Protective Effects of Loofah Leaf-extract on Nerve Cell Injury

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ABSTRACT
Alzheimer’s disease (AD) is a debilitating neurodegenerative disorder characterized by increased β-amyloid (Aβ) deposition and neuronal dysfunction leading to impaired learning and recall. Ageing and induced oxidative stress are among proposed risk factors. We studied the protective effect of Loofah leaf-extract (LL) on the injury of PC12 cells induced by amyloid β protein (Aβ25-35). Neurons injury model was induced by Aβ25-35. MTT assay was used to detect the cellular viability after different concentrations of Loofah leaf-extract. The activity of superoxide dismutase (SOD), malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) were detected. The expression of Bcl-2 and Bax were detected by Western Blotting. Results: Compared with the control group, the cellular viability of Loofah leaf-extract group is increased, the activity of GSH-Px and SOD are significantly increased (P<0.01, P<0.05), MDA content decreased significantly, the expression of Bax is decreased and Bcl-2 is increased (P<0.05, P<0.01). These results indicate that Loofah leaf-extract can protect PC12 cells damage.

Key words: Alzheimer’s Disease, Loofah Leaf-extract, PC12 Cells, Aβ25-35
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Introduction
Luffa, a plant of Cucurbitaceae, Loofah leaves can be used as medicine (Su et al., 2014), been proved in recent years to have such physiological functions as enhancing immunity, anti-stress, detoxification, hemostasis, and promoting growth. Modern pharmacological studies found that loofah leaves contain a terpenoid saponin substance called L-6a, which can increase blood supply to the brain, protect brain cells, and promote the release of somatostatin in the brain neuron, and prevent Alzheimer’s disease (Qi et al., 1999; Li et al., 2001). The present study aims to investigate the neuroprotective effect of Loofah leaf-extract on PC12 cells injury induced by β-amyloid protein (Aβ25-35)( Zeng et al., 2004; Choi et al., 2010), and to explore the neuroprotective mechanism of Loofah leaf-extract on AD (Ghofrani et al., 2015).

Methods
Drugs and reagents
The Loofah leaf-extract was provided by Xi’an SR Bio-Engineering Co, Ltd. The PC12 cells were purchased from Beijing BeNa Chuanglian Biotechnology Research Institute, MTT and Aβ25-35 from Sigma, MDA, SOD and GSH-Px detection kit from Nanjing Jiancheng Bioengineering Institute, DMEM medium, fetal bovine serum, and horse serum from Hangzhou Sijiqing Engineering Materials Research Institute, and Bcl-2 and Bax antibodies were purchased from Santa Cruz Biotechnology. All other reagents are analytical reagents produced in China.

Instruments
Carbon dioxide incubator (Thermo), EPOCH microplate reader (BioTek Instruments, Inc), high speed freezing centrifuge (Shanghai Canspec

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Effects of Loofah leaf-extract on antioxidant capacity of PC12 cells induced by oxidative stress

Select the cells in the logarithmic growth period, for the preparation of suspension of 2×10^5 cell/ml with trypsin cells, which was then inoculated into the 6-cell culture plate. After being pretreated with final concentrations (25μg/ml, 50μg/ml, and 75μg/ml) for 30 minutes, the Loofah leaf-extract was then incubated with final concentration of 20μmol/L for 24 hours. After completion of administration and Aβ25-35 treatment, the collected cells were ultrasonicated and centrifuged at 4000r/min at 4°C for 10 minutes. The supernatant was collected and the activity of SOD, MDA and GSH-Px in cell homogenate was tested by kit method.

Western blotting analysis

After 24-hour drug treatment, the cells were harvested and washed. The total protein was separated by SDS-PAGE electrophoresis. Then the protein was transferred to PVDF membrane, which was sealed with 5% skim milk powder for 2 hours at room temperature. After being washed 3 times with TBST, the sealed membranes were placed in hybridization bags. Rat Bcl-2, Bax and beta-actin- were incubated overnight at 4°C, and then washed 3 times with TBST. Horseradish peroxidase-labelled rabbit anti rat IgG was incubated at 37°C for 1 hour, washed with TBS and developed by ECL method. Then the gray value of each band was analyzed by gel imaging system and the gray ratio of the target band to the internal reference calculated.

Statistical analysis

The experimental data were expressed as X±S. SPSS16.0 software was used for statistical analysis, and t test to compare the differences between groups. If P<0.05, then there considered to be significant differences between groups.

Results

Protective effect of Loofah leaf-extract on PC12 cells induced by Aβ25-35

Compared with the blank group, the viability of the cells treated with 20μmol/L of Aβ25-35 is significantly lower, which is only 59.20%. Thus there is significant difference (P<0.01). When PC12 cells are pretreated with Loofah leaf-extract and then treated with Aβ25-35, the viability of PC12 cells is significantly increased, and shows a trend of dose-dependent tolerance. The cellular viability reaches 76.11%, 69.27% and 61.12% respectively.
after treatment with Loofah leaf-extract (75μg/ml, 50μg/ml, and 25μg/ml). The results are as shown in Figure 1.

Figure 1. MTT Testing of protective effects of different concentrations of Loofah leaf-extract on cytotoxicity of PC12 cells induced by Aβ25-35
Notes: when compared with the blank group, ##P < 0.01; when compared with the model group, *P < 0.05, **P < 0.01

The effect of Loofah leaf extract on MDA content in Aβ25-35 induced PC12 cells
Compared with the blank group, the content of MDA in PC12 cells treated with 20μmol/L of Aβ25-35 is significantly increased (P<0.01). Compared with model group, groups with different doses of Loofah leaf-extract can significantly decrease MDA content (P<0.01, 0.05), with a certain dose correlation, as shown in Figure 2.

Figure 2. Effect of Loofah leaf-extract on, MDA of PC12 cells induced by Aβ25-35
Notes: when compared with the blank group, #P < 0.05, ##P < 0.01; when compared with the model group, *P < 0.05, **P < 0.01.

Effect of Loofah leaf-extract on the activity of SOD in PC12 cells induced by Aβ25-35
Compared with the blank group, the activity of SOD in PC12 cells treated with 20μmol/L of Aβ25-35 is significantly decreased (P<0.01). Compared with model group, groups with different doses of Loofah leaf-extract can significantly raise SOD activity (P<0.01, 0.05), as shown in Figure 3.

Figure 3. Effect of Loofah leaf-extract on, SOD of PC12 cells induced by Aβ25-35
Notes: when compared with the blank group, #P < 0.05, ##P < 0.01; when compared with the model group, *P < 0.05, **P < 0.01.

Effect of Loofah leaf-extract on the activity of GSH-PX in PC12 cells induced by Aβ25-35
Compared with the blank group, the activity of GSH-PX in model group is significantly decreased (P<0.01). Compared with model group, groups with different doses of Loofah leaf-extract can significantly raise GSH-PX activity (P<0.01, 0.05), as shown in Figure 4.

Figure 4. Effect of Loofah leaf-extract on, GSH-PX of PC12 cells induced by Aβ25-35
Notes: when compared with the blank group, #P < 0.05, ##P < 0.01; when compared with the model group, *P < 0.05, **P < 0.01.

Effect of Loofah leaf-extract on the expression changes of Bcl-2 and Bax in PC12 cells Induced by Aβ25-35
Compared with the blank group, the expression changes of Bcl-2 and Bax in PC12 cells induced by Aβ25-35.
Compared with the blank group, in the PC12 cells treated with 20µmol/L of Aβ_{25-35}, Bax expression is significantly increased, Bcl-2 expression is significantly decreased (P<0.01), and Bcl-2/Bax expression ratio declines. Compared with the model group, in the groups with high, medium and low doses of Loofah leaf-extract, Bax expression is significantly decreased (P<0.01, 0.05), and Bcl-2 expression ratio is significantly raised (P<0.01, 0.05), The ratio of Bcl-2/Bax expression is significantly raised compared with model group, as shown in Figure 5 and Figure 6.

**Figure 5.** Effect of Loofah leaf-extract on protein expression induced by Aβ_{25-35}

**Figure 6.** Effect of Loofah leaf-extract on expression changes of Bcl-2/Bax in PC12 cells induced by Aβ_{25-35}

Notes: when compared with the blank group, ##P <0.01; when compared with the model group, *P <0.05, **P <0.01.

Conclusions
The pathological feature of Alzheimer's disease (AD) is senile plaque (SP) (Ong et al., 2015), whose core is β-amyloid (Aβ). In normal physiological conditions, Aβ has the function of nutritional factor, but high concentration of long chain Aβ tends to be neurotoxic (Bagheri et al., 2011; Zhu et al., 2009). Aβ production and increased accumulation is the main mechanism for causing neuronal degeneration and death. Aβ can produce peroxide and free radicals through a variety of ways, thus aggravating the damage of oxidative injury to nerve cells (Liu et al., 2015).

Recent studies have shown that the degeneration of human body with age is the result of the side effects of excessive free radicals produced during normal metabolism of cells. MDA is a product of lipid peroxidation. If the body's SOD, GSH-PX and other antioxidant enzyme decrease, the free radical formation in the body will decline, the peroxidation will be strengthened, and the tissue and the cell will be destroyed (Li et al., 2014; Andersen et al., 2003), so that the function of the tissue will decline and organism age (Khan et al., 2012). This study shows that Aβ_{25-35} can lead to MDA level increase and SOD, GSH-PX level decrease in PC12 cells. However, the PC12 cells pretreated with Loofah leaf-extract significantly increases SOD, GSH-PX and decrease MDA in cells induced by Aβ_{25-35}, thereby reducing the level of oxidative stress within the cell. Reducing or blocking the action of lipid peroxides can protect the body from the damage of hydrogen peroxide and delay the injury of cell senescence, thereby preventing the diseases caused by free radicals.

The balance of anti-apoptotic gene Bcl-2 and pro-apoptotic gene Bax in Bcl family plays an important role in deciding whether or not the cells enter the apoptosis pathway, which means, the ratio of Bc1-2/Bax determines whether or not apoptosis occurs (Wu and Li 2010). The experimental results show that the expression of Bc1-2 protein is decreased, the expression of Bax increased and the ratio of Bc1-2/Bax decreased in the model group. Thus the apoptosis of cells is exacerbated. After the intervention with Loofah leaf-extract, Bcl-2 expression is increased, Bax expression decreased, and the ratio of Bcl-2/Bax increased, thus apoptosis is inhibited. It indicates that Loofah leaf-extract can inhibit cell apoptosis. The protective effect of Loofah leaf-extract on brain cells of mice with AD may be achieved by up-regulating Bcl-2 protein, down-regulating the expression of Bax protein and inhibiting apoptosis, thereby relieving Alzheimer's disease (Goodsell 2002; Paul-Samojedny et al., 2005).

In conclusion, Loofah leaf-extract has obvious protective effect on PC12 cell injury induced by Aβ_{25-35}, which may be realized by enhancing the anti-oxidative stress and anti-apoptosis effect of PC12 cells, but its mechanism of AD prevention and treatment remains to be further studied.
References


