Study on Anti-radiation Effect of Ya'an Tibetan Tea

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Abstract. To explore the protective effects of Ya'an Tibetan Tea on 60Coγ irradiation damage in mice, forty-eight male SPF mice were randomly divided into six groups, including normal control group, irradiation control group, positive control group and Ya'an Tibetan tea low, medium and high-dose groups. Each group of mice were administrated with corresponding test samples for continuously 15 days. All mice were irradiated by 60Coγ-rays at the dose of 5 Gy except normal control group on the 6th day. After gavage, the mice were dissected and blood was taken from the eyeballs. The total antioxidant indexes of liver tissues, peripheral blood cells, and bone marrow DNA content of the mice were measured. The results showed that compared with the radiation control group, the contents of peripheral blood cells in the three dose groups of Ya'an Tibetan Tea increased in varying degrees, with the largest increase in the medium-dose group. In the medium-dose group, WBC were significantly increased (P<0.01), and RBC were increased (P<0.05), and the content of bone marrow DNA of the medium-dose group was significantly different from the radiation control, and MDA significantly decreased (P<0.01), and T-SOD activities and T-AOC activities significantly increased (P<0.01). The study shows that Ya'an Tibetan tea has a strong protective effect on radiation-damaged mice, and the medium-dose group has the best effect.

1. Introduction

With the development of society and economy, at the same time science and technology have brought convenience to human beings, they have also brought about radiation hazards that cannot be ignored [1]. Studies have shown that long-term low-dose ionizing radiation can increase the number of red blood cells and the proportion of eosinophils, reduce the proportion of monocytes, and increase the detection rate of giant platelets; And high doses of ionizing radiation can hinder the growth of lymphocytes: High-dose ionizing radiation will cause the generation of free radicals, and induce a chain reaction of a series of reactive oxygen species and free radicals, causing damage to cell biofilms, DNA, proteins, and a series of small molecule glycolipids [2], thus making the body immune Reduced ability, and may even cause the occurrence of malignant tumors [3-4]. Therefore, exploring effective radiation protection measures is of great significance to human health [5].

Ya'an Tibetan tea belongs to dark tea, with 1 bud, 3 to 5 new shoots as raw materials, and the basic processing steps include mainly killing greens, rolling, vineyards and drying [6], among which vineyards are the key process for the quality of Tibetan tea. A series of oxidation, condensation, and hydrolysis reactions led by tea polyphenols under the action of damp heat and microorganisms [7],
which make Ya'an Tibetan tea rich in a variety of organic acids, tea polyphenols, and their oxidation products, tea polysaccharides and other biologically active substances [6]. Studies have shown that Ya'an Tibetan Tea has good health effects, can effectively remove free radicals, increase the body's antioxidant function [8], and also has weight loss, lipid reduction, anti-cancer and laxative effects [9-11]. No research on its radiation resistance has been reported.

This article intends to explore the protective effect of Ya'an Tibetan tea on ionizing radiation-damaged mice through animal experiments, to provide relevant evidence for future research on the health effects of Ya'an Tibetan tea, to develop the Ya'an Tibetan tea deep-processed products and to promote the revitalization of Sichuan's border tea industry has great significance.

2. Material and methods

2.1. Materials and instruments

6 to 8-week-old male SPF Kunming mice, weighing 22-24 g, were selected for the experiment. SCXK (Sichuan) 2013-24 was purchased from Chengdu Dashuo Biotechnology Co., Ltd. Ya'an Tibetan Tea (with 1 bud, 4, 5 leaves, produced in 2014) was provided by Ya'an Yixing Tea Factory. Reagent: Total Antioxidant Capacity (T-AOC), Total Superoxide Dismutase (T-SOD) Kit (purchased from Nanjing Jiancheng Biotechnology Research Institute); all chemical reagents used were of analytical grade.

Radiation source $^{60}$Coγ (Institute of Biochemistry, Sichuan Academy of Agricultural Sciences), HH-6 type digital constant temperature water bath (Changzhou), DHG-9245A electric constant temperature blast dryer (Shanghai), DZF-6090 vacuum drying oven (Shanghai), V-1600 visible spectrophotometer (Shanghai), UV-1800 ultraviolet spectrophotometer (Shanghai), 2-16K high-speed refrigerated centrifuge (Japan), RE-2000 rotary evaporator (Shanghai).

2.2. Experiment method

2.2.1. Experimental animal group processing. Forty-eight healthy male Kunming mice of SPF were selected in the experiment, weighing 22-24 g. After 5 days of adaptive feeding, they were randomly divided into 6 groups of 8 mice each, including normal control group, radiation control group, and positive control group (amifostine, 150mg / kg), Ya'an Tibetan tea low, medium and high dose groups (0.5g / kg, 1.0g / kg, 2.0g / kg). According to the research on Tokyo Japan's Sangye study, the daily tea dosage for adults is 0.1g / (kg • bw) [13-14], and the mice's gavage dose is 5 times the human's dosage [15]. The medium and high doses of tea are two or four times the base dose. After grouping, gavage was started. The control group was gavage distilled water, and the other groups were gavage corresponding test samples.

2.2.2. Gavage and radiation treatment. After 5 days of continuous intragastric administration of the corresponding test samples in each group, except for the normal control group, all groups were irradiated with $^{60}$Coγ-rays for a whole-body irradiation on the 6th day. The total dose was 5 Gy, the dose rate was 1.0 Gy / min, and the irradiation time was 5min. The positive control group was injected intraperitoneally with amifostine solution (150mg / kg) 30 minutes before the irradiation [16]. After irradiation, the corresponding test substance was administered to the stomach for 10 days.

2.2.3. Determination of DNA content in peripheral blood cells and bone marrow. Blood samples were collected by eyeball blood collection. The collected blood samples were stored in anticoagulation tubes, and the content of white blood cells (WBC) and red blood cells (RBC) in the blood was measured with a blood cell analyser.

DNA content determination [17]: The mouse femurs were taken and immersed in 75% alcohol, the right bone and femoral meat was removed and dried, and the femoral heads was cut off at both ends and was weighed with an electronic balance. All bone marrow was rinsed into a centrifuge tube with 10mL 0.005 mol / L CaCl2, and it was put in a refrigerator at 4°C for 30min, and taken out, and
centrifuged for 15min at a speed of 2500 r / min. The lower liquid was removed and added to 5mL 0.002 mol / L HClO₄ and shaken and mixed, and put in a 90°C water bath for 15min, then and cooled with running water, and then centrifuged in a centrifuge for 10 minutes at a speed of 3500 r / min. Finally, the absorbance OD value of the supernatant was measured with a UV spectrophotometer at 68 nm.

Coefficient of DNA = DNA absorbance (A268) / femoral weight (g)                      (1)

2.2.4. Determination of total antioxidant activity in liver tissue. 0.2 g of liver tissue of each group of mice was weighed with an electronic balance, and prepared a 10% homogenate with an electric homogenizer, and then centrifuged in a centrifuge for 10 minutes at a speed of 2000r / min. The supernatant was taken and measured with reference to the kit Liver T-SOD, T-AOC activity and MDA content in mice of each group.

2.3. Data recording and processing
SPSS19.0 was used for data analysis. The comparison between different dose groups and the control group was performed by analysis of variance. All experimental data were expressed as mean ± standard deviation. The results were significantly different at P <0.05 and extremely significant at P <0.01.

3. Results and Analysis

3.1. Effect of Ya'an Tibetan Tea on Peripheral Blood Cells of Radiation Damaged Mice
As can be seen from Table 1, compared with the normal control group, the levels of WBC and RBC in the blood of the mice in the radiation control group significantly reduced (P <0.01), which indicated that radiation reduced the hematopoietic function of the mice and the radiation damage model was successfully established. Compared with the radiation control group, the contents of three groups of Ya'an Tibetan tea increased in varying degrees, among which the medium-dose group had the largest increase, and the WBC content increased significantly (P <0.01), and the RBC content increased significantly (P <0.05) which was not significant compared with the positive control group (P > 0.05). The results showed that Ya'an Tibetan tea could protect the mice from damage to hematopoietic function to a certain extent, and the effect of the medium-dose group was the most obvious, achieving the effect of a positive drug.

| Group                        | WBC(10⁹/L)   | RBC(10¹²/L)   |
|------------------------------|--------------|--------------|
| Normal control               | 5.77±0.30    | 8.04±0.61    |
| Radiation contrast           | 2.00±0.13    | 7.02±0.34    |
| Positive control             | 2.62±0.19    | 7.53±0.48    |
| Ya'an Tibetan -low dose      | 2.36±0.17    | 7.4±0.33     |
| Ya'an Tibetan-medium dose    | 2.60±0.23    | 7.52±0.28    |
| Ya'an Tibetan-high dose      | 2.18±0.08    | 7.29±0.39    |

Note: *. Compared with the normal control group, the difference was significant (P <0.05). **. Compared with the normal control group, the difference was extremely significant (P <0.01). #. Compared with the radiation control group, the difference was significant (P <0.05). ###. Compared with the radiation control group, the difference was extremely significant (P <0.01); Δ. Compared with the positive control group, the difference was significant (P <0.05). ΔΔ. Compared with the positive control group. The difference was extremely significant (P <0.01). The below was the same.
3.2. Effect of Ya'an Tibetan Tea on the DNA Content of Bone Marrow of Radiation Damaged Mice

As shown in Table 2, compared with the normal control group, the bone marrow DNA content of the radiation control group significantly reduced (P <0.01), which indicated that the radiation damage mouse model was successfully established. Compared with the radiation control group, Ya'an Tibetan tea was administered differently. The DNA content of the dose group increased in varying degrees, and the coefficients of the medium=dose group and the radiation control group were significantly different (P <0.01), and there was no significant difference compared with the positive control group (P> 0.05). The above results showed that the dose of Ya'an Tibetan Tea could significantly increase the bone marrow DNA content in mice, and the effect was comparable to that of the positive control group (P> 0.05).

Table 2. Effects of Ya'an Tibetan tea on DNA content in bone of irradiation damage in mice

| Group                    | DNA coefficient |
|--------------------------|-----------------|
| Normal control           | 0.85±0.04*##△△  |
| Radiation contrast       | 0.50±0.04**△△   |
| Positive control         | 0.65±0.01*##    |
| Ya'an Tibetan -low dose  | 0.62±0.05**##   |
| Ya'an Tibetan-medium=dose| 0.63±0.08**##   |
| Ya'an Tibetan-high dose  | 0.56±0.02**     |

3.3. Effects of Ya'an Tibetan Tea on Total Antioxidant Activity on Liver Tissues of Radiation Damaged Mice

As shown in Table 3, compared with the radiation control group, the T-AOC and T-SOD activities of the Ya'an Tibetan tea group and the positive control group at different doses increased in the gavage group, and the high-dose group showed a significant increase (P <0.01). There was no significant difference with the positive control (P> 0.05). Compared with the radiation control group, the MDA content in the gavage Ya'an Tibetan tea group significantly reduced (P <0.01), and the content in the medium=dose group was closer to the normal control group, which showed that Ya'an Tibetan tea could enhance the antioxidant capacity of radiation-injured mice to a certain extent, and the effect of the medium=dose group is the most obvious.

Table 3. Effects of Ya'an Tibetan tea on activity of liver of irradiation damage in mice

| Group                        | T-AOC (U/mgprot) | T-SOD (U/mgprot) | MDA (μmol/mgprot) |
|------------------------------|------------------|------------------|-------------------|
| Normal control               | 1.048±0.0733*##△△| 167.63±3.95*##△△| 0.99±0.10*##△△   |
| Radiation contrast           | 0.356±0.028**△△ | 109.21±5.44**△△ | 2.01±0.17*##     |
| Positive control             | 0.664±0.066***## | 126.59±7.69***##| 1.538±0.0737***##|
| Ya'an Tibetan -low dose      | 0.511±0.039**△   | 116.33±2.12**△  | 1.51±0.21**##    |
| Ya'an Tibetan-medium=dose   | 0.609±0.126***## | 127.29±6.11***##| 1.46±0.09**##    |
| Ya'an Tibetan-high dose      | 0.514±0.068***## | 114.08±4.34**△  | 1.60±0.04**##    |

4. Conclusion

Radiation causes the polar macromolecules in the body to vibrate and generate heat through thermal effects, increasing tissue temperature, protein denaturation, and inactivation of enzymes in the body [18], while inhibiting the growth of thymocytes, reducing their differentiation ability, and significantly increasing S-phase cells. Decreased, while the proportion of G0, G1 phase cells increased. Autologous T cell proliferation ability is significantly reduced, and the immune system is disordered [19]. In addition, studies have suggested that external radiation fields cause vibration of molecules and ions in the body, leading to changes in cell membrane fluidity, potential, and permeability, resulting in DNA single-strand breaks and double-strand breaks [20]. In this experiment, the number of peripheral blood
cells and DNA content in the blood of mice decreased rapidly after irradiation, indicating that their cell proliferation ability may be inhibited to a certain extent, thereby affecting the blood circulation of the mice. However, compared with the radiation group, the red and white blood cells of the mice were significantly increased, and the DNA content of the mice was also higher than that of the radiation group, which indicated that the Ya'an Tibetan tea had a certain recovery effect on the hematopoietic and immune ability of the irradiated mice, and it could well reduce the DNA breakage and stabilize its content, and had a protective effect on the damage of hematopoietic stem cells in the bone marrow. Enhance the mouse's immune system, indicating that its effect is equivalent to that of the drug. The best dose is the medium-dose of Ya'an Tibetan Tea.

Antioxidant system of mouse liver is divided into antioxidant enzyme system and non-enzyme system. T-AOC reflects the overall level of the two systems [21], T-SOD is a kind of enzyme system that can remove superoxide anion free radical Specific enzymes [22]; MDA is a lipid peroxide produced by free radicals attacking phospholipid molecules on biofilms, which can indirectly reflect the level of lipid peroxidation in the body [23]. After the mice were irradiated with 60Coγ rays, a large amount of free radicals were generated in the body, attacking unsaturated fatty acids on the membrane, leading to aging and canceration of the body [16]. The experiment showed that the liver T-AOC and T-SOD activities of the mice increased correspondingly after administering Ya'an Tibetan tea, and the MDA content decreased, indicating that Ya'an Tibetan tea has a certain protective effect on the antioxidative damage of mice. It is related to the effect of water extracts of Ya'an Tibetan tea on scavenging superoxide anion radicals and hydroxyl radicals [8], and the specific related active ingredients and their mechanism of action in water extracts need further research.

To sum up, the radiation of 60Coγ-ray could cause the mice's anti oxidative function and hematopoietic function to decline to a certain extent, and the administration of Ya'an Tibetan tea could significantly increase the peripheral blood cell content and liver tissue T-AOC and T-SOD activities reduce the MDA content in the liver of mice and increase the bone marrow DNA content. The results showed that Ya'an Tibetan tea had a strong protective effect against 60Coγ-ray radiation damage, and the medium-dose group had the best effect.

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