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The Effect of Different Chicken Ovalbumin Extracts on Cell Proliferation

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ABSTRACT

Background: Protein extracts from chicken egg whites were prepared by utilizing different solvents and we studied the differences in proliferation.

Methods: The egg white extract was prepared by using lysate, phosphate-buffered saline (PBS), saline and pure water. The *in vitro* mixture experiment was carried out to observe the effect of different egg white preparations on the proliferation of cells. The samples were divided into the following groups: the control group with media, the original lysate group, the new lysate group, the PBS group, the saline group and the pure water group.

Result: The study found that at final concentrations of 10%, 20%, 30%, 40% and 50%, the differences among the six groups were statistically significant ($P<0.01$, $F>100$). The results of pairwise comparison showed that the proliferative effect of chicken egg albumin extract prepared by PBS was significantly higher than that of the medium at final concentrations of 20%, 30% and 50% ($P<0.05$). When the fetal bovine serum concentration was only 8%, 7% and 5%, the cell proliferation effect was better than that of the control group with 10% fetal bovine serum, indicating that the chicken egg white extract promoted cell proliferation. This result indicated that the best chicken egg albumin extract was obtained through PBS addition. The quantity of expensive fetal bovine serum could be considerably reduced by supplementing the media with chicken egg albumin extract. Among the solvents tested, PBS was the best solvent for preparing chicken egg albumin extract.

Key words: Cell proliferation, Chicken egg white, Extract.

INTRODUCTION

Eggs are not only a nutritious food but are also provide a model for ated that there are ingredients in the chicken ovalbumin extract that promote cell proliferation (Mizunoya *et al*. 2015; Ramshini *et al*. 2015; Ruan *et al*. 2020). Our previous studies have also shown that egg white extract promotes cell proliferation (Ruan *et al*. 2015; Ruan *et al*. 2016). However, in a previous study, the lysate was used to prepare the chicken egg white protein extract and the lysate formula was complicated. There were many factors influencing the preparation process and the obtained chicken egg white extract was unstable. In this study, lysate, phosphate buffered saline (PBS), sodium chloride solution and sterile water for injection were used as solvents to prepare chicken egg white extract.

Among the solvents tested, PBS was the best solvent for preparing chicken egg albumin extract. When the fetal bovine serum concentration was only 8%, 7% and 5%, the cell proliferation effect was better than that of the control group with 10% fetal bovine serum, indicating that the chicken egg white extract promoted cell proliferation.

MATERIALS AND METHODS

Methods

Preparation of lysate

The lysate used was prepared with the following formula: 50 mol/L NaCl, 5 mM MgCl2, 100 mM HEPES (pH 8.2), 1 mM dithiothreitol (DTT), 0.1 mM phenylmethylsulfonyl fluoride (PMSF) and protease inhibition (Aprotinin, Shanghai Shenggong.

In this experiment, we used original lysate and freshly prepared lysate. The original lysate was a three-month-old lysate and was stored in a refrigerator at 4 degrees. In addition, in the study, PBS (HyClone), sodium chloride injection (Kunming Nanjiang Pharmaceutical Co., Ltd.) and sterile water for injection (Sichuan Kelun Pharmaceutical Co., Ltd.) were used as the solvents for the chicken protein extract.

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Preparation of chicken egg white extract

A total of 15 local chicken eggs were bought from the market and a small hole was made aseptically. Approximately 20 ml of egg white was removed from each egg and added to a 50 ml sterile centrifuge tube and then an equal volume of the original lysate (the lysate was stored for three months at 4°C), the self-prepared lysate (freshly prepared lysate), PBS, sodium chloride injection and sterile water were added for injection. Three chicken eggs were used for each solvent. The samples were mixed thoroughly, frozen at -20°C, thawed on the second day, frozen and thawed three times and centrifuged at 4000 rpm for 10 minutes. The supernatant was removed to obtain the final chicken ovalbumin extract. The experiment was repeated 3 times and similar results were obtained.

293T cell proliferation experiment

The 293T standard cell line was purchased from the cell bank of the Kunming Institute of Zoology, Chinese Academy of Sciences. The cells were first digested with trypsin and counted. The number of cells in the well was 5×10^3. A total of 100 µl of saline was added to each hole around the 96-well plate to prevent the liquid from evaporating. A total of 6 rows of cells were added, with 10 holes in each row. A total of 50 µl of medium (medium group) was added to the first row, the chicken ovalbumin extract, which was prepared with the lysate that was previously prepared in this room (the original lysate group), was added to the second row and the lysate that was prepared more recently was added to the third row chicken egg white protein extract (new lysate group), the chicken egg white extract prepared with PBS (PBS group) was added to the fourth row, the chicken egg white extract prepared with saline (saline group) was added to the fifth row and the egg white extract prepared with pure water (pure water group) was added to the 6th row. There was a total of 5 96-well plates and one plate had 50 µl of 20% extract prepared with medium, one had 50 µl of 40% extract with medium, one had 50 µl of 60% extract with medium and one had 50 µl of the 80% extract prepared by the medium. A total of 50 µl of extract stock solution was added together and the final concentrations of each group of chicken protein extract were 10%, 20%, 30%, 40% and 50%. The concentration of the medium group was unchanged and it was DMEM-F12 medium with 10% fetal bovine serum. After culturing for 3 d, the reagents in the kit were added at 20 µl/well to detect the cell proliferation activity (CellTiter 96 Aqueous One Solution Cell Proliferation Assay, purchased from Promega), were placed at 37°C for 3 h and were placed in a microplate reader (model RT-6000, manufacturer Rayto) to compare the color at a 490 nm wavelength. The higher the OD value, the higher the cell proliferation activity.

Materials	Factory
NaCl	Domestic analytical pure
$MgCl_2$	Domestic analytical pure
HEPES	Domestic analytical pure
Dithiothreitol	Domestic analytical pure
Phenylmethylsulfonyl fluoride	Domestic analytical pure
Aprotinin	Shanghai Shenggong
PBS	HyClone
Sodium chloride injection	Kunming Nanjiang Pharmaceutical Co., Ltd.
Sterilized water for injection	Sichuan Kelun Pharmaceutical Co., Ltd.
Chicken eggs	Local property
293T st andard cell line	Cell Bank of Kunming Institute of Zoology, Chinese Academy of Sciences
CellTiter 96 Aqueous One Solution Cell Proliferation Assay	Promega

Statistical analysis method

Using SPSS 21.0 statistical software, the data (OD value) are expressed as the mean ± standard deviation, one-way ANOVA was used for comparisons between multiple groups and the LSD and SNK methods were used for pairwise comparisons between the culture group and each group. The difference was statistically significant at $P<0.05$.

RESULTS AND DISCUSSION

Proliferation of 293T cells was promoted with a 10% final concentration of extract

The extract with a 10% final concentration was cocultured with 293T cells for 3 days. Because the final concentration of the chicken ovalbumin extract was low, it did not show a significant proliferation effect (Table 1), but it can be seen from the table that PBS and saline were prepared. The proliferation-promoting effect of the chicken egg white extract was slightly better than that of other solvents. The PBS group and the saline group had no statistically significant difference in promoting cell proliferation compared with that of the medium. The original lysate group, the new lysate group and the pure water group exhibited less cell proliferation than that of the medium and the difference was statistically significant ($P<0.05$).

Proliferation of 293T cells was promoted by a 20% final concentration of extract

The extract with a 20% final concentration was cocultured with 293T cells for 3 days. The proliferation effect of the PBS group and the saline group increased and the difference between the PBS group and the saline group and the medium group was statistically significant ($P=0.001$) (Table 1).

Table 1: Comparison of the effects of different final concentrations of chicken protein extracts on promoting cell proliferation (n=10).

Groups	Final concentration of chicken ovalbumin extract				
	10%	20%	30%	40%	50%
Medium group	1.80±0.05	1.60±0.07	1.69±0.08	1.67±0.08	1.63±0.05
Original lysate group	0.73±0.01[a]	0.63±0.02[a]	0.84±0.04[a]	0.71±0.02[a]	0.73±0.048[a]
Newly equipped lysate group	1.67±0.08[a]	1.56±0.07	1.57±0.08[a]	1.38±0.08[a]	1.38±0.088[a]
PBS group	1.73±0.08	1.69±0.06[a]	1.85±0.08[a]	1.69±0.10	1.77±0.168[a]
Salt water group	1.73±0.10	1.69±0.04[a]	1.85±0.14[a]	1.66±0.05	1.62±0.05
Pure water group	1.60±0.14[a]	1.57±0.07	1.66±0.11	1.39±0.06[a]	1.13±0.088[a]
F value	208.23	494.13	156.87	275.31	193.81
P value	0.001	0.001	0.001	0.001	0.001

Note: A compared with the medium group, P<0.05.

Effect of the extract with a 30% final concentration on the proliferation of 293T cells

The extract with a 30% final concentration was cocultured with 293T cells for 3 days. The proliferation-promoting effect of the chicken albumen extract prepared with PBS and saline was higher than that of the culture medium group and the cell proliferation effect of the original lysate group and the newly prepared lysate group was lower than that of the culture. In the basic group, except for the pure water group, the differences between the other groups and the culture medium were statistically significant (P<0.05, Table 1).

Promotion effect of the extract with a 40% final concentration on 293T cell proliferation

The extract with a 40% final concentration was cocultured with 293T cells for 3 days. The proliferation effect of the PBS group was slightly higher than that of the medium group (Table 1), but the difference between the two was not statistically significant. The original lysate group, the new lysate group and the pure water group had less proliferation effects than those of the medium group and the differences were statistically significant compared with that of the medium group (P<0.001).

Promotion of 293T cells proliferation by extracts at a final concentration of 50%

The extract with a 50% final concentration was cocultured with 293T cells for 3 days. The proliferation effect of the PBS group was increased (Table 1) and the difference was statistically significant compared with that of the culture group (P=0.002). Except for the saline group, the differences between the other groups and the medium group were statistically significant (P<0.001).

Earlier studies have shown that the chicken ovalbumin extract promotes cell proliferation (Ruan *et al*. 2016; Shimazaki *et al*. 2018; Shimazaki *et al*. 2018; Sidorova *et al*. 2018). In the past, the chicken ovalbumin extract was prepared with the lysate (Ruan 2021; Ruan 2022). The lysate was prepared and filtered for sterilization. There are many influencing factors and in the chicken ovalbumin extract prepared in liquid, only components less than 3 kDa can promote the proliferation of 293T cells (Yoshinuma *et al*. 1989; Wang *et al*. 2014). In this study, the commercial solvents PBS, sodium chloride injection and sterile water injection were used as solvents to prepare chicken protein extracts. These liquids are sterile, do not need to be prepared, do not need to be filtered and sterilized and can be used over time. The results showed that the proliferation effect of the PBS group was greater than that of the medium group when the final concentration was 20%, 30% and 50%, especially when the final concentration was 50%; the fetal calf serum concentration of the PBS group was only 5%; and the effect of promoting cell proliferation was still observed for the medium group with more than 10% fetal bovine serum (P=0.002). In addition, the cell proliferation effect of the lysate stored at 4 degrees for 3 months was less than that of the newly prepared lysate. This shows that the time need to prepare the lysate is too long and this affects the activity of the egg white extract.

Fetal bovine serum is an essential supplement for culturing cells, usually at a concentration of 10% to 20%. However, fetal bovine serum is expensive, leading to an increase in the cost of culturing cells. In this study, chicken protein extract was added to the PBS group and the fetal bovine serum concentration was only 8% and 7%; however, the proliferation-promoting effect was greater than that of the medium group supplemented with 10% fetal bovine serum, which reduced the cost of culture and had a greater effect, promoting the application value of the serum.

The traditional MTT involves cumbersome steps and many influencing factors, which make the test results unstable. Therefore, this study used the imported Promega cell viability test kit to test the cell proliferation activity. This method simplifies the operation steps and does not require the supernatant to be aspirated or the precipitate to be dissolved, but it directly compares the colors after cocultivation, making the results more accurate and reproducible. Due to the edge effect of the 96-well plate, to prevent the hole on the side from being inaccurate due to evaporation, this study excluded the hole on the side and added 100 µl PBS to prevent the edge effect.

This study found that the PBS group had the best proliferation-promoting activity. At final concentrations of

20%, 30% and 50%, the proliferation-promoting effect was higher than that of the medium group, while the fetal bovine serum concentration was only 8% and 7%, respectively. 5%, which can greatly reduce the consumption of expensive fetal bovine serum.

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Author contribution

Jie He, Guang-ping Ruan and Xiang Yao, made substantial contributions to the study conception and design, data acquisition, or data analysis and interpretation. Jie He, Guang-ping Ruan, Kai Wang and Xiang Yao conducted the experiments. Xiang Yao and Jie He agree to be accountable for all aspects of the work and ensure that questions related to the accuracy or integrity of any part of the study will be appropriately investigated and resolved. Xing-hua Pan and Guang-ping Ruan provided final approval of this version of the manuscript for publication. Guang-ping Ruan, Xing-hua Pan, Jie He, Xiang-qing Zhu and Rong-qing Pang were involved in drafting the manuscript or critically revising it for important intellectual content. All authors read and approved the final manuscript.

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