Effect of the Extract from Fungus Garden of Termite on Drosophila Model of Parkinson’s Disease

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Abstract
Traditional Chinese medicine (TCM) was successfully used in many cases of chronic disease treatment. The fungus garden of termite, a mixture of leaves, growing fungi, and secretion of termite, is often used for protection of the liver, and treatments for disorder diseases, Parkinson’s disease (PD), and so on. However, the mechanism is unknown. To reveal the effective component, we applied the ethanol extract from fungus garden of termite treat Drosophila model
of PD. The result showed that the extract rescued the climbing defect, increased the movement activity, and extended the lifespan of the PD Drosophila. Histological observation of brain tissue of PD Drosophila indicated that the number of nerve cells increased and vacuoles reduced in the group treated with termite fungus garden. It implied that PD Drosophila model is effective in screening of TCM for treatment of PD disease, and the fungus garden of termite is helpful for cure of PD disease.

Keywords: fungus garden of termite, Drosophila, model of Parkinson’s disease

Introduction

Termites, a group of eusocial insects, were classified at the order of Isoptera. Until now, they are accepted as the epifamily Termitoidae, of the cockroach order Blattodea [1, 2]. They are major detritivores and mostly feed on dead plant material, particularly in the subtropical and tropical regions, and their recycling of wood and other plant matter is of considerable ecological importance. About 10% of the estimated 4,000 species are economically significant as pests that can cause serious structural damage to buildings, crops or plantation forests [3, 4]. All termites eat cellulose in its various forms as plant fiber. Some species of termites rely primarily upon microbes to digest the cellulose for them and absorb the end products for their own use. They maintain fungal gardens of specialized fungi of genus Termitomyces, which are nourished by the excrement of the insects [5]. When the fungi are eaten, their spores pass undamaged through the intestines of the termites to complete the cycle by germinating in the fresh faecal pellets. In the forests of Middle China, a specific kind of termite, Odontotermes formosanus, feeds only on wilted leaves and produces fungal gardens which are used as a kind of Traditional Chinese medicine (TCM). In Asian countries, TCM is an essential part of the healthcare system, and is considered a complementary or alternative medical system in most Western countries [6, 7]. The fungus garden of termite was used as a TCM to prevent bacteria infection through raising the ability of human immunity and to protect the liver [8, 9]. However, the mechanism is still unknown.

To get insight into the functions of fungus garden of termites, we collected the fungus garden of termite and confirmed its function on the model of Parkinson's disease (PD). PD is a chronic degenerative neurological disease, the cause of PD and its protection mechanisms are not clear. Oxidative stress may be related to PD and still lack of an effective treatment method for its pathogenesis [10, 11]. At current, drugs treatment of PD is the primary method in clinical-based therapy. The main approach is to inhibit cholinergic neurotransmitter and to enhance dopaminergic neurotransmitter. However, the treatments can only relieve symptoms but with some side effects. In our experiments, we investigated fungus garden of termite to prove if it has an effect on PD.
Material and Methods

Materials: Fungus garden of termite was collected and identified by the Termite Control Center of Jurong Forest. Drosophila UAS-wild-type, α-synuclein/+, and UAS-DdC were the gifts from Professor Jing Gao of Jiangsu University, and all reagents and experimental equipments were supplied by Institute of Life Science, University of Jiangsu.

Preparation of ethanol extract from termite fungus garden: Ten grams powder of termite fungus garden were soaked in 150 mL ethanol, and were heated under reflux extraction for 2 h. 150 mL of ethanol were added in the left powder to extract for 2 h again. After that, all the distillates were mixed together.

Maintaining and crossing of Drosophila: The flies were reared on standard medium and were kept at 25 degree according to the previous method [12]. Crosses were conducted using virgin females collected no longer than eight hours after eclosion at 25 degree. The crosses were performed at 29 degree. All adult offspring (F1) were collected up to 9 days after their eclosion at 25 degree to avoid offspring from the next generation (F2). The male flies carrying a driver \( \text{elav}^{c155} \)-Gal4 on their X chromosome were crossed to the females carrying the UAS-regulated A53T alpha-synuclein transgene on X chromosome. As a result, first generation (F1) female offspring expressing A53T alpha-synuclein in their nervous system which served as PD fly model [11, 12].

Special fly feeding: The Drosophila flies were divided into five groups, 240 /group, for the wild-type (WT) Drosophila (Non-PD), PD Drosophila (Ddc-the GAL4/+; the UAS-wild-type α-synuclein in/+), and PD Drosophila treated with 1mg, 10 mg, 100 mg of the ethanol extract powder of termite fungus garden (per 100g medium), respectively. The Drosophila were placed in medium which containing agar, corn meal, sugar, water and yeast extract at 25 degree, with propionic acid to inhibit fungal infections in the breeding process [11, 12].

Locomotive (climbing) assay and lifespan analysis: The method of Drosophila climbing experiment was described previously [13]. Briefly, ten flies were reared on medium supplemented with the tested compound in a vial, which was marked with red scaffold drawing an 80 mm line left from the vial bottom. The climbing ability was detected at room temperature (25 degree and 60-70% humidity undisturbed conditions). At the beginning of the test, all the flies were tapped lightly to the bottom of the vial. The percentage of flies which climbed along the test tube over the marker line in 10 s was monitored. Each class had 10 independent vial repeats. Lifespan analyses were performed using groups of 10–20 age-matched flies. The flies from each genotype were transferred to fresh standard food or supplemented food every 3-7 d, and the number of dead flies was recorded during each transfer. Transfers were continued until all flies died, and each experiment was performed in triplicate.

Histology analysis of brain tissue of PD fly: To compare the brain tissues of flies, 36-day old adult flies were dissected and their brains were selected in the test. After incubation in Paraformaldehyde, the specimens of whole brain were cut into slices and stained with Kongo red dye and were observed under microscope.
Data analysis: All the data obtained using SPSS 17.0 statistical software for test. ANOVA analysis of variance between the two groups for the q test. Differences for all data were considered statistically significant at $P < 0.05$.

Results

Climbing ability of Drosophila: The climbing ability of the flies was monitored for 48 days (Table 1). The results showed that the locomotion of PD flies deteriorated significantly from day 18 onwards. The untreated flies became almost immobile by day 24, while the control groups were active at this time. In contrast, the treated flies displayed dramatic improvement, behaving almost identical to the control group, presenting an increase in their climbing ability in comparison with the untreated group. In PD Drosophila groups with different concentrations of termite fungus garden, climbing ability of the group treated with 1 mg per 100 g medium were improved compared with the PD control group, and the climbing ability was apparently higher when the concentration increased. The climbing ability of PD Drosophila in three concentrations of garden alcohol extract was improved in different degrees, especially from 18-48 days (Table 1). This result indicated that the extract of termite fungus garden partially rescued the climbing defect and increased the movement activity of PD Drosophila.

Impact of termite fungus garden on Drosophila lifespan: We tested whether the protective effects of termite fungal garden would extend to the lifespan of PD Drosophila. We found that the maximum lifespan of PD Drosophila increased from 58.40 d on nonsupplemented food to 60.96 d on food containing termite fungal garden. The lifespan of PD male Drosophila decreased 6.4% comparing to Non-PD Drosophila, and the lifespan extended from 1.82%-3.34% ($p<0.01$) by treatment with termite fungal garden. Similar to male Drosophila, the lifespan of PD female Drosophila decreased 5.45% comparing with NON-PD Drosophila, and by the treatment with termite fungal garden, the lifespan extended from 1.47%-3.34% ($p<0.01$) (Table 2). This result indicated that termite fungal garden can prolong the Drosophila life with a dose-dependent manner.

Histological observation: The 36 d Drosophila brains were selected to compare the cells stained with Kongo red dye. The edge layer cells of brain tissue became thinning and disorganized, and the vacuoles increased in nerve cells in PD Drosophila, while the number of nerve cells increased and vacuoles reduced in the Drosophila flies treated with termite fungus garden (Figure 1).

Discussion

Termite fungus garden is composed of termite saliva, other secretions and leaf debris, richen in amino acids and other biological active substances. Its medical
properties were recorded in the "Compendium of Materia Medica" (a Chinese classic medicine book). It has functions in treatment of various inflammations, and is widely used in modern medicine. Researchers successfully developed a drug made of termite fungus garden for cough cure [8]. Pharmacology studies have shown that termite fungus garden also can improve the phagocytosis of murine macrophage and transformation of lymphocyte. This result provided a pharmacological basis for the conventional termite fungus garden used for the treatment of diseases.

PD pathological mechanism is not completely clear yet. It is probably caused by a combination of risk factors like the aging process, oxidative stress, genetic propensity, and environmental exposure [14]. Fundamental cellular processes of fruit flies are quite similar to humans”, including regulation of gene expression, subcellular trafficking, synaptic transmission, synaptogenesis, and cell death. Many signaling pathways are as well conserved between human and flies. Flies have simpler genetics but capable of performing complex biological behaviors, like walking, climbing, and flying. Furthermore, it has a short reproductive and developmental cycle. These advantages of Drosophila make it ideal for creating animal models of complex disorders that can individually model a subset of phenotypes associated with a disease in order to simplify analysis of the disease [15]. We successfully set up a Drosophila model of PD and used extract of termite fungus garden to treat the flies. This is the first report that the PD flies were treated with the components of Chinese herb.

Over-expression of α-synuclein in cells of neurodegenerative disease induced the change of mitochondria membrane potential (MMP), and affected mitochondrial structure and function, leading to tissue degradation [16]. Our results suggest the termite fungus gardens can change the PD Drosophila climbing ability, lifespan as well as brain tissue pathology. The results indicated that the ethanol extracts of termite fungus garden can improve the movement ability of the flies with PD, and prolong the lifespan of the flies, with a concentration and time dependent manner. It indicated that the disease treatment should maintain a long time to improve the health. However, the effective components should be further identified.

Conclusion

The ethanol extract of termite fungus garden can be applied in the clinical treatment of diseases of PD and aging. Development and application of termite fungus garden can provide a protection of PD related diseases.

Acknowledgment

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References


Table 1 The effect ethanol extract of termite fungus garden on climbing ability of PD flies (X±S, n=12)

<table>
<thead>
<tr>
<th>Group</th>
<th>3d (cm)</th>
<th>6d (cm)</th>
<th>12d (cm)</th>
<th>18d (cm)</th>
<th>24d (cm)</th>
<th>36d (cm)</th>
<th>48d (cm)</th>
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<tr>
<td>Non-PD control</td>
<td>9.054±0.818</td>
<td>9.070±0.817</td>
<td>8.820±0.916</td>
<td>9.117±0.856</td>
<td>8.184±1.056</td>
<td>4.685±1.674</td>
<td>0.694±1.056</td>
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<td>PD control</td>
<td>8.678±8.863</td>
<td>8.382±6.057</td>
<td>6.734±3.088</td>
<td>1.023±1.023</td>
<td>1.737±0.949</td>
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<tr>
<td>Treatment 1 mg</td>
<td>8.682±8.765</td>
<td>8.730±8.262</td>
<td>7.124±4.132</td>
<td>1.263±1.023</td>
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<tr>
<td>Treatment 10 mg</td>
<td>8.650±8.761</td>
<td>8.761±8.407</td>
<td>7.434±3.944</td>
<td>1.215±1.072</td>
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<tr>
<td>Treatment 100 mg</td>
<td>8.751±8.967</td>
<td>8.834±8.506</td>
<td>7.607±4.275</td>
<td>1.446±1.882</td>
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*comparing with Non-PD flies, P<0.05; **comparing with PD flies, P<0.05

Table 2 The effect of ethanol extract of termite fungus garden on lifespan of PD flies (X±S, n=240)

<table>
<thead>
<tr>
<th>Type</th>
<th>Non-PD control</th>
<th>PD control</th>
<th>treatment 1 mg</th>
<th>treatment 10 mg</th>
<th>treatment 100 mg</th>
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<tr>
<td>male</td>
<td>62.19±10.92</td>
<td>58.40±12.02</td>
<td>59.46±11.64</td>
<td>59.74±13.10</td>
<td>60.35±12.87</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>1.15*</td>
<td>1.63**</td>
<td>1.761*</td>
</tr>
<tr>
<td>female</td>
<td>62.39±9.83</td>
<td>58.99±12.46</td>
<td>59.86±11.81</td>
<td>60.18±13.43</td>
<td>60.96±13.45</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>1.15*</td>
<td>1.63**</td>
<td>1.761*</td>
</tr>
</tbody>
</table>

*comparing to PD flies, P<0.01; **comparing to Non-PD flies, P<0.01
Figure 1  The brain tissue stained with Kongo red dye. The arrows indicate the β-amyloid protein (β-AP).

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