 5 rats in each cage. The temperature is 20-25 °C, the relative humidity is 40% - 60%, the ventilation is good, the light / dark condition is 12h / 12h, free drinking and eating. After 18 months, the natural aging rat model was established. 20 rats were randomly divided into natural aging group and pine needle

proanthocyanidins extract group. One week before the experiment, 10 healthy SD rats (from the Second Affiliated Hospital of Harbin Medical University) were purchased. They were 3 months old, half male and half female, with a weight of (160 ± 20) g. they were the blank control group.

2.2.2 Administration method and dosage

The Songzhen proanthocyanidins extract group was administrated by gavage once a day, 3 ml each time. The blank control group and the natural aging group were administrated by gavage with the same volume of normal saline. The experimental period was 4 weeks.

2.2.3 detection of serum indexes of rats

The rats were anesthetized with ether, the eyeball was removed, blood was taken out, put into 5ml EP tube, put into a low-speed desktop centrifuge, 3500 rpm, centrifuged for 10 minutes, and the supernatant was taken for determination, that is, serum. The contents of total antioxidant capacity (T-AOC), glutathione peroxidase (GSH PX) and total superoxide dismutase (SOD) were determined.

2.2.4 Data analysis

Data are analyzed and processed by spss20.0 statistical software. Data are expressed by means of mean \pm standard deviation. Statistical method is single factor analysis of variance. $P < 0.05$ is statistically significant, $P < 0.01$ is extremely significant.

3. Results and discussion

3.1 Effect of pine needle proanthocyanidins extract on T-AOC in rats

Table 1 results of the effect of pine needle proanthocyanidins extract on T-AOC in rats ($\bar{x} \pm S$)

Group	Number	T-AOC($\bar{x} \pm S$ mol/L)
Blank control group	10	16.135 \pm 2.934
Natural aging group	10	9.631 \pm 2.116*
Gastric administration of extract	10	13.649 \pm 3.121* Δ

Note: compared with the blank control group, * P < 0.05; compared with the natural aging group, Δ P < 0.05.

3.2 Effect of pine needle proanthocyanidins extract on GSH PX in rats

Table 2 results of GSH PX effect of pine needle proanthocyanidins extract on rats ($\bar{x} \pm S$)

Group	Number	GSH-Px (\bar{x} mol/L)
Blank control group	10	213.2 \pm 75.32
Natural aging group	10	123.2 \pm 59.2*
Gastric administration of extract	10	181.1 \pm 81.6* Δ

Note: compared with the blank control group, * P < 0.05; compared with the natural aging group, Δ P < 0.05.

3.3 Effect of pine needle proanthocyanidins extract on SOD in rats

Table 3 results of the effect of pine needle proanthocyanidins extract on SOD in rats ($\bar{x} \pm S$)

Group	Number	SOD (\bar{x} mol/L)
Blank control group	10	353.28 \pm 8.24
Natural aging group	10	205.87 \pm 4.12*
Gastric administration of extract	10	240.29 \pm 3.98* Δ

Note: compared with the blank control group, * P < 0.05; compared with the natural aging group, Δ P < 0.05.

3.4 Discussion

Because the activity of (T-SOD) is closely related to aging, the reasonable decrease of its content in a certain range is considered as an important index of aging. Many researchers at home and abroad have carried out a lot of scientific experiments from different angles and different aspects on the reason why the activity of (T-SOD) decreases with the increasing of age, but failed to make a satisfactory explanation and reach a unified conclusion. The research shows that the analysis of the activity of

T-SOD after aging shows that, on the one hand, with the increase of age, the content of oxygen free radical [14], hydroxyl free radical and other active oxygen in the body is constantly increasing, which increases the consumption and damage of T-SOD. On the other hand, with the increase of age, the physiological functions of various organs of the body are also declining, and the regulatory capacity of endocrine system is declining, which can not guarantee the source of new enzymes, resulting in the rapid decline of (T-SOD) activity.

In addition, the content and antioxidant capacity of GSH Px, vitamin C and vitamin E absorbed or stored in the body are decreased. Because these substances and their functions are the same as those of (T-SOD), and they can protect each other, coordinate with each other and complement each other. The decrease of their content and activity can greatly increase the rate of (T-SOD) deactivation.

4. Conclusion

After intragastric administration of the pine needle extract of naturally aging rats, the data show that the pine needle extract effectively acts as an antioxidant and has the ability to scavenge free radicals. Therefore, it is of great scientific significance to develop and use pine needles for further research.

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