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Effect of white lion goose serum on gastric cancer cells

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Abstract

Goose blood is rich in nutrients and a variety of bioactive ingredients, it have the effect of prevent cancer in humans, but the mechanism is not clear. So, we studied the effects of White Lion Goose serum on gastric cancer cells. The *in vitro* cell proliferation of human gastric cancer cells (AGS) was analyzed by a CCK-8 assay; cell morphology was observed using an inverted microscope; the wound healing ability of cells was detected by a scratch and abrasion test; and cell cycle distribution and cell apoptosis were detected by flow cytometry. The results showed that with increased concentration and prolonged incubation time, the serum of White Lion Goose reduced the viability, changed the morphology, and reduced the migration rate of gastric cancer cells. The proportion of gastric cancer cells in the early (G0/G1 phase) and late (G2/M phase) stages of DNA synthesis was decreased, the proportion of cells in stationary phase (S phase) was increased, and the apoptosis rate was increased accompanied by a decrease in the number of viable cells. The serum of White Lion Goose had a significant inhibitory effect on the proliferation of human gastric cancer cells, changed the distribution of the cell cycle and induced apoptosis in both a dose- and time-dependent manner. The results showed that the blood of the White Lion Goose contained highly bioactive components, which had an obvious inhibitory effect on the proliferation of gastric cancer cells cultured in vitro.

Key words: White Lion Goose serum; gastric cancer cells; the apoptosis; the migration.

Practical Application: Goose blood has strong health care value, contains a variety of cytokines and metabolites, is a kind of food with high nutritional value. It contains 8 kinds of essential amino acids and abundant trace elements needed by human body. Geese have high blood protein content, including immunoglobulin G, which can regulate the body's immunity, promote lymphocyte phagocytosis and resist virus, and transferrin, which can regulate the body's iron balance. In addition, geese also contain a lot of superoxide dismutase, It is the only metallosphere proteinase that can scavenge superoxide anion radical in aerobic organisms. Whether goose serum can induce cell apoptosis has not been studied. In this study, the blood of Lioness goose was used as raw material to detect the content of biological active ingredients and nutrients, explore the particularity of the blood component content of lioness goose, and further establish in vitro cell experiments to study the inhibitory effect of lioness goose serum on the proliferation of cancer cells, the distribution of cell cycle and the effect of inducing apoptosis.

1 INTRODUCTION

Geese are one of the earliest fowls domesticated by humans. In china, Goose breeding dates back more than 3,000 years. When a Goose is killed, a large amount of blood can be obtained through assassination and bloodletting. The blood generally accounts for about 6%–8% of the body weight of the animal. In China, animal blood is increasingly used to develop high-end biochemical products and drugs, such as goose blood sausage, blood biscuits, goose blood paste, goose blood powder, black pudding and goose blood series of health food, etc (Chen et al., 2011; Fan et al., 2006; Li et al., 2014). The blood can also be made into fodder. However, the current utilization of Goose blood is fairly limited (Qi et al., 2017). Previous studies have confirmed that The blood of white lion goose contains rich nutrients, including 1.69 mmol/L calcium, 3.23 mmol/L magnesium, 18.52 μmol/L copper, 93.71 μmol/L zinc and 9.16 mmol/L iron. Biochemical indices Total protein 56.23 g/L, albumin 18.83 g/L, glucose 14.01 mmol/L, total cholesterol 4.82 mmol/L, triglyceride 1.12 mmol/L, high density lipoprotein-cholesterol 3.08 mmol/L; Total amino acid 17.18 g/100g, essential amino acid content 7.04 g/100 g (Zhou et al., 2019). Goose blood can enhance the immune function of lymphocyte, which is very suitable for people with weak body and low immunity. It has the function of enhancing human immunity. Goose blood also has the function of clearing heat, lowering blood pressure, antibacterial, antioxidant, beautification and preventing cancer.

Gastric cancer is a malignant tumor with a high incidence rate (Jiao et al., 2022; Li et al., 2022). Symptoms are difficult to detect in the early stages and gastric cancer is therefore usually diagnosed in the advanced or metastatic stage. At present, the treatment of gastric cancer is mainly surgical resection and preoperative adjuvant chemical therapy (Amiri et al., 2015; Garaci et al., 2003). However, the prognosis following surgical treatment for patients with advanced gastric cancer is poor

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and chemotherapy aggravates coagulation dysfunction. Therefore, there is an urgent need for the development of low toxicity, effective anti-tumor drugs (Meng et al., 2022). Studies have found that Goose blood contains a large number of bioactive components, such as immunoglobulin, thymosin and superoxide dismutase, which can be used clinically as adjuvant therapeutic drugs for digestive tract tumors such as gastric cancer (Liu et al., 2001). The white goose blood was used to treat gastric cancer and after 10 days of treatment the patient showed partial recovery (Russo & Strong, 2017). Goose blood contains many bioactive substances such as immunoglobulin and superoxide dismutase, which can be used clinically as adjuvant therapeutic drugs to treat digestive tract tumors such as gastric cancer, esophageal cancer and liver cancer. The above results indicated that Goose blood has anti-tumor effects against early-stage gastric cancer (Peng et al., 2017). Taihu Goose blood was used to a raw material to explore the effective protein components in Goose serum and their effects on the proliferation of gastric cancer cells, and they identified the composition of effective components in Goose blood by mass spectrometry (Xu et al., 2018). The results showed that the growth of gastric cancer cell lines HGC-27 and SGC-7901 was significantly inhibited by Goose blood components, showing a cell survival rate of less than 40%, and a 10-kD component played a major role in this effect. The research results provided evidence that Taihu Goose serum had an inhibitory effect on the proliferation of gastric cancer cells, but whether Goose serum can induce apoptosis has not been studied.

In this study, we used White Lion Goose serum as a raw material to cultivate human gastric cancer AGS cells *in vitro*. The 5-Fu uracil was included as a positive control, and cells without any drug added were included as a negative control. Gastric cancer cells were cultivated with different concentrations of White Lion Goose serum for a set time to observe the inhibitory effects of White Lion Goose serum on the growth of gastric cancer cells *in vitro*. The CCK-8 assay method was used to determine the effects of different concentrations of White Lion Goose serum on cancer cell activity at different times. A scratch test was used to detect cell migration ability and calculate the cell migration inhibition rate. Inverted microscopy was used to observe the changes in cell morphology and images were recorded. Flow cytometry combined with PI staining was used to determine the cycle changes and distribution of cancer cells. Flow cytometry combined with Annexin V-PI double staining was used to detect the apoptosis of cancer cells and to calculate the apoptosis rate. Based on these studies, we analyzed the inhibitory effect of White Lion Goose serum on the proliferation of cancer cells, the distribution of the cell cycle and the induction of apoptosis.

2 MATERIALS AND METHODS

2.1 Animals

Ten 80-day-old White Lion geese were randomly selected from the North Goose Breeding Farm in Datong District, Daqing City, Heilongjiang Province, which were healthy, disease-free and showed no significant differences in body shape. The body mass of female geese was about (3.75 ± 0.2) kg and that of male geese was about (4.50 ± 0.2) kg.

2.2 Procedure for feeding

White Lion geese were raised from birth. The experiment lasted for 11 weeks. The fattening mode of captive brooding was adopted at the age of 1–2 weeks (main silage supplementation + corn silage supplementation). After 2 weeks, the goslings were stocked in corn fields with a plant height of more than 70 cm at a stocking density of 30/Mu. The goslings fed on weeds and the lower leaves of corn as roughage, and on maturity, geese were fed on corn straw supplemented with silage as roughage. The test geese used an automatic drinking device to obtain water and were allowed to feed freely. The animals were immunized according to the normal immunization procedure of Goose farms.

2.3 Collection and processing of blood samples

The experimental animals were transported to the animal breeding laboratory 10 days prior to the experiment and fed a conventional diet. Before euthanization, animals were fasted for 12 h, then 10 mL of blood was collected from the leg bone vein and placed in a 15 mL centrifuge tube, which was kept at 4°C for 2 h. The serum was separated and immediately centrifuged at 1,500 ×g and 4°C for 20 min. The upper layer of serum was aspirated and placed into 1.5 mL centrifuge tubes to immediately be tested for various indexes. Hemolysis was avoided as much as possible by not freezing-thawing samples. In addition, 10 mL of blood from the leg bone vein of another test Goose was collected and placed in a 15 mL centrifuge tube filled with anticoagulant. After thorough mixing, the blood sample was stored at -80°C prior to the determination of various indexes.

2.4 Preparation of white lion goose serum powder

Fresh White Lion Goose blood was collected and stored at 4°C for 2 h, then centrifuged at 1,500 ×g for 20 min. The supernatant containing the serum was aspirated and stored at -20°C overnight, then dried to a particular weight in a vacuum freeze dryer. The dried serum was crushed using a high-speed universal crusher, then passed through a 100-mesh sieve, and stored for later use.

2.5 Determination of IgG, SOD, GSH, thymosin and TRF in serum powder of White Lion goose

According to the experimental method of Wang et al. (2017). ELISA kit were used to determine 5 immune indexes in the serum of White Lion goose.

2.6 Resuscitation of cancer cell lines

Before cell resuscitation, laboratory surfaces were disinfected with 75% alcohol and materials to be used were irradiated with an ultraviolet germicidal lamp for more than 30 min. All of the necessary precautions were taken to ensure that these experiments were carried out under sterile conditions in a ventilated cabinet. The culture medium and PBS were prewarmed in a 37°C water bath for 30 min, and the cancer cells were also removed from the liquid nitrogen and defrosted in a 37°C water bath with continuous shaking. The contents of the cell cryopreservation tube were then transferred to a 15 mL centrifuge

tube and centrifuged at 1,000 ×g for 4 min. After discarding the supernatant, 3 mL of PBS was used to resuspend the cells by gently blowing. The cells were again centrifuged at 1,000 ×g for 4 min, then the cell pellet was resuspended in 1 mL of DMEM or RPMI-1640 medium. This cell suspension was then added to 9 mL of DMEM or RPMI-1640 medium containing 10% fetal bovine serum, 10,000 U/mL penicillin and 10,000 μg/mL streptomycin in a 10 cm petri dish, which was shaken horizontally using the cross method. After observing the cell density under a microscope, the dish was placed in a saturated humidity incubator at 37°C and 5% CO_2 for observation and culture. After 24 h, the cells were washed with PBS and resuspended in fresh culture media. When the cell density reached 70%–85%, subculturing was carried out.

2.7 Culture of cancer cell lines

After culturing in the petri dish for 48 h, the cells appeared to form a dense monolayer (70%–85% confluence), as assessed under an inverted microscope. The human gastric cancer AGS cell line was cultured in prewarmed RPMI-1640 medium with 10% fetal bovine serum (Zhang et al., 2022), 10,000 U/mL penicillin and 10,000 μg/mL streptomycin. The culture medium, PBS and pancreatin were prewarmed in a 37°C water bath. The culture medium was then discarded from the petri dish and the cells were washed by the addition of 3 mL of prewarmed PBS followed by gently shaking. Then the PBS was removed and discarded. Next, 1 mL of trypsin containing 0.25% EDTA was added dropwise to the culture dish, followed by incubation with gentle shaking for 3 min. The morphology of the cells was observed under a microscope and the cells became round, with larger intercellular spaces. At this point, 3 mL of culture medium was added to terminate the reaction, and the cells were completely detached from the dish wall by gentle blowing. The cells were then collected into a 15 mL centrifuge tube and centrifuged at 1,000 ×g for 4 min. The supernatant was discarded and the cells were resuspended in 3 mL of PBS by gentle blowing, followed by centrifugation at 1,000 ×g for 4 min. The cell pellet was resuspended in 3 mL of medium by gentle blowing. To each of three 10 cm culture dishes, 9 mL of culture medium was added, followed by 1 mL of culture medium containing cells, to passage the cells at a 1:3 ratio. The cells were shaken horizontally using the cross method to ensure even mixing. The cell morphology was observed under a microscope, and the cells were then incubated at 37°C and 5% CO_2 in a saturated humidity incubator for continuous culture. The culture media and passage were adjusted according to the growth phase of the cells.

2.8 Cryopreservation of cancer cell lines

The test materials were prepared according to the above methods, and an ultraviolet lamp was used for sterilization. After sterilization, the growth status of the cells was observed through an inverted microscope, and cells in logarithmic growth phase were selected for freezing. The culture medium was then removed from the cultured cells and the cells were washed with 3 mL of PBS. After removal of the PBS, 1 mL of trypsin containing 0.25% EDTA was added to the culture dish, and mixed by gentle shaking in an incubator for 3 min. Next, 3 mL of fresh medium was added to resuspend the cells by gentle blowing and terminate the reaction. The culture medium containing cells was transferred to a 15 mL centrifuge tube and centrifuged at 1,000 ×g for 4 min, then the supernatant was discarded. Next, 3 mL of PBS was added and the resuspended cells were centrifuged at 1,000 ×g for 4 min, then the supernatant was discarded. Following this, 1 mL of prepared cryopreservation solution was added, and the cells were mixed to disperse any aggregates. The cells were transferred to a 1.5 mL cryopreservation tube and stored at three different temperatures: 4°C for 1 h, -20°C for 1 h, and -80°C for overnight storage. They were then placed into liquid nitrogen for freezing.

2.9 Detection of the inhibitory effect of white lion goose serum powder on human cancer cells

2.9.1 CCK-8 assay to detect cell proliferation

White Lion Goose serum powder and 5-Fu were prepared as 1,000 μg/mL and 300 μg/mL stock solutions, respectively. After filtering through an aseptic filter membrane to remove any impurities, the stock solutions were stored at 4°C. Before use, the stock solution was serially diluted in PBS.

Logarithmic growth phase cancer cells were inoculated into a 96-well plate at a density of 2×10^4 cells/mL and incubated at 37°C and 5% CO_2 until the cell density of the monolayer reached 70%–85% confluence. To the drug treatment group, 10 μL of White Lion Goose serum of different concentrations was added. To the positive control group, 5-Fu uracil at a concentration of 300 μg/mL was added. Finally, to the blank control, cell culture medium alone with no cells was added. Five wells per concentration were set up, and the cells were cultured to the indicated time point. Cell morphology was observed using an inverted microscope, the cell survival was assessed using the CCK-8 method. Briefly, 10 μL of CCK-8 solution was added to each well. After incubation for 2 h, the absorbance values were measured at 450 nm, with 630 nm as the reference wavelength for the enzyme marker, and the cell viability was calculated according to Equation 1:

$$\text{Cell viability (\%)} = [(A_{\text{medicated group}} - A_{\text{blank control}})/(A_{\text{non-administered group}} - A_{\text{blank control}})] \times 100 \quad (1)$$

2.9.2 Inverted microscopy to observe changes in cell morphology

Cancer cells in logarithmic growth phase were used to inoculate a six-well plate at a density of 2×10^5 cells/mL, with 2 mL added to each well. The plate was then incubated at 37°C and 5% CO_2 until the cells adhered to the walls of the plate. The culture medium was then aspirated and the wells were gently washed with 3 mL of PBS, which was then discarded. To the drug treatment group, 10 μL of White Lion Goose serum of different concentrations was added. To the positive control group, 300 μg/mL of 5-Fu uracil was added. Finally, to the blank control group, cells were cultured alone. Cell morphology was observed under an inverted microscope after culture at 37°C, and images were recorded.

2.9.3 Scratch test

Six-well plates were marked on the back with uniform lines at intervals of 0.5–1 cm so as to fix the observation points. Logarithmic growth phase cancer cells were inoculated into the six-well plate at a density of 2×10^5 cells/mL, 2 mL per well, and were mixed evenly by the cross method. The plate was incubated at 37°C and 5% CO_2 until the cells reached more than 70%–85% confluence. A scratch was then created using a 200 μL pipette head at an angle perpendicular to the plate. The culture medium was aspirated and discarded, and the cells were washed with 3 mL of PBS 1–2 times. After discarding the PBS, cells were cultured in medium containing 1% fetal bovine serum. To the drug treatment group, 10 μL of different concentrations of White Lion Goose serum were added. Culture medium containing 1% fetal bovine serum was used as a negative control, and to the positive control group, 300 μg/mL of 5-Fu uracil was added. After culturing for the indicated time, cell growth at the scratch was observed under an inverted microscope. Five areas within the field of vision were randomly selected, and the cell migration distance was measured to assess cell mobility.

2.9.4 PI staining to detect the cell cycle distribution

Logarithmic growth phase cancer cells were inoculated into six-well plates at a density of 5×10^5 cells/mL, with 2 mL added per well. The cells were incubated at 37°C with 5% CO_2 until the density of the cell monolayer reached 70%–85% confluence. The culture medium was discarded and the cells were washed with 3 mL of PBS. After discarding the PBS, 10 μL of different concentrations of White Lion Goose serum were added to the drug treatment group. To the positive control group, 300 μg/mL of 5-Fu uracil were added. Cells cultured alone were included as a blank control. All groups of cells were incubated at 37°C and 5% CO_2. Samples of the cell culture solutions were stored for later use. The cells were then digested with 0.25% trypsin and harvested by centrifugation at 1,000 ×g for 3–5 min. Cells were washed with pre-cooled PBS and fixed in 70% ethanol for 8 h. After centrifugation, the cells were resuspended in 0.5 mL of propidium iodide (PI) staining solution per tube, and incubated at 37°C for 30 min. Red fluorescence was detected by flow cytometry at an excitation wavelength of 488 nm and light scattering was also detected.

2.9.5 Annexin V-FITC staining to detect apoptotic cells

Logarithmic growth phase cancer cells were inoculated into six-well plates at a density of 5×10^5 cells/mL, with 2 mL added per well. The cells were incubated at 37°C and 5% CO_2 until the density of the monolayer reached 70%–85% confluence. The culture medium was then replaced with 3 mL of PBS to wash the cells. After removal of the PBS, 10 μL of different concentrations of White Lion Goose serum were added to the drug treatment group. To the positive control group, 300 μg/mL of 5-Fu uracil was added, whereas cells cultured alone were included as the blank control group. All cells were incubated at 37°C and 5% CO_2. Samples of the cell supernatants were retained in 15 mL centrifuge tubes for later use and the remaining cells were washed with PBS. The cells were harvested by centrifugation at 1,000 ×g for 3–5 min, then washed with pre-cooled PBS for 3–5 min. After discarding the supernatant, the cells were re-suspended in 195 μL of Annexin V-FITC binding solution, then 5 μL of Annexin V-FITC staining solution was added, along with 10 μL of PI. The cells were mixed, incubated at room temperature for 10–20 min, then immediately detected by flow cytometry.

2.10 Effect of White Lion Goose serum powder on human gastric cancer cells

2.10.1 Effect of White Lion Goose serum powder on the proliferation of human gastric cancer cells

Human AGS gastric cancer cells were treated with White Lion Goose serum at concentrations of 200, 400, 800 and 1,000 μg/mL for 24 h, followed by culturing at 37°C and 5% CO_2 for 8, 12, 24 and 48 h. Then, the absorbance value (OD450) was measured and cell viability was calculated.

2.10.2 Effect of White Lion Goose serum powder on the morphology of human gastric cancer cells

Human AGS gastric cancer cells were treated with White Lion Goose serum powder at concentrations of 200, 400, 800, and 1,000 μg/mL for 24 h, followed by culturing at 37°C and 5% CO_2 for 8, 12, 24, and 48 h. The morphology of the cells was then observed and photographed under an inverted microscope.

2.10.3 Effect of White Lion Goose serum powder on the migration of human Gastric cancer cells

Human AGS gastric cancer cells were cultured at 37°C and 5% CO_2 for 8, 12, 24, and 48 h. The migration of cells across the scratch was observed under an inverted microscope and cell motility was assessed.

2.10.4 Effect of White Lion Goose serum powder on the human gastric cancer cell cycle

Human AGS gastric cancer cells were cultured at 37°C and 5% CO_2 for 8, 12, 24, and 48 h. The growth phase of cells was detected by flow cytometry and PI staining.

2.10.5 Effect of White Lion Goose serum powder on the apoptosis of human gastric cancer cells

Human AGS gastric cancer cells were treated with White Lion Goose serum powder at concentrations of 200, 400, 800, and 1,000 μg/mL for 24 h. After 8, 12, 24, and 48 h incubation at 37°C and 5% CO_2, the apoptosis of AGS cells was detected by Annexin V-FITC double staining and flow cytometry.

3 RESULTS AND DISCUSSION

3.1 The contents of IgG, SOD, GSH, thymosin and TRF in serum powder of White Lion goose

The content of immunoglobulin IgG, superoxide dismutase SOD, glutathione GSH, thymosin and transferrin TRF in serum was 533.71 μg/mL, 387.32 U/mL, 4.54 μg/mL, 309.13 pg/mL and 1,532.39 μg/mL respectively.

3.2 Effect of White Lion Goose serum on the activity of human AGS gastric cancer cells

As shown in Table 1, AGS cells cultured *in vitro* were treated with different concentrations of White Lion Goose serum. The absorbance value of each group decreased with the increased concentration, which indicated that White Lion Goose serum may significantly inhibit the proliferation of human gastric cancer cells in a dose-dependent manner. With an extended incubation time, the absorbance value of the blank control group and each dose group increased, showing a significant difference compared with the blank control group ($P < 0.05$); whereas, the absorption value of the drug-treated group decreased with increased incubation time. Different doses of White Lion Goose serum had different effects on the viability of gastric cancer cells. The levels of viability of AGS cells were 145.35, 43.64, 21.43, 19.46, and 184.47% when treated with 200 μg/mL of serum and 5-Fu for 8 h. The viability of AGS cells treated with 200 μg/mL serum was higher than that of the blank control group, which may be due to the fact that the low concentration of Goose serum played a similar role to that of fetal bovine serum in the medium, promoting cell proliferation. However, after 48 h of continuous treatment, cell activity decreased to 54.58, 22.53, 7.10, 5.96, and 23.71%, respectively. This indicated that an increased concentration and incubation time with White Lion Goose serum inhibited the growth of gastric cancer cells and gradually reduced cell activity, showing strong dose and time dependence. The activity of 800 and 1,000 μg/mL of White Lion Goose serum was superior to that of the positive control drug.

3.3 Effect of White Lion Goose serum on the morphology of human AGS gastric cancer cells

As shown in Figure 1, with extended incubation time, the cells in the blank control group showed clear cell contours, good adherence, polygonal or irregular shapes, and almost no cells with a full cell body. However, after treatment with different concentrations of White Lion Goose serum and 5-Fu, the morphology of AGS cells was roughly the same. When the treatment time was 8–24 h, only a few cells became smaller and the cell morphology did not change significantly. However, with an increased concentration of Goose serum powder and an extended incubation time, the deformation of AGS cells was obvious. In particular, after being treated with 800 and 1,000 μg/mL White Lion Goose serum and 5-Fu for 48 h, the volume of AGS cells and the number of adherent cells gradually decreased, while the amount of particulate matter in the cells increased. The large number of non-adherent cells resulted in turbidity in the culture solution. This shows that White Lion Goose serum induces changes in the morphology of AGS cells.

3.4 Effect of White Lion Goose serum on the migration of human AGS gastric cancer cells

Cell migration refers to the movement of cells after receiving migration signals or stimulation from certain substances. During the migration process, cells repeatedly protrude forward, and detecting the level of AGS cell migration can provide insight into the diffusion speed of cancer cells (Zhang et al., 2022). In this experiment, to avoid false positive migration results caused by excessive cell proliferation, cancer cells were cultured in serum-free or low-concentration serum for the scratch test. As shown in Figure 2, AGS gastric cancer cells have strong migration ability. After treatment with different concentrations of White Lion Goose serum, cell migration was significantly inhibited. The 5-Fu also had some effect on the mobility of gastric cancer cells. When AGS cells were treated with 200, 400, 800, and 1,000 μg/mL White Lion Goose serum and 5-Fu for 8 h, the cell mobility levels were 9.50, 8.57, 7.89, 6.78, and 8.13%, respectively. When the culture was continued to 48 h, their cell mobility increased to 36.59, 23.85, 13.37, 12.96, and 23.12%, respectively. It is therefore evident that White Lion Goose serum can inhibit the activity of cancer cells and slow their proliferation. With an increased White Lion Goose serum concentration and an extended incubation time, the mobility of AGS cells gradually decreased and this inhibitory effect gradually increased. The inhibitory effects observed with 800 and 1,000 μg/mL White Lion Goose serum were superior to those observed with 5-Fu. As indicated in Figure 2, after 48 h of migration of untreated cancer cells, the scratched area became covered by cells compared with the migration of only a small number of cells in the drug-treated group with different concentrations of White Lion Goose serum and 5-Fu added. These results showed that White Lion Goose serum has a significant inhibitory effect on the migration of AGS cells, and this effect was more prominent than with 5-Fu.

3.5 Effect of White Lion Goose serum on the cell cycle distribution of human AGS gastric cancer cells

The cell cycle refers to the process through which a cell completes one division. The cell cycle is divided into two stages:

Table 1. Absorbance of White Lion goose serum at different times after AGS cells.

Group	Drug dose μg/mL	OD			
		8 h	12 h	24 h	48 h
Blank group	0	0.73 ± 0.043	0.95 ± 0.026	1.39 ± 0.145	2.02 ± 0.087
Positive group(5-FU)	300	1.159 ± 0.028*	1.004 ± 0.010 *	1.042 ± 0.029*	0.637 ± 0.024*
Serum dose group of white lion goose	200	0.960 ± 0.022*Δ	0.969 ± 0.006	1.230 ± 0.034*Δ	1.195 ± 0.044*Δ
	400	0.442 ± 0.031*Δ	0.446 ± 0.038*Δ	0.545 ± 0.028*Δ	0.615 ± 0.024*
	800	0.329 ± 0.006*Δ	0.333 ± 0.020*Δ	0.326 ± 0.017*Δ	0.336 ± 0.022*Δ
	1000	0.319 ± 0.005*Δ	0.310 ± 0.004*Δ	0.316 ± 0.004*Δ	0.316 ± 0.001*Δ

*Compared with the blank group, the difference was significant, $P < 0.05$; ΔCompared with the positive group, the difference was significant, $P < 0.05$.

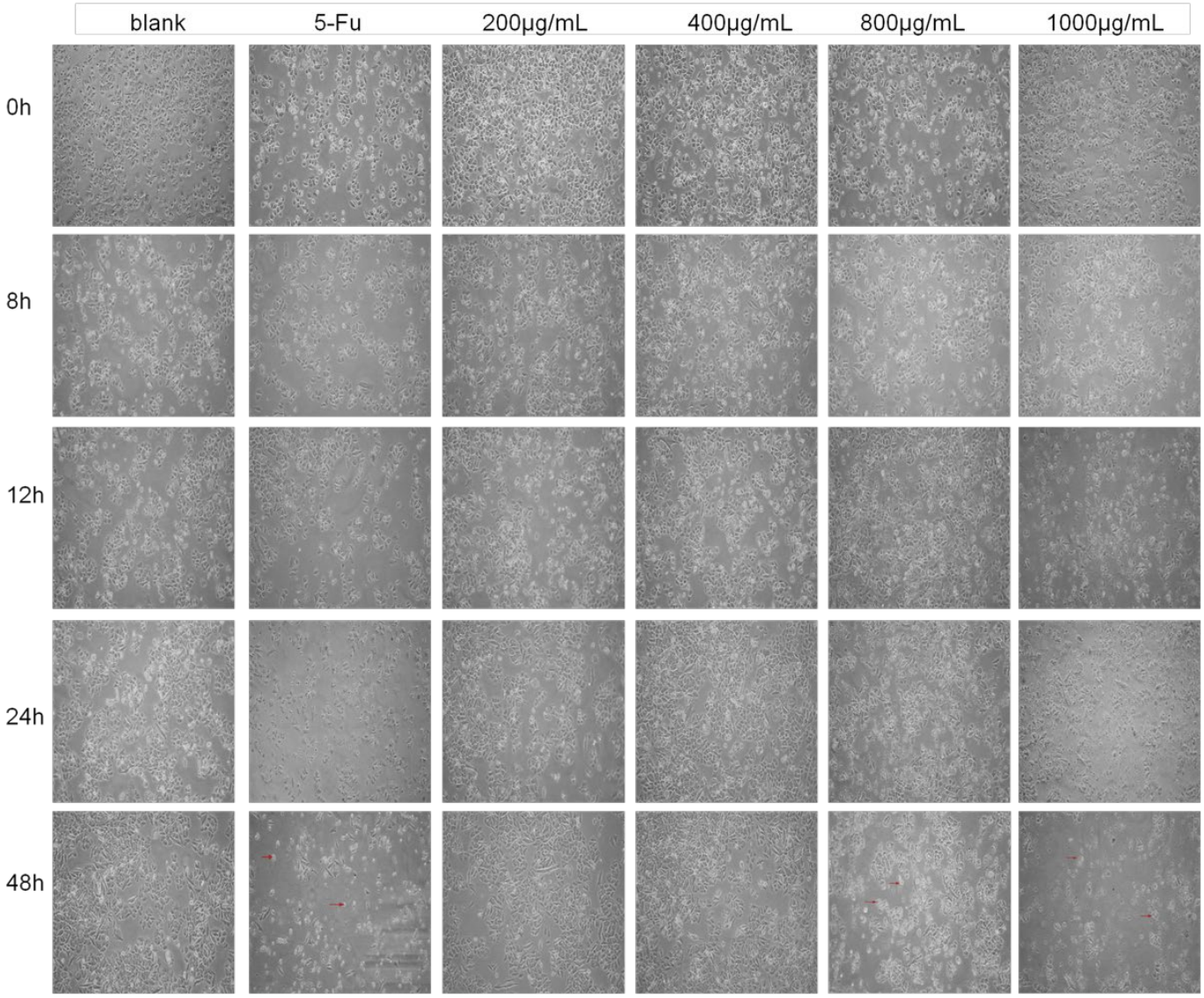


Figure 1. Cell morphology of White Lion goose serum after 8 to 48 hours of AGS gastric cancer cells (inverted microscope×10).

the intercellular phase and the cell division phase (M phase), and the intercellular phase can further be divided into the prophase (G1), anaphase (S), anaphase (G2), and mitotic phase. Because it is a cycle, adjacent phases are often grouped, i.e., G0/G1 phase, S phase and G2/M phase, and the DNA content in each phase differs. The DNA content in G0/G1 phase is 2N, that in G2/M phase is 4N, and that in S phase is between the two. Propidium iodide (PI) is a double-stranded DNA fluorescent dye that produces red fluorescence when combined with double-stranded DNA, and the fluorescence intensity is proportional to the amount of double-stranded DNA. Therefore, analysis of the cell cycle and cellular apoptosis can be carried out by detecting the DNA content and distribution by flow cytometry. In addition, due to the phenomena of nucleus concentration and DNA fragmentation in apoptotic cells, some genomic DNA fragments are lost in the staining process, resulting in weak staining. In flow cytometry, a so-called sub-G1 peak, i.e., an apoptotic cell peak, can be observed. As seen in Figure 3, compared with the blank control group, AGS cells treated with 200 μg/mL White Lion Goose serum exhibited no obvious effect on the cell cycle. When the concentration was increased to 400 μg/mL, the number of S phase cells increased, while the number of G0/G1 and G2/M cells decreased. However, the effect of White Lion Goose serum on cell cycle arrest of gastric cancer cells correlated with the drug concentration and the incubation time. Compared with the positive control drug, the 800 g/mL and 1,000 μg/mL dose groups all showed cell cycle arrest in S phase when AGS cells were cultured for 8–12 h, and the effect was stronger than that of 5-Fu, which indicated that the 800 g/mL dose group had a certain promoting effects on the cell cycle process, as well as anti-tumor effects. When 5-Fu was used to treat AGS cells, the number of S phase cells increased, but the number of G0/G1 phase and G2/M phase cells decreased, and sub-G1 apoptosis peaks appeared at 24 and 48 h. This may be due to the toxic effects of 5-Fu anti-cancer drugs on normal cells, thus disturbing the cell cycle and inducing apoptosis.

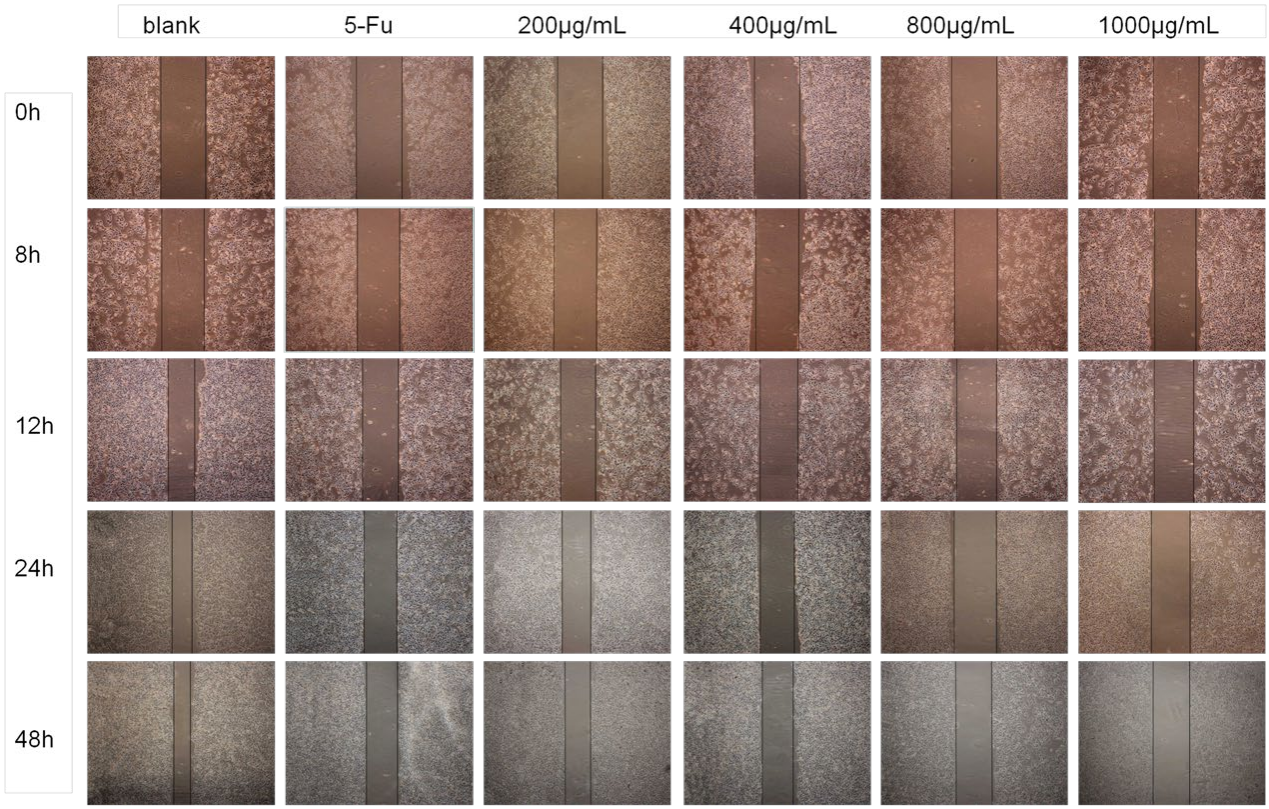


Figure 2. Effect of White Lion goose serum on migration level of AGS cells for 8-48 hours.

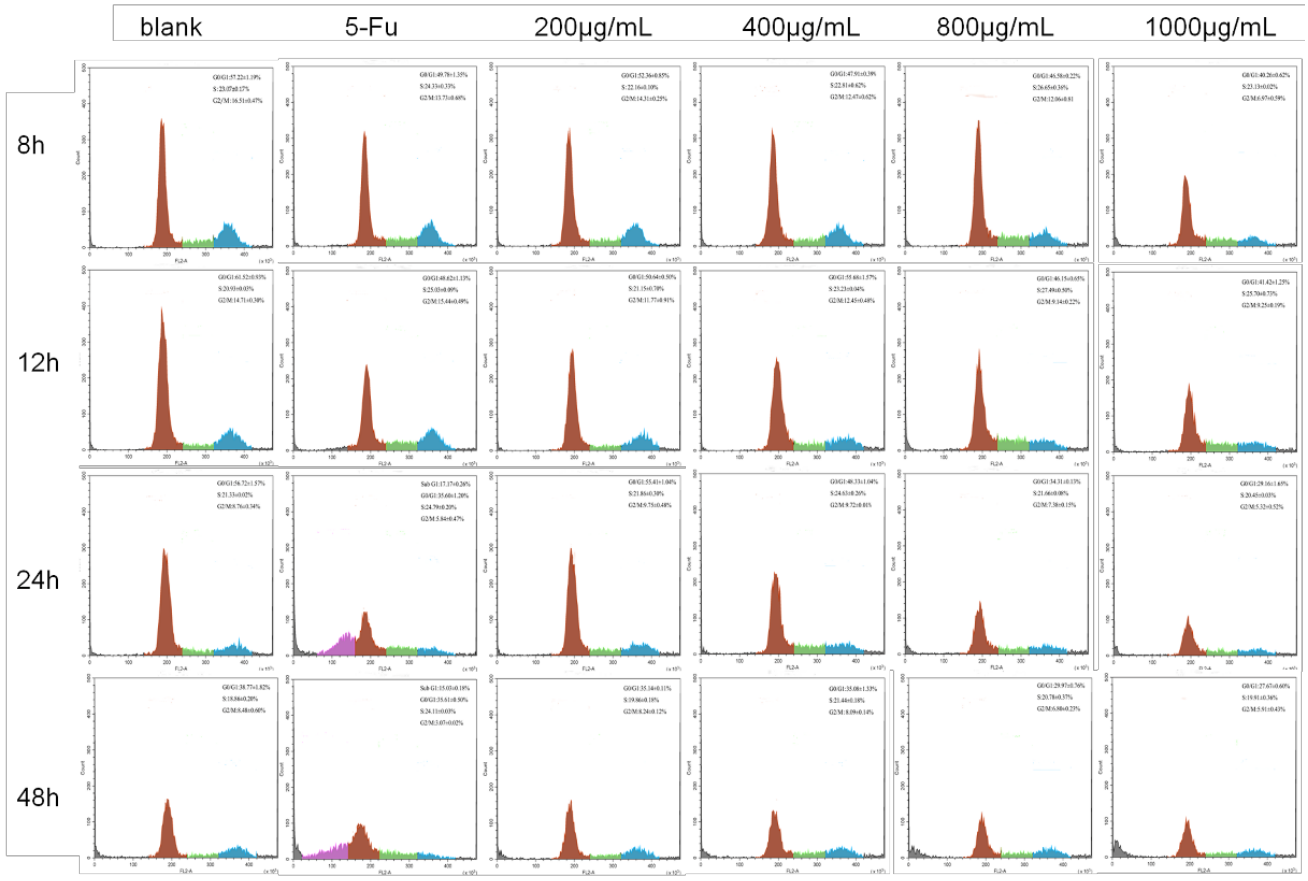


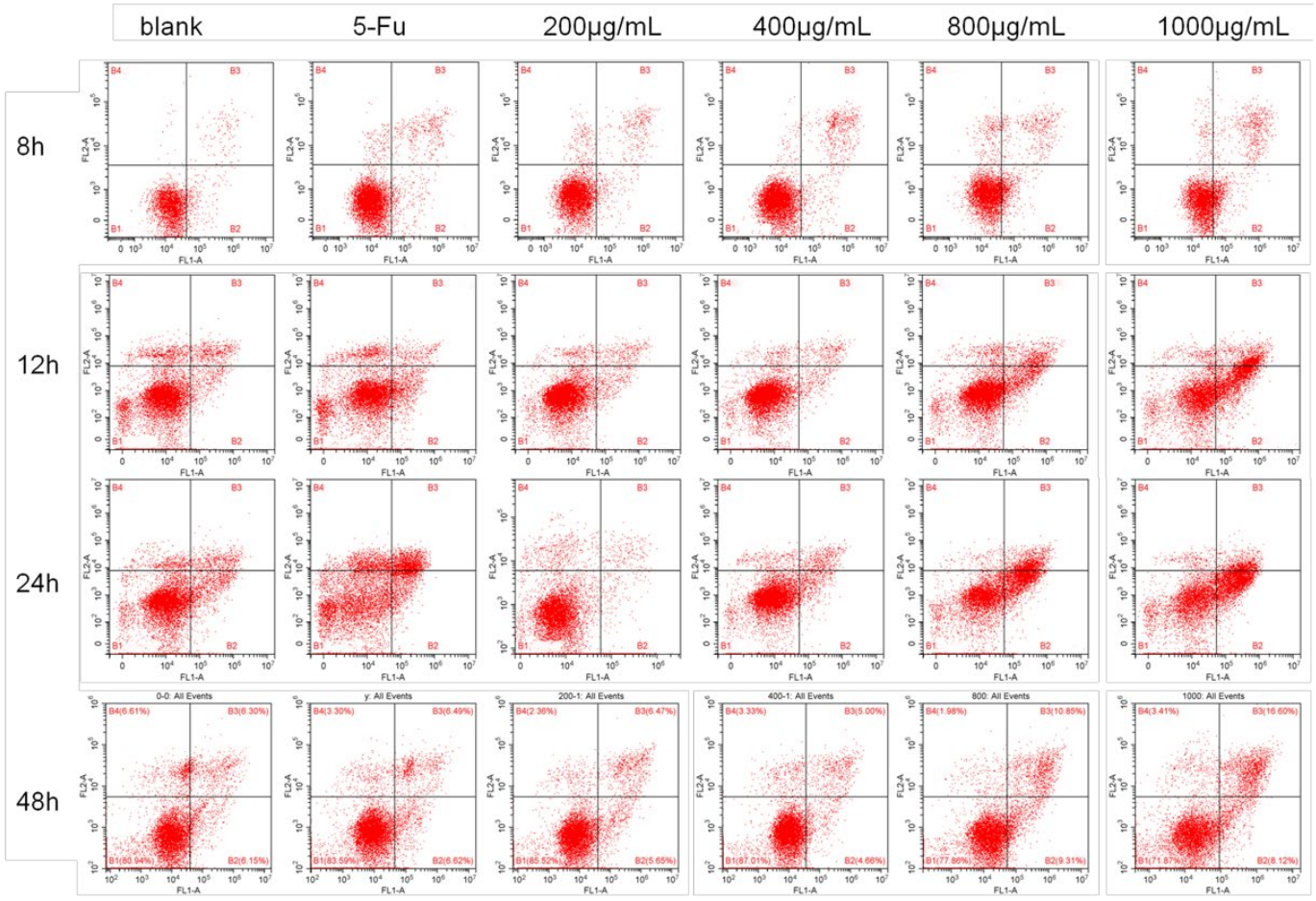
Figure 3. Cell cycle distribution of AGS gastric cancer cells treated with White Lion goose serum for 8-48 hours.

3.6 Effect of White Lion Goose serum on the apoptosis of human AGS gastric cancer cells

Annexin V is a calcium-dependent phospholipid binding protein widely distributed in the cytoplasm of eukaryotic cells, which can selectively bind to phosphatidylserine (PS). PS is mainly distributed on the inner side of the cell membrane. In the early stage of cell apoptosis, different types of cells evert PS to the cell surface, outside of the cell membrane (Bernhard et al., 2000). PS exposure on the cell surface promotes coagulation and inflammatory reactions, while Annexin V can block the procoagulation and proinflammatory activity of PS after combining with the everted cell surface PS. Propidium iodide (PI) is a fluorescent dye that can bind to DNA. It dyes necrotic cells and cells that lose cell membrane integrity in late apoptosis (An et al., 2022), as visualized by red fluorescence. For necrotic cells, Annexin V can enter the cytoplasm because of the lack of cell membrane integrity and combine with PS located inside the cell membrane, as visualized by green fluorescence. As seen in Figure 4, the apoptosis rate of gastric cancer cells was significantly increased after treatment with 5-Fu and different concentrations of White Lion Goose serum for 8–48 h, it indicates that compared with the blank control group (Deswaerte et al., 2018), the number of living cells decreased significantly ($P < 0.05$) after treatment with different concentrations of White Lion Goose serum for 8 h, and this effect was dose- and time-dependent. When treated with 200 and 400 μg/mL of White Lion Goose serum for 8–24 h, no distinct populations of early or late apoptotic cells were evident. However, when the White Lion Goose serum concentration was increased to 800 μg/mL, the apoptosis rate increased from 16.51% at 8 h to 57.32% at 48 h, and when it was further increased to 1,000 μg/mL the apoptosis rate increased from 26.10% at 8 h to 64.71% at 48 h, an increase of 40.81 and 38.61%, respectively, with no obvious change in the number of necrotic cells. AGS cells treated with 5-Fu showed the same trend. The number of living cells decreased with extended incubation time and the apoptosis rate gradually increased, but this effect was lower than that induced by White Lion Goose serum.

4 DISCUSSION

Goose blood is rich in bioactive substances such as immunoglobulins, superoxide dismutase, transferrin, thymosin and other nutrients such as vitamins and trace elements. These components contribute to the effects of goose blood on eliminating sclerosis and tumors, invigorating Qi and nourishing Yin, and soothing the liver and stomach. However, the nutrient content



B1: living cells; B2: early apoptotic cells; B3: late apoptotic cells; B4: necrotic cells.

Figure 4. Effect of White Lion goose serum on apoptosis of AGS gastric cancer cells for 8-48 hours.

of goose blood is dependent on the breed, the nutrition level and the feeding environment.

Some studies have suggested that goose serum contains high concentrations of immune antigen substances, which can scavenge free radicals in vivo, increase the white blood cell concentration in blood, effectively inhibit the growth of cancer cells, sharply reduce cell nuclear fission, and induce tumor cell apoptosis and the resolution of tumor tissue atrophy (Garaci et al., 2012; Storm et al., 2015). Previous studies have shown that B lymphocytes can recognize antigens in tumor tissues and produce IgG antibodies to inhibit the growth of cancer cells (Li et al., 2009). Serum IgG can also activate the phagocytosis of macrophages in vivo. Macrophages are an important component of the body's non-specific immunity. They can actively kill and digest antigenic substances such as tumor cells, and inhibit their malignant proliferation, thus neutralizing tumor cells. In addition, IgG can also alter the adhesive and migratory properties of tumor cells, thus inhibiting their proliferation (Guillem & Sampsel, 2006). The serum IgG concentration was negatively correlated with tumor volume in squamous cell carcinoma patients, confirming the important role of IgG in tumor occurrence and development (Mizukami et al., 2005). The occurrence of cancer is closely related to the level of reactive oxygen species (ROS) in vivo. A high level of ROS can cause oxidative damage in the body, resulting in the activation of proto-oncogenes and the inactivation of tumor suppressor genes, thereby interfering with the normal expression of cytokines and metabolic enzymes. However, superoxide dismutase (SOD) can catalyze the disproportionation reaction of superoxide anion radicals and decompose them to generate water molecules and oxygen molecules to protect cells against oxidative damage (Strassburger et al., 2005). SOD can also induce apoptosis by downregulating the Bcl-2 gene, upregulating the Bax gene, increasing the Bax/Bcl-2 ratio and increasing Caspase-3 activity. When the Bax protein is induced by apoptosis, it interacts with mitochondrial channel protein, triggering the activation of Caspase-9, which in turn catalyzes Caspase-3 and induces cell apoptosis. In addition, SOD can also induce apoptosis through the death receptor (Fas) pathway. When apoptosis signals occur, SOD triggers the apoptosis of target cells where Fas is located, and then continues to transmit apoptosis signals to Caspase-8, activating a series of enzyme-linked reactions of the Caspase-8 family, thus leading to apoptosis and morphological changes of cancer cells (Tian et al., 2017). The Mn-SOD functions by inhibiting the growth of tumor cells and has an inhibitory effect on malignant tumor cells (Millikin et al., 1991). The thymus is the most important immune organ of the body. A variety of thymosin enzymes extracted from the thymus have anti-tumor activity. Thymus peptide, which exists as a polypeptide, is reported to promote lymphocyte growth, further regulate T cell differentiation and maturation, and promote the production of antibodies by B cells. At the same time, thymus peptide can also promote the production of cytokines, such as IL-2 and interferon (IFN), and enhance the ability of natural killer (NK) cells and macrophages to improve the immune function of the body, whilst also exerting an anti-tumor role. Schulof et al. (1985) found that thymosin-1 can significantly improve the recurrence and overall survival rate of patients with non-small cell carcinoma. In recent years, studies have found that using transferrin as a targeted ligand to deliver drugs to malignant tumors with high expression of transferrin receptor has achieved effective results. Transferrin receptor (TfR) is an important component protein necessary for iron metabolism in vivo. Under its mediation, iron metabolism, immune function and cell regulation of the body can operate normally. Therefore, TfR plays a role in the metabolic activities of rapidly proliferating cancer cells, and it is generally combined with other drugs for the targeted therapy of tumor diseases. Yeh e Faulk (1984) and colleagues used glutaraldehyde to combine TfR with the anticancer drug Adriamycin through its Schiff base, and found that this TfR drug combination could selectively kill leukemia cells and had no toxicity against normal blood cells. Yang et al. (2011) found that transferrin liposome can inhibit the cell proliferation of tumor cell lines and induce the apoptosis of cancer cells through ROS-related mitochondrial pathways.

Previous research results showed that IgG, SOD, TRF and thymosin all have anti-cancer effects, mainly directed by activating antibodies, promoting phagocytosis of macrophages, and protecting cells from oxidative damage. In our study, White Lion Goose serum was found to be rich in IgG, SOD, TRF and thymosin, contributing to its anti-cancer properties, which include its ability to inhibit proliferation, slow migration, block the cell cycle, and promote the apoptosis of cancer cells.

5 CONCLUSIONS

White Lion Goose serum can reduce the activity of AGS gastric cancer cells by inhibiting their proliferation, and this effect was dose- and time-dependent. White Lion Goose serum also changed the morphology of cancer cells, increasing their propensity to rupture with increased serum concentration and incubation time. A scratch assay revealed that White Lion Goose serum has a significant inhibitory effect on the migration ability of AGS cells, and this effect was also dose- and time-dependent. By flow cytometry, White Lion Goose serum was found to block the cell cycle of AGS cells in DNA synthesis phase (S phase) and induce the apoptosis of cancer cells. The apoptosis rate gradually increased with increased White Lion Goose serum concentration and an extended incubation time. According to the above experimental results, we concluded that White Lion Goose serum inhibited the proliferation, disrupted the cell cycle and promoted the apoptosis of cancer cells.

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