Effects of the *Coreopsis tinctoria* extracts on anti-aging in the aging model mice

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**ABSTRACT**

The effects of the *Coreopsis tinctoria* extracts on anti-aging were observed by investigating the cerebral index and visceras indexes, the contents of hydrogen peroxide (H₂O₂) and malondialdehyde (MDA) in the sera, the activities of glutathione peroxidase (GSH-Px) in the brain tissues and the ones of catalase (CAT) and superoxide dismutase (SOD) in the liver tissues of the aging model mice. The aging model mice were injected subcutaneously with D-galactose in vivo and intragastric administrated with the *Coreopsis tinctoria* extracts at doses of low (0.5g/kg), medium (1g/kg) and high (2g/kg) once daily for 6 weeks. The results showed that all the cerebral index, spleen index, thymus index, liver index and kidney index of the three groups dosed of the *Coreopsis tinctoria* extracts increased, the activities of GSH-Px in the brain tissues and the ones of CAT and SOD in the liver tissues increased to different degree while the contents of H₂O₂ and MDA in the sera decreased extremely and significantly (P<0.01) compared with the aging model mice. All of these results suggested that the *Coreopsis tinctoria* extracts might possess anti-aging effects by improving antioxidant capacity of the mice.

**Key words:** Aging model mice, Anti-aging, Antioxidase, *Coreopsis tinctoria* extracts.

**INTRODUCTION**

Being an important member of the Chrysanthemum family, *Coreopsis tinctoria* Nutt. is widely distributed in North America and Southern Xinjiang, China. It grows in the Karakorum Mountainous Regions at altitudes of 3000 meters or even higher above sea level in the Southern Xinjiang (Guo et al., 2015), known as “snow chrysanthemum” or “snow tea” locally. It has been reported that *Coreopsis tinctoria* possessed many important biological activities, such as hypolipidemic activity (Li et al., 2014; Liang et al., 2009, 2010a, 2010b), hypoglycaemic activity (Mao et al., 2014; Zhang et al., 2011a, b; Dias et al., 2010), antioxidant activity (Wang et al., 2015; Yao et al., 2016; Okada et al., 2014; Zalaru et al., 2014), anti-inflammatory activity (Zhang et al., 2013), anti-neurodegenerative properties (Li et al., 2015) and the reduction of blood pressure and vasorelaxant effects (Yang et al., 2014; Sun et al., 2013; Ming et al., 2012; Liang et al., 2010b). Despite those extensive studies, the anti-aging effects of the *Coreopsis tinctoria* extracts have not been reported. In this work, the anti-aging effects of the extracts from *Coreopsis tinctoria* were investigated by the aging model mice induced by D-galactose. The research might provide scientific evidence for the further application of *Coreopsis tinctoria* in pharmacology or bromatology.

**MATERIALS AND METHODS**

**Chemicals and reagents:** The flowers of *Coreopsis tinctoria* were provided by Xinjiang Hetian Shamo Meigu Co., Ltd, China and air-dried at room temperature. D-galactose and triton X-100 were purchased from Sigma Chemical Co., USA. Vitamin E and hydrogen peroxide (H₂O₂) were obtained from Fluka Chemie AG, Switzerland. Ammonium molybdate was from Shanghai Hushi Chemical Industry Co., Ltd, China. Commercial kits used for the determination of H₂O₂, malondialdehyde (MDA), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide dismutase (SOD) and Coomassie blue protein were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). All other chemical agents used were of analytical grade.

**Preparation of the *Coreopsis tinctoria* extracts:** The extracts of *Coreopsis tinctoria* was prepared as follows: 10 g *Coreopsis tinctoria* in 100 mL distilled water were autoclaved at approximately 120°C for 20 min, then filtrated and freeze dried, and the high (2g/kg) doses of *Coreopsis tinctoria* extracts were obtained. In this experiment, the animal dose of *Coreopsis tinctoria* extracts was the dose of the crude drug.

**Animals and experimental protocol:** Male and female Kunming mice (body weight 18-22g, n=36 for each sex), were provided by the Experimental Animal Center, Tarim University (Alar, Xinjiang, China). The animals were bred and housed under conventional experimental animal facilities with free access to food and water. All the animal procedures were approved by the Institutional Animal Ethical Committee. All Kunming mice were randomly divided into...
six groups, and each group consisted of 12 animals. The groups were categorized as normal (normal saline s.c. and i.g.), model (D-galactose s.c. and normal saline i.g.), positive control groups (D-galactose s.c. and Vitamin E i.g.) and low (D-galactose s.c. and 0.5g/kg•d Coreopsis tinctoria extracts i.g.), medium (D-galactose s.c. and 1g/kg•d Coreopsis tinctoria extracts i.g.), high (D-galactose s.c. and 2g/kg•d Coreopsis tinctoria extracts i.g.) doses of Coreopsis tinctoria-extracts-treated (CTET) groups. The normal group was administered with 0.20mL/10g normal saline s.c. and 50mL/kg normal saline i.g. daily. All other groups were administered with 0.20mL/10g (i.e. 300 mg/kg) D-galactose s.c. once daily for 6 weeks to establish the aging model. The three CTET groups were administered with low, medium and high doses of Coreopsis tinctoria extracts i.g. once daily for 6 weeks, the positive control group was Vitamin E (50mg/kg•d), the model group was normal saline (50mL/kg•d). The animals were weighed weekly to adjust the dosage. Twenty-four hours after the last dose, the animals were sacrificed by decapitation to collect the blood into dried tubes, the blood was thereafter centrifuged at 4°C, 3500 rpm for 10 min to obtain the serum. The brains, spleens, thymuses, livers and kidneys were rapidly excised and thoroughly washed to clear off blood, and then weighed. The indices were expressed as the brain, spleen and thymus weights relative to the body weight. Cerebral (viscera) index is equal to brain (viscera)/body weight (in mg/g). The brain and liver tissues were immediately transferred to ice-cold normal saline and homogenized. The suspended tissues were centrifuged at 4°C, 3500 rpm for 10 min. GSH-Px, CAT, SOD, MDA, H$_2$O$_2$, and protein assays in the supernatants were detected according to the respective detection kits (Nanjing Jiancheng Institute of Bioengineering, Nanjing, China).

**Statistical analysis:** Experimental results were processed using SPSS 13.0 (SPSS Inc.). The data were expressed as the mean plus or minus standard deviation (SD). Dunnett’s t-test was used to compare the differences between the treated and control groups. Differences were regarded as significant at $P<0.05$, extremely significant at $P<0.01$.

**RESULTS AND DISCUSSION**

**Effects of the Coreopsis tinctoria extracts on cerebral index and viscera indexes in the aging model mice:**

As shown in Table 1, the cerebral index and viscera indexes in the aging model mice were substantially lower than in the normal mice, for example, the kidney index in the aging model mice significantly decreased compared with the normal group ($P<0.05$), the cerebral index, spleen index, thymus index and liver index significantly decreased compared with those in the normal group ($P<0.01$). The cerebral index and viscera indexes of the three CTET mice were higher than those of the aging model mice and the administration of Coreopsis tinctoria extracts was dose-depended. For example, compared with those of the aging model mice, the spleen index of the three CTET groups, the cerebral index and thymus index of the high-doses group, the liver index and kidney index of the medium and high-doses groups were all significantly increased ($P<0.01$). Moreover, the kidney index of the low-doses group, the cerebral index and thymus index of the medium-doses group were all significantly increased compared with the aging model mice ($P<0.05$).

**Effects of the Coreopsis tinctoria extracts on the contents of free radicals in the serums, the activities of antioxidase in the tissues of the aging model mice:**

As shown in Table 2, in the aging model group, the contents of H$_2$O$_2$ and MDA in the serums significantly increased while the activities of GSH-Px in the brain tissues and the activities of CAT and SOD in the liver tissues significantly decreased compared to those of the normal group ($P<0.01$). The activities of GSH-Px in the brain tissues and the activities of CAT and SOD in the liver tissues of the three CTET groups were higher than those of the aging model mice and the administration of Coreopsis tinctoria extracts were dose-depended. First, the activities of CAT and SOD in the liver tissues of the three CTET groups, the activities of GSH-Px in the brain tissues of the medium-doses and high-doses groups were all significantly increased compared with those of the aging model mice ($P<0.01, P<0.05$). Compared with the aging model group, the contents of H$_2$O$_2$ and MDA in the serums of the three CTET groups and the positive control group were all markedly decreased ($P<0.01$). There were very significantly increases in the activities of GSH-Px in the brain tissues and in the ones of CAT and SOD in the liver tissues of the positive control group compared with those of the aging model mice ($P<0.01$).

**Table 1:** Effects of the Coreopsis tinctoria extracts on cerebral index and viscera indexes in the aging model

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (g/kg)</th>
<th>Cerebral index (mg/g)</th>
<th>Spleen index (mg/g)</th>
<th>Thymus index (mg/g)</th>
<th>Liver index (mg/g)</th>
<th>Kidney index (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>—</td>
<td>16.30±3.58</td>
<td>6.55±1.84</td>
<td>1.59±0.49</td>
<td>56.57±6.22</td>
<td>11.95±1.82</td>
</tr>
<tr>
<td>Aging model</td>
<td>—</td>
<td>12.03±1.25**</td>
<td>2.62±0.29**</td>
<td>0.52±0.21**</td>
<td>43.19±0.48**</td>
<td>9.45±0.68*</td>
</tr>
<tr>
<td>Positive control group</td>
<td>0.05</td>
<td>14.42±1.09**</td>
<td>4.10±0.67**</td>
<td>1.07±0.40**</td>
<td>49.77±5.59*</td>
<td>10.64±1.15</td>
</tr>
<tr>
<td>Low doses group</td>
<td>0.5</td>
<td>12.83±0.73</td>
<td>5.79±0.84**</td>
<td>0.81±0.30</td>
<td>46.85±6.96</td>
<td>10.52±0.64*</td>
</tr>
<tr>
<td>Medium doses group</td>
<td>1</td>
<td>13.39±0.36**</td>
<td>7.00±0.36**</td>
<td>0.92±0.31**</td>
<td>51.32±1.72**</td>
<td>12.26±0.78**</td>
</tr>
<tr>
<td>High doses group</td>
<td>2</td>
<td>14.00±0.36**</td>
<td>7.28±1.52**</td>
<td>1.38±0.24**</td>
<td>58.45±6.72*</td>
<td>13.05±0.93*</td>
</tr>
</tbody>
</table>

Note: Compared with the normal groups, $\*P<0.05$, shows significant difference; Compared with the aging model groups, $\*\*P<0.01$, shows extremely significant difference. The same as below.
D-galactose is a simple monosaccharide that can be metabolized in the human body as a normal nutrient. However, D-galactose may be further metabolized to hydrogen peroxide and superoxide anion when its concentration is too high (Yu et al., 2015), excess oxygen radicals can cause oxidative damage of normal cells. Therefore, the mice treated with D-galactose are usually used as an experimental aging model for the study of anti-aging effects. The results shows that the cerebral index and viscera indexes in the aging model mice are substantially lower than the normal mice (P<0.05 or P<0.01). The significant atrophy of the brain and viscera indicates that the aging models were successful, meanwhile the three doses of the Coreopsis tinctoria extracts could resist the atrophy of the brain and viscera induced by D-galactose.

Table 2: Effects of the Coreopsis tinctoria extracts on the contents of free radicals in the sera, and the activities of antioxidase in the tissues of the aging model mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Doses (g/kg)</th>
<th>H$_2$O$_2$ (nmol/g)</th>
<th>MDA (nmol/ml)</th>
<th>GSH-Px (U)</th>
<th>CAT (U/ml)</th>
<th>SOD (U/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal group</td>
<td>—</td>
<td>0.57±0.05</td>
<td>4.06±0.41</td>
<td>285.93±34.95</td>
<td>0.12±0.03</td>
<td>3.60±0.08</td>
</tr>
<tr>
<td>Aging model group</td>
<td>—</td>
<td>1.09±0.05</td>
<td>6.53±0.52</td>
<td>146.75±18.32</td>
<td>0.02±0.01</td>
<td>2.72±0.13</td>
</tr>
<tr>
<td>Positive control group</td>
<td>0.05</td>
<td>0.71±0.04</td>
<td>5.10±0.45</td>
<td>257.14±45.39</td>
<td>0.19±0.04</td>
<td>4.17±0.10</td>
</tr>
<tr>
<td>Low doses group</td>
<td>0.5</td>
<td>0.87±0.01</td>
<td>5.68±0.39</td>
<td>160.62±21.13</td>
<td>0.14±0.01</td>
<td>4.28±0.13</td>
</tr>
<tr>
<td>Medium doses group</td>
<td>1</td>
<td>0.69±0.02</td>
<td>5.09±0.37</td>
<td>228.35±29.53</td>
<td>0.17±0.06</td>
<td>4.91±0.16</td>
</tr>
<tr>
<td>High doses group</td>
<td>2</td>
<td>0.65±0.04</td>
<td>4.66±0.34</td>
<td>261.39±31.73</td>
<td>0.23±0.02</td>
<td>6.55±0.04</td>
</tr>
</tbody>
</table>

The cumulative radicals generated from aerobic respiration would cause oxidative damage, then further result in aging and death. Antioxidants may postpone aging by reacting directly with free radicals, or by enhancing the activity or expression of antioxidant enzymes, such as GSH-Px, CAT, SOD (Lü et al., 2010) and so on. GSH-Px can scavenge reactive oxygen species and prevent H$_2$O$_2$-induced hydroxyl radical formation. The primary role of CAT is to abolish H$_2$O$_2$, which has been generated by free radicals or SOD in removal of superoxide anions and then convert it to water (Urso and Clarkson, 2003). SOD dismutates superoxide radicals into hydrogen peroxide, which in turn is decomposed into water and oxygen by GSH-Px and CAT, thus preventing the formation of hydroxyl radicals (Yao et al., 2005). MDA is one of the end products in the lipid peroxidation process that leads to an increase in phospholipids rigidity (Esterbauer et al., 1991). The results showed that the three doses of the Coreopsis tinctoria extracts could increase all the activities of GSH-Px in the brain tissues and the ones of CAT and SOD in the liver tissues to different degree and decrease the contents of H$_2$O$_2$ and MDA in the sera extremely and significantly (P<0.01) compared with the aging model mice. The findings were consistent with previous reports (Liu et al., 2015; Yu et al., 2015; Farahmand et al., 2013; Liu et al., 2012; Ji et al., 2009). In conclusion, all results suggested that the Coreopsis tinctoria extracts possessed prominent anti-aging effects, and could be a promising adjuvant agent for preventing aging, the anti-aging properties might be related with its abilities of decreasing the product of lipid peroxide and increasing the activities of antioxidative enzymes. Therefore future studies will be needed to determine the mechanism of the anti-aging effects.

DECLARATION OF INTEREST

The authors have declared that there were no conflicts of interest.

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