JOURNAL OF PURE AND APPLIED MICROBIOLOGY, FEBRUARY 2014. Vol. **8**(1), p. 635-639

Protective Effects of Ethanol Extract from Termite Fungus Garden on the SH-SY5Y Cell against Oxidative Damage

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(Received: 08 April 2013; accepted: 25 May 2013)

To determine the protective effects of ethanol extract from termite fungus garden on the SH-SY5Y cell against oxidative stress induced by H_2O_2. SH-SY5Y cells were cultured in the medium as the normal control group. H_2O_2 was added into SH-SY5Y cells to establish the cell model of oxidative damage as the model control group. Different concentrations of ethanol extract from termite fungus garden and the reagent H_2O_2 were added into the culture medium of SH-SY5Y in sequence as the prevention groups. Ethanol extract from termite fungus garden added into the cell culture medium induced by H_2O_2 was considered as the protection groups. Cell activity of SH-SY5Y was determined with MTT assay, and the activities of SOD (Superoxide Dismutase), GSH (Glutathione), and content of MDA (Malondialdehyde) were also detected in each group. Cell suruvival rate in the prevention group had been increased by 27.2% to 44.5% as compared with that in the model control group. All of the protection groups had 24.2% to 32.5% improvement on survival rate as compared with that in the model control group. The ethanol extract from termite fungus garden plays an important role in protecting SH-SY5Y cell through its anti-oxidative effect on H_2O_2.

Key words: termite fungus garden; antioxidant; SH-SY5Y.

Free radical theory is one of the most important mechanisms of aging. It is known that free radical is apparently participated in arteriosclerosis, cornoary heart disease, diabetes, Alzheimer's disease, Parkinson's disease, ect[1-6]. The oxidant-antioxidant balance is of great significance in the prevention and protection of age-related diseases[7]. We have used termite fungus garden (a free radical scavenger) to make antioxidant research because of scavenging superoxide radicals and resisting lipid peroxidation[8]. This study was further to explore the protective effects of termite fungus garden on the SH-SY5Y cell against oxidative stress.

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MATERIALS AND METHODS

Materials

Termite fungus garden was collected and identified by the Termite Control Center of Jurong Forest. Human neuroblastoma cells (SH-SY5Y) were donated by Institute of Life Science in Jiangsu University. DMEM culture medium and trypsin, fetal bovine serum (FBS) were bought from Gibco Company and Lanzhou Minhai Biological Engineering CO, Ltd. respectively. MTT (Methylthiazolyldiphenyl-tetrazolium bromide), all other reagents and experimental equipments were supplied by Institute of Life Science in Jiangsu University. The kit of SOD (Superoxide dismutase), MDA (Malondialdehyde), GSH (Glutathione) were provided by Nanjing Jiancheng Biological Engineering Insitite.

Preparation of ethanol extract from termite fungus garden

Ten grams of termite fungus garden powder were socked in 150 mL ethanol, and were heated and extracted for 2-hr. One hundred and fifty millmiter of ethanol were added in the residue to extract for another 2-hr. After that, the distillates were all mixed together.

Cell culture

Human neuroblastoma cells (SH-SY5Y) were cultured in DMEM with 10% FBS, and placed in the incubator with conditon of 5% CO_2, saturated humidity and 37°C. Culture medium was replaced every 2^ÿ3 days, and digested with 0.25% trypsinthe when cells were generated.

Effect of ethanol extract from termite fungus garden on SH-SY5Y

Model establishment of SH-SY5Y oxidative damage

H_2O_2, which final concentration was 300 µmol/L, was cultured with SH-SY5Y for an hour, cell activity was dectected by MTT and SOD, MDA and GSH were also measured respectively.

u$ Preventive effects of ethanol extract on SH-SY5Y oxidative damage

SH-SY5Y cells were cultured in 96-well plate, injected with different doses (200, 175, 150, 125, 100 µL) of termite fungus garden ethanol extract after 24-hr, and then added H_2O_2 into it after another 24-hr, while those in the model control group injected with DMSO solution and final concentration was adjusted to 0.1%. After 1-hr, cell activity was detected by MTT assay, so did the activities of SOD, GSH and content of MDA.

Protective effect of ethanol extract on SH-SY5Y oxidative damage

The experimental procedures of anti-oxidative damage were pretty much the same as former one except that both termite fungus garden ethanol extract and H_2O_2 were added into the SH-SY5Y cells simultaneously.

Measurement of cell activity with MTT assay

Twenty microlitre MTT Reagent was added into each well, then placed the plate under the condition of 5% CO_2, saturated humidity and 37°C for 4-hr, removed supernatant and added DMSO 150 uL/well, which were set it on shaking bed with low speed until the crystals dissolved compelely. Absorbance was recorded at 490 nm and survial rate of cells caculated in different time period according to OD (optical density). Survial rate of cells" (model control group OD prevention group OD or protection group OD)/ model control group OD×100%. Reapted it for 3 times.

Measurement of SOD, MDA and GSH

The prosess of experiment was strictly mesasured in accordance with the procedure of the kit.

Data analysis

All the data obtained using SPSS 17.0 statistical software for test. ANOVA analysis of variance between the two groups was for the *q* test. Differences for all data were considered statistically significant at $P<0.05$.

RESULTS

Effect of termite fungus garden on SH-SY5Y oxidative damage

Preventive effect of the termite fungus garden ethanol extract on the SH-SY5Y induced by H_2O_2

Different doeses of termite fungus garden ethanol extract and H_2O_2 were injected into the culture medium of the SH-SY5Y cells in sequence, then detected the cell activity. The result showed

Table 1. Activity of SOD in the culture media of SH-SY5Y cell ($\bar{x} \pm s$, n = 5)

Concentrations of ethanol extract of termite fungus garden	Culture medium (U /mL)	
	Prevention	Protection
0 uL (added nothing)	14.65±0.24	14.27±0.19
0 uL (with H_2O_2)	6.43±0.13 *	6.54±0.21*
200 uL	10.89±0.09 **	9.68±0.12**
175 uL	10.23±0.16 **	9.57±0.23**
150 uL	9.98±0.21 **	9.47±0.22**
125 uL	9.58±0.28 **	8.87±0.12**
100 uL	9.27±0.23 **	8.48±0.17**

Table 2. Content of MDA in the cell culture media of SH-SY5Y cell (±*s*, n = 5)

Concentrations of ethanol extract of termite fungus garden	Culture medium (U /mL)	
	Prevention	Protection
0 uL (with H_2O_2)	0.64±0.03*	0.65±0.02*
200 uL	0.32±0.09**	0.42±0.05**
175 uL	0.39±0.06 **	0.48±0.03**
150 uL	0.40±0.03 **	0.50±0.02**
125 uL	0.44±0.02 **	0.54±0.02**
100 uL	0.48±0.03 **	0.55±0.17**

Table 3. Content of GSH in the cell culture media of SH-SY5Y cell (±*s*, n = 5)

Concentrations of ethanol extract of termite fungus garden	Culture medium (U /mL)	
	Prevention	Protection
0 uL (added nothing)	0.875±0.042	0.817±0.111
0 uL(with H_2O_2)	0.176±0.021*	0.154±0.025*
200 uL	0.631±0.09**	0.499±0.121
175 uL	0.591±0.016**	0.457±0.123**
150 uL	0.553±0.027**	0.411±0.202**
125 uL	0.491±0.018**	0.387±0.112**
100 uL	0.427±0.023**	0.348±0.176**

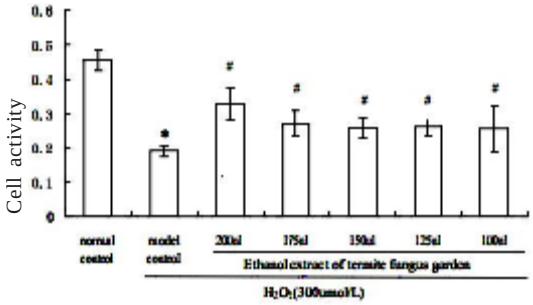
that 300 μmol/L H_2O_2 led to an obviouse decrease in cell activity and H_2O_2 had effect of oxidative damage on SH-SY5Y cells. Compared with H_2O_2-injury group, cell sruvival rate in the ethanol extract pretreatment group was increased by 27.2% to 44.5%. (Fig. 1)

Protective effect of the termite fungus garden ethanol extract on the SH-SY5Y induced by H_2O_2

Both the termite fungus garden ethanol extract and H_2O_2 were simultaneously added into the culture medium of the SH-SY5Y cells for 1-hr, then detected the cell activity. The result showed the protection group had a 24.2% to 32.5% improvement on survival rate compared with the model control group. (Fig.2) Decreased cell death indicated that termite fungus garden of the ethanol extract had inhibitory activities against oxidative injury in SH-SY5Y cells induced by H_2O_2.

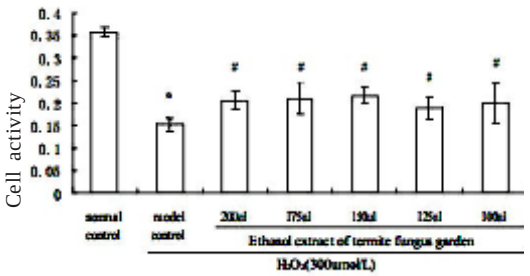
Activities of SOD, GSH and content of MDA

The activities of SOD and GSH were detected when SH-SY5Y cells cultured with H_2O_2 (final concentation: 300 μmol/L) in an hour. The result showed that activities of SOD and GSH significantly decreased, but the content of MDA



(Average ± SD, n=5;*p< 0.05 vs control, #p< 0.05 vs 300 μmol/L H_2O_2)

Fig. 1. Preventive effect of the termite fungus garden ethanol extract on the SH-SY5Y induced by H_2O_2 (300 μmol/L)



(Average ± SD, n=5;*p< 0.05 vs control, #p< 0.05 vs 300 μmol/L H_2O_2)

Fig. 2. Protective effect of the termite fungus garden ethanol extract on the SH-SY5Y induced by H_2O_2 (300 μmol/L)

increased compared with the normal control group ($P \leq 0.05$). To be treated with termite fungus garden with different concentration, we found that the content of MDA is changing corresponded with it. (Table 1-3)

DISCUSSION

Hydrogen peroxide (H_2O_2), the most reactive and destructive reactive oxygen species, oxidizes fatty acids, DNA and proteins, thus lipid peroxidation and membrane fluidity reduction[9, 10]. Also, H_2O_2 increases oxidative damage to mtDNA, eventually leads to cell death[11]. However, these effects of H_2O_2 can be delayed or restrained by antioxidants. Studies have shown that oxidative damage plays an important role in some degeneration diseases including Parkinson's disease and Alzheimer's disease. SH-SY5Y cell line, derived from neuroblastoma cells, is the most common cell model which used to explore the mechanism of neurodegenerative disease[12].

Injury from H_2O_2 in SH-SY5Y cells was used as oxidative damage model. The results showed that 300 µmol/L H_2O_2 led to an obviouse decrease in cell activity and H_2O_2 had effect of oxidative damage on SH-SY5Y cells. Cell sruvival rate in the ethanol extract prevention group was increased by 27.2% to 44.5% compared with model control group, which suggested termite fungus garden of the ethanol extract had characteristic of preventing oxidation. The protection group had a 24.2% to 32.5% improvement on survival rate compared with the model control group which indicated that termite fungus garden of ethanol extract were observed to be protected strongly from oxidation damage.

After SH-SY5Y cells were cultured with H_2O_2 at concentation of 300 µmol/L for an hour, the activty of antioxidant enzymes SOD and GSH were detected. Result showed that activity of SOD and GSH were significantly decreased, but the content of MDA was increased with the change of different concentration of termite fungus garden.

Superoxide dismutase (SOD) acts as an important member of keeping the balance between oxidation and anti-oxidation, which removes superoxide anion radical and protects cell from damage. Decreased activity of SOD induces peroxide which caused by unsaturated fatty acids, forms lipofuscin and alters the activites of proteins and enzymes[13,14]. Therefore, the activity and content of SOD reflect abilities of clearing free radicals. Malondialdehyde (MDA) is a product of lipid peroxides, often measured together with SOD. It indirectly reflects the extents of cell menbrane damage attacked by free radical[15]. Reactive oxygen species are produced through enzyme and non-enzyme system, promote lipid peroxidation of polyunsaturated fatty acids (PUFA) of biomembranes, leading to formation of lipid peroxides[16]. Researches have shown that glutathione peroxidase (GSH) has effect on shifting redox status of vitamine E towards deoxidation. Shortage or depletion of GSH promotes chemicals to produce or aggravate toxic effect. This study showed that GSH specifically catalyzed to eliminate harnful products and block the chain reactions of lipid peroxide within cells. In addition, GSH also acts as a scavenger and clears away low molecular including O_2-, H_2O_2, LOOH which may be related to an increase of oxidative damage[17]. Consequently, activity of GSH is a crucial factor of antioxidant capacity.

In this experiment, positive effects of termite fungus garden ethanol extract have been found on the prevention and protection of age-related diseases, but its mechanisms are still unknow. More research is needed to further study.

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