

## Study on Antioxidant Activity of *Typhonium giganteum* Engl. Tuber Extracts *in vitro* and *in vivo*

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### Abstract

To study the antioxidant effect of the aqueous extracts of *Typhonium giganteum* Engl. tuber (AEoTGE) and ethanol extracts of *Typhonium giganteum* Engl. tuber (EEoTGE) *in vitro* and *in vivo*, which may be the possible mechanism of its bioactivity efficacy. The antioxidant activity *in vitro* was evaluated by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging rate with Vitamin C as a positive control. The antioxidant activity *in vivo* was carried out to investigate the effects of AEoTGE and EEoTGE on D-galactose caused aging model mice by determining liver tissues' superoxide dismutase (SOD) activities. The results indicated that AEoTGE and EEoTGE have good antioxidant activity *in vivo* and *in vitro*. The antioxidant effect of AEoTGE is better than that of EEoTGE.

**Keywords:** *Typhonium giganteum* Engl.; Antioxidant; Bioactivity

### Introduction

*Typhonium giganteum* Engl. belongs to the family Araceae, which is a Chinese endemic plant. The dried root tuber of which has been used as a Chinese traditional medicine (Baifuzi) recorded in Chinese Pharmacopoeia [1]. *Typhonium giganteum* Engl. has many chemical constituents including glycosides, amino acids, proteins, sterols, sugars, flavonoids, trace elements and other substances [2-8]. Its rich constituents determine its pharmacological effects such as anti-bacterial, anti-inflammatory and anti-cancer etc [9-11]. With the development of herbal drugs bioactivity mechanism research, it was discovered that the efficacy of herbal medicine is closely related to its antioxidant effects. In this study, the antioxidant activity of *Typhonium giganteum* Engl. tuber extracts *in vitro* and *in vivo* is mainly discussed.

### Materials and Reagents

#### Plants and reagents

*Typhonium giganteum* Engl. was collected from Tonghua Country, Jilin Province, P.R.China; 1,1-Diphenyl-2-picrylhydrazyl (DHHP) were purchased from Sigma Chemical Co., Ltd.; SOD test kit were bought from Nanjing Jiancheng Bioengineering Institute. All the other reagents were analytical grade.

#### Experiment animals

Fifty female Kunming mice (20 ± 2 g) were purchased from Laboratory Animal Center of Liaoning University of Traditional Chinese Medicine, certificate number: SCXK 2008-0005.

### Methods

#### Extraction of *Typhonium giganteum* Engl. tuber

100 g *Typhonium giganteum* Engl. tuber were washed and dried at 40-45°C. Subsequently, the dried roots were crushed to 20-mesh. 500 ml distilled water or 95% ethanol was added for soaking overnight at room temperature (25 ± 2°C). Heated to reflux for 3 hours and then filtered. The extraction procedure was repeated twice. The aqueous extracts of *Typhonium giganteum* Engl. tuber (AEoTGE) and ethanol extracts of *Typhonium giganteum* Engl. tuber (EEoTGE) were combined respectively, concentrated and dried at 60°C in vacuum. At the end, 9.6 g AEoTGE and 8.5 g EEoTGE were obtained and stored at 4°C until used.

#### Scavenging DPPH radical test *in vitro* [12]

Accurately weighted 1.97 g DPPH, dissolved in methanol and in 10 mL volumetric flask, DPPH concentration was 0.5 mmol/mL, which was stored at 4°C and protected from light. 0.5 ml of 0.5 mmol/mL DPPH solution and 2 mL of 1 mg/mL AEoTGE, EEoTGE or Vitamin C solution were mixed and rest for 30 min at 37°C, the absorbance of solution was measured at 510 nm and the value was recorded as A<sub>1</sub>. When using methanol instead of extract, the measured absorbance values were recorded as A<sub>0</sub>. When using methanol instead of DHHP, the measured absorbance values were recorded as A<sub>2</sub>. Then the scavenging rate (%) of sample could be expressed as:

$$SR = 1 - (A_1 - A_2) / A_0 \times 100\%$$

#### Animal grouping and administration

Fifty female Kunming mice were divided into five groups randomly (10 mice/group), including I: negative control group; II: AEoTGE group (1.0 g/kg); III: EEoTGE group (1.0 g/kg); IV: positive control group (Vitamin C, 1.0 g/kg); V: model group (D-galactose, 1.0 g/kg). All the Kunming mice were adapted in animal room for 1 week before they were administration. Model group, positive control group, AEoTGE group and EEoTGE group were injected of D-galactose, 1.0 g/kg on the nape for 42 days to cause aging model. AEoTGE and EEoTGE were dissolved in pure water to make 1.0 g/ml solution. The AEoTGE, EEoTGE and Vitamin C solution were orally administered into mice with the dose of 0.1 ml/10 g body weight, once per day for 15 d using a feeding atraumatic needle. After administration they were sacrificed by cervical dislocation and liver was taken. The liver was given an ice-bath in 0.9% iced normal saline and made into 10% tissue homogenate, centrifuged at 3500 rpm at 4°C for 20 min, then kept at -20°C for next testing.

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### Superoxide dismutase (SOD) test *in vivo*

Superoxide anion ( $O_2^-$ ) can promote the oxidation of hydroxylamine into its nitrite forms. Superoxide dismutase (SOD) is a special inhibitor to  $O_2^-$ . The assay for total superoxide dismutases (SOD) was based on its ability to inhibit the oxidation of oxyimine by the xanthine-xanthine oxidase system [13]. SOD activity was determined using SOD Assay Kit. The operation was in accordance with the specification requirements of SOD kit.

### Statistical analysis

All tests were carried out in 3 times. All data are expressed as s mean  $\pm$  standard. Statistical analysis was carried out using SPSS software (SPSS, version 10.0). Data were analyzed using by Student's t-test and were considered to be statistically significant if  $P \leq 0.05$ .

## Results and Analysis

### DPPH radical scavenging rate *in vitro*

The results were shown in Figure 1. According to the analysis of t-test, AEoTGE exhibited a similar anti-oxidation activity compared with Vitamin C ( $P < 0.01$ ). The radical scavenging rate of anti-oxidation of AEoTGE is higher than EEOtGE, the difference of them is 13.77%, which means that the anti-oxidation activity of AEoTGE is better than EEOtGE.

### Activity of SOD *in vivo*

As shown in Figure 2, compared with the normal group, SOD activities in liver of model group decreased significantly ( $P < 0.01$ ). This suggested that antioxidant capability of organism under D-galactose condition was evidently weakened. It was found the antioxidant activity increased after the administration of AEoTGE ( $P < 0.01$ ), EEOtGE ( $P < 0.01$ ) and Vc ( $P < 0.01$ ) compared with the model group. The result indicated that AEoTGE and EEOtGE could improve SOD activity of mice, and the AEoTGE group is better than EEOtGE group.

## Conclusion

In the present study, antioxidants regulate the body metabolism and promote the antioxidant capability of the organism, to further exert other functions and help to cure various diseases [14]. In order to study the possible relationship between antioxidant properties and the other effect such as anti-bacterial, anti-inflammatory and anti-cancer of *Typhonium giganteum* Engl., we examined the antioxidative potential of it *in vitro* and *in vivo*. Our data demonstrated that AEoTGE have good antioxidant effect compared with Vitamin C *in vitro* and *in vivo*. It could be a natural antioxidant eroxidation and its antioxidant activity should make contributions to its other aspect activities.

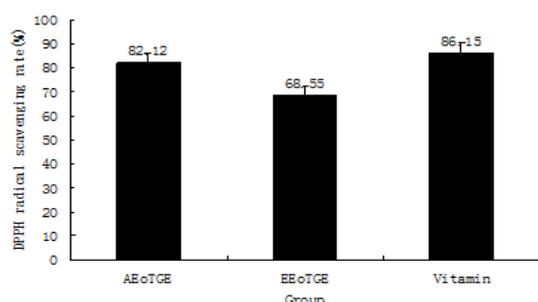


Figure 1: DPPH radical scavenging rate of AEoTGE and EEOtGE *in vitro*.

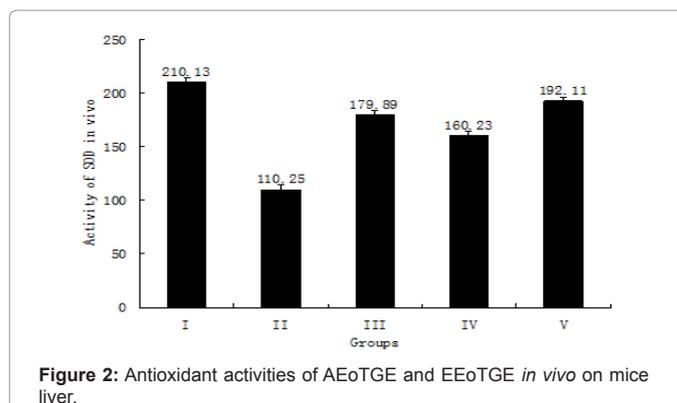


Figure 2: Antioxidant activities of AEoTGE and EEOtGE *in vivo* on mice liver.

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