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Preliminary Study on the Anti-oxidative Damage Substances of Ginkgo Flavone Components

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Abstract. Ginkgo flavonoids are extracts of Ginkgo biloba leaves with precise antioxidant activity. In this study, a model of human umbilical vein endothelial cell chemical injury was established. Based on the cell viability, nitric oxide (NO) content and lactate dehydrogenase (LDH) content, the preliminary antioxidant bases of different ginkgo flavonoids were discussed. The study found that 45% ethanol component can effectively improve cell survival rate, has damaged cell repair function, and there is synergy and antagonism between different components, which provides reference and guidance for subsequent product development.

Key words: Ginkgo biloba; flavonoids; oxidative stress; material basis.

INTRODUCTION

Ginkgo flavonoids can be divided into three types of compounds: flavonoids, diflavonoids and catechins, and there are more than 40 kinds of chemical components [1~2]. At present, the research on the anti-oxidation of flavonoids in Ginkgo biloba at home and abroad focuses on the activity of total flavonoids, and there are relatively few studies on different flavonoids. The establishment of a chemically damaged endothelial cell model to study its role in the anti-cell damage and its preliminary mechanism, from the effective site and monomer composition level to clarify the characteristics of Ginkgo oxidative stress and its material basis [3~4].

MATERIAL

Homemade ginkgo flavonoids (25% ethanol, 45% ethanol, 65% ethanol, 85% ethanol); vitamin C (Sigma); Ginaton (HC20090014, Taiwan); fetal bovine serum, RPMI-1640 cell culture medium, pancreas Enzyme cell digestive solution (Thermo Fisher Bio Beijing Co., Ltd.); MTT, DMSO (AMRESCO); PBS buffer solution (pH 7.4, Beijing Zhongshan Jinqiao Biological Co., Ltd.); human umbilical vein endothelial cells (HUVEC) from Shandong Province Provided by the hospital.

METHOD AND RESULT

Establishment of HUVEC Model

HUVEC was inoculated into 96-well plates as required, and divided into zero-adjusted group, blank control group, positive control group and drug-administered group. The final concentration of 0.6 mmol/L hydrogen peroxide was

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added for 2 h to induce HUVEC chemical damage model. 100 μL of RPMI-1640 medium containing 100 μg/mL and 50 μg/mL of the final drug concentration was added to each well of the drug-administered group, and the drug-free RPMI-1640 medium was used as a blank control group; The positive control group was added with 100 μL of serum-free RPMI-1640 medium with different concentrations of Ginaton. The absorption values of human umbilical vein endothelial cells were observed by MTT method at 492 nm. The effects of drugs on human umbilical vein endothelial cell injury were observed. The mechanism of different components was preliminarily elucidated by using NO and LDH kits.

Basic Research on Antioxidant Damage Substances of Different Components of Flavonoids

Four intervention factors were selected, namely 25% ethanol, 45% ethanol, 65% ethanol, 85% ethanol, each factor was divided into 2 levels, ie 100 μg/mL and 0 μg/mL, using L_{16} (2^{15}) orthogonal Arrange the experiment, see Table 1. The medicinal cells were cultured for 24 h at 37°C in a 5% CO_2 incubator. The cell viability was determined by MTT assay, and the contents of NO and LDH were detected by the kit, as shown in Table 2.

TABLE 1. Table of different flavonoid component factors.

Factor	A	B	C	D
Medicine	25% ethanol	45% ethanol	65% ethanol	85% ethanol
1	100	100	100	100
2	0	0	0	0

TABLE 2. Cell evaluation experiments and data analysis of different flavonoid components (n=6).

No	A	B	AB	C	A*C	B*C	D	A×D	B×D	C×D	Survival (%)	NO (μmol/L)	LDH (ng/mL)
1	1	1	1	1	1	1	1	1	1	1	37.32	15.69	24.31
2	1	1	1	1	1	1	2	2	2	2	35.52	16.49	20.80
3	1	1	1	2	2	2	1	1	1	2	44.13	13.27	22.62
4	1	1	1	2	2	2	2	2	2	1	33.41	17.53	17.16
5	1	2	2	1	1	2	1	1	2	1	42.02	13.94	21.58
6	1	2	2	1	1	2	2	2	1	2	32.08	18.25	16.51
7	1	2	2	2	2	1	1	1	2	2	34.51	16.97	17.68
8	1	2	2	2	2	1	2	2	1	1	28.48	20.56	14.56
9	2	1	2	1	2	1	1	2	1	1	44.37	13.20	22.75
10	2	1	2	1	2	1	2	1	2	2	45.54	10.86	23.40
11	2	1	2	2	1	2	1	2	1	2	38.81	15.09	19.89
12	2	1	2	2	1	2	2	1	2	1	35.92	16.30	18.46
13	2	2	1	1	2	2	1	2	2	1	37.21	15.74	19.11
14	2	2	1	1	2	2	2	1	1	2	31.81	18.41	16.38
15	2	2	1	2	1	1	1	2	2	2	34.51	16.97	17.68
16	2	2	2	2	2	2	2	2	2	2	29.96	19.55	15.34
F ratio	114.18	1060.2	259.04	400.27	148.72	0.329	509.53	182.68	119.08	95.65	Survival (%)		
P		⁎#	⁎	⁎			⁎#	⁎					
F ratio	150.33	1674.4	249.67	647.89	114.39	10.78	1012.9	271.33	295.17	133.5		LDH (ng/mL)	
P		⁎#	⁎	⁎#			⁎#	⁎	⁎				
F ratio	2.4	455.7	1.0	224.7	0.4	11.3	258.5	62.5	6.0	1.4			LDH (ng/mL)
P		⁎#		⁎			⁎						

*P<0.05, #P<0.01; F-boundary value (0.05) = 161; F-boundary value (0.01) = 405.

Effects of Different Flavonoid Components on Cell Viability

According to the cell survival rate, 65% ethanol was statistically significant ($p < 0.05$), and 45% ethanol was significantly different from 85% ethanol ($p < 0.01$); different flavonoids were ranked as high to low according to survival rate. B>D>C>A; A*B and A*D have a first-order interaction ($p < 0.05$), indicating that 45% ethanol, 65% ethanol and 85% ethanol flavonoids have a certain protective effect on the damage model; A and C, B and D, C and D have a certain antagonistic effect [5~7].

Effect of Different Flavonoids on Cellular NO Content

With NO content as the evaluation index, 45% ethanol, 65% ethanol and 85% ethanol were statistically significant ($p < 0.01$), and the different flavonoid components were repaired from high to low as B>D>C>A; A*B, A*D, B*D have a first-order interaction ($p < 0.05$), indicating that 45% ethanol, 65% ethanol and 85% ethanol components have a repairing effect on damaged cells, and the repair effect is enhanced after group distribution; It has antagonistic effects with C, B and C, C and D.

Effect of Different Flavonoids on LDH Content in Cells

LDH content was used as the evaluation index, 45% ethanol, 65% ethanol and 85% ethanol were statistically significant ($p < 0.05$), of which 45% ethanol group was significant ($p < 0.01$), indicating 45% ethanol, 65% ethanol It is resistant to LDH exudation with the 85% flavonol component and has the function of protecting damaged endothelial cells. The purified components of different flavonoids are arranged according to the protection function of damaged cells from high to low as B>D>C>A.

Preliminary Study on Anti-Oxidative Stress Mechanism of Ginkgo Flavonoids

Under normal conditions, HUVEC has a low proliferation rate and apoptotic rate, and there is a dynamic balance between apoptosis and proliferation [8]. H_2O_2 easily penetrates into the cell membrane and enters the cell freely, transforming into a highly active hydroxyl radical in the nucleus, causing DNA strand breakage and oxidative stress reaction, resulting in damage of HUVEC cells. The HUVEC survival activity decreased after H_2O_2 oxidative damage, and the LDH content in HUVEC culture solution increased significantly, and the NO content decreased significantly.

After administration, 45% ethanol group B, 65% ethanol group C, 85% ethanol group D, A*B group, A*D group can reduce cell death rate after H_2O_2 injury; 45% ethanol group B, 65% ethanol group C, 85% ethanol group D, A*B group, A*D group, B*D group can increase NO content; 45% ethanol group B, 65% ethanol group C, 85% ethanol group D can resist LDH exudation.

The drug-administered group can increase the NO content in the supernatant of H_2O_2 injured cells and reduce the release of LDH after HUVEC injury, suggesting that the flavonoid molecules containing reducing hydroxyl groups in the drug-administered group can directly remove O^{2-}, OH•, H_2O_2 and capture peroxidation. Free radicals and alkane radicals, which prevent oxygen free radicals and lipid peroxidation, participate in the regulation of antioxidant enzyme activity, resist the membrane damage of oxygen free radicals to HUVEC, reduce cellular antioxidant stress, and increase HUVEC cell survival rate [9].

CONCLUSION

An in vitro oxidative stress cell model was established, and different flavonoid components were screened for activity. It was found that 45% ethanol component can improve cell survival rate, repair damaged cell function, and have certain synergistic and antagonistic compatibility between different flavonoid components. Give full play to the comprehensive effects of multi-component, multi-target and multi-channel of traditional Chinese medicine. On this basis, the separation and purification methods were used to find the qualified flavonoid lead compounds from Ginkgo biloba, which provided reference and guidance for the next step of research and development of Ginkgo biloba products.

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