



Effects of Kangbingdu oral liquid on immunological function of normal mice

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ABSTRACT

To explore the effects of Kangbingdu Oral Liquid (KOL) on immunological function of normal mice. The effects of the large, middle and small dosage KOL and Hornsey brand KOL were investigated on normal mice by macrophage percentage and index of phagocytosis, the formation of hemolysin and hemolytic plaque and lymphocyte transformation. Compared with physiological saline group, the large, middle and small dosage KOL group and Hornsey brand KOL group could significantly improve the macrophage percentage of normal mice abdominal cavity, the large, middle dosage KOL group and Hornsey brand KOL group could significantly improve the phagocytic index of normal mice abdominal cavity. The large, middle and small dosage KOL group and Hornsey brand KOL group could significantly promote the formation of hemolysin of normal mice, the large dosage KOL group and Hornsey brand KOL group could significantly promote the formation of hemolytic plaque, the middle and small dosage KOL group could significantly promote the formation of hemolytic plaque. The large, middle and small dosage KOL group and Hornsey brand KOL group could significantly improve the lymphocyte transformation of normal mice, the middle dosage KOL group had the strongest effect. KOL could enhance the immune function of normal mice.

Key words: Kangbingdu Oral Liquid, Normal mice, Immunological function.

INTRODUCTION

Kangbingdu Oral Liquid (KOL) was a compound preparation of traditional Chinese medicine, was composed of Radix Isatidis, gypsum, Anemarrhena, Forsythia and other components [1], the prescription was from the Eastern Han medical written by Zhang Zhongjing in "Treatise on Febrile Diseases", KOL has heat clearing and dampness, cooling blood detoxification, anti-inflammatory, antiviral, heat clearing and detoxifying pharmacological effects [2], used for influenza, foot and mouth disease, upper respiratory tract infection, viral influenza, eye disease. Clinical for the treatment of infantile viral pneumonia, acute bronchitis, mumps, cold and so on [3]. To investigate the characteristics of KOL, we observed the effect on immune function in normal mice.

EXPERIMENTAL SECTION

Instruments and reagents drugs: UV-2000 UV VIS spectrophotometer: Unico (Shanghai) instruments Co.Ltd.; BI-2000 medical image analysis system, Chengdu Taimeng Electronic Co.Ltd. KOL, confidence in Zhengzhou Pharmaceutical Company Limited production, batch number 0607001; the country medicine accurate Z41022403; KOL (Hornsey card), Guangzhou Xiangxue pharmaceutical Limited by Share Ltd production, batch number: 0606001; the country medicine accurate Z10890017; saline: Zhengzhou Chemical Pharmaceutical Company Limited production, batch number: 050409; Rapid Wright's stain, Mike limited company of science and technology production, batch number: 040602; phytohemagglutinin (PHA), Shanghai Yihua production, batch number: 050101; glucose, Tianjin Municipality kemi'ou technology limited production, batch number: 20040702.

Animals: Kunming, grade II, provided by experimental animal center of Hebei Province, the certificate number: 704168.

EXPERIMENTAL SECTION

Effects on the phagocytic function of normal mouse peritoneal macrophage

Took 50 mice, weighing 18~21g, half male and half female, were randomly divided into 5 groups, respectively, were gavage of large, middle and small dose group (KOL concentrations were 1.5 times dose, 0.75 times dose, 0.375 times dose, intragastric volume was 0.1ml/10g, 30, 15, 7.5 times the amount equivalent to in clinical medication) and Hornsey brand KOL group (positive control, concentration was 0.75 times dose, intragastric volume was 0.1ml/10g, 15 times the amount equivalent to the clinical medication) and saline of the same volume (volume of perfusion for 0.1ml/10g). Administered 1 times daily, continuous administration for 7 days. The morning of the seventh day of each rats were injected 5% chicken red blood cell (CRBC) saline solution 0.5ml, on the seventh day after intragastric administration of 2h, to chicken red blood cells after 4h, the mice were sacrificed by cervical dislocation [4]. Intraperitoneal injection of Han's fluid 2.5ml, gently rubbed the mice abdomen, open the mouse abdominal skin, cut a small hole in the peritoneum, absorbed the peritoneal fluid 2ml in a test tube with straw, mixed, learned a little of peritoneal fluid drops on the slide, liquid point about the size of 1.5cm×2cm. The slides on a shop to have the porcelain plate wet gauze, 37°C incubating for 30min, normal saline flush to attached cells, Wright's staining, water washing to dry, phagocytosis of peritoneal macrophages of mice were observed under microscope, and calculated the percentage and index of phagocytosis.

Influence on the formation of normal mouse hemolysin and hemolysis

Number of animals, groups, administration and dosage were the same as before, on the first day of administration, each mouse was injected intraperitoneally 5% suspension of chicken erythrocytes saline 0.2ml/each, immunization, administered at the seventh day after 2h, blood samples in mice [4], centrifugal, serum; with physiological saline 1:100 diluted, the 1ml dilution and 5% CRBC suspension 0.5ml, complement 0.5ml 10% (Guinea pig serum, using chicken red blood cells pre saturated 6H) mixed, incubated at 37°C, ice water termination reaction; the blank additional without complement tube as control, the supernatant from 540nm colorimetric tube, determination of hemolysin formation groups. Mice were sacrificed by cervical dislocation after blood, anatomy from spleen, the two together with the spleen homogenate machine, and adjust the spleen cell suspensions of spleen cells in the liquid density was 5×10^6 /ml; spleen cell suspensions of 0.5ml, and 0.2% chicken red blood cell suspension and 1:10 of guinea pig serum 0.5ml blending; the blank additional without complement tube, 1H was incubated at 37°C, centrifugal, the supernatant by 413nm colorimetry, the test group hemolytic plaque formation.

Influence of transformation of normal mouse lymphocytes

Number of animals, groups, and doses were administered as the same with the former, before the 3d administration, each mouse were added daily intramuscular PHA 8mg/kg, the last one after the first dose 2h, tails of mice blood, push tablets Swiss dye staining, oil immersion observation, calculation lymphocyte transformation rate [5].

Statistical Analysis

Data analyzed using SPSS13.0 for windows statistical package for statistical data, the measurement data with the mean \pm SD.

RESULTS AND DISCUSSION

Effects on the phagocytic function of normal mouse peritoneal macrophage

Observation of mouse peritoneal macrophages on phagocytic percentage and phagocytic index in BI-2000 medical image analyzer, the results can be seen from Table 1.

Table 1 Effect of KOL on phagocytic function of normal mouse peritoneal macrophages ($\bar{x} \pm s$)

Groups	n	Dose (mg/kg)	Phagocytic percentage(%)	Phagocytic index
Physiological saline group	10		46.62±2.1	0.62±0.03
Hornsey brand KOL group	10	7.5	54.8±4.3**	0.71±0.06**
Large dose of KOL group	10	15	56.9±3.9**	0.70±0.03**
Middle dose of KOL group	10	7.5	56.7±4.8**	0.73±0.05**
Small dose of KOL group	10	3.75	52.6±4.0**	0.66±0.06*

Note: * compared with normal saline group $P < 0.05$, ** compared with normal saline group $P < 0.01$

From the chart can be seen, compared with normal saline group, the large, middle and small dosage KOL group and Hornsey brand KOL group could significantly improve the phagocytic function of peritoneal macrophage phagocytosis in normal mice, which was increased obviously ($P < 0.01$), the large, middle dose of KOL group and Hornsey brand KOL group could significantly increase the phagocytic index of peritoneal macrophages of normal mice to chicken red blood cells ($P < 0.01$).

Influence on the formation of normal mouse hemolysin and hemolytic plaque.

The experimental method according to the hemolysin and hemolytic plaque formation, to detect the hemolysin and hemolytic plaque formation, a method for direct determination of OD, the results can be seen from Table 2.

Table 2 Effect of KOL on formation of normal mouse hemolysin and hemolytic plaques ($\bar{x} \pm s$)

Groups	n	Dose (mg/kg)	Formation of hemolysin(OD)	Number of cases	Formation of hemolytic plaque(OD)
Physiological saline group	10		0.130±0.009	5	0.326±0.046
Hornsey brand KOL group	10	7.5	0.175±0.021**	5	0.410±0.042**
Large dose of KOL group	10	15	0.177±0.017**	5	0.438±0.044**
Middle dose of KOL group	10	7.5	0.174±0.013**	5	0.401±0.052*
Small dose of KOL group	10	3.75	0.157±0.026**	5	0.382±0.036*

Note: * compared with normal saline group $P < 0.05$, ** compared with normal saline group $P < 0.01$

From the chart can be seen, the large, middle and small dose of KOL group and Hornsey brand KOL group could significantly promote the formation of hemolysin in normal mice, which was significantly improved ($P < 0.01$), the large dose of KOL group and Hornsey brand KOL group could significantly promote the formation of normal mice hemolytic plaques ($P < 0.01$), the middle and small dose of KOL group could significantly promote the formation of normal mouse hemolytic plaques ($P < 0.05$).

Influence of transformation of normal mouse lymphocytes.

Observation of the lymphocyte transformation in BI-2000 medical image analyzer, the results can be seen from Table 3.

Table 3 Effect of KOL transformation of normal mouse lymphocytes ($\bar{x} \pm s$)

Groups	n	Dose(mg/kg)	Lymphocyte transformation rate (%)
Physiological saline group	10		40.8±2.1
Hornsey brand KOL group	10	7.5	45.9±1.9**
Large dose of KOL group	10	15	46.2±2.4**
Middle dose of KOL group	10	7.5	47.5±2.2**
Small dose of KOL group	10	3.75	44.8±2.0**

Note: * compared with normal saline group $P < 0.05$, ** compared with normal saline group $P < 0.01$

From the chart can be seen, compared with normal saline group, the large, middle and small dosage KOL group and Hornsey brand KOL could significantly improve the group lymphocyte transformation of normal mice ($P < 0.01$), the middle dose of KOL group had the strongest effect.

CONCLUSION

The main components of KOL are gypsum, Radix Isatidis, Rhizoma Phragmitis, raw land, ageratum, forsythia, composition and so on. Traditional Chinese Medicine believes that the good governance plague fever, sore throat, scarlet fever. Modern research confirms it has the antiviral, antibacterial and strengthen the body defense function. From the perspective of modern pharmacology of traditional Chinese medicine, the components combined, played a total of dampness, cooling blood detoxification effect, were found for wind and heat. This drug can inhibit the replication of the virus, but also to enhance the body immunity, bitter, spicy, cold, heat Reduce Pathogenic Fire and do not hurt the Yin, KOL has the effect of antiviral, antibacterial, anti-inflammatory and antipyretic[6]. Modern pharmacy studies of these drugs can also prove the above drugs of Radix Isatidis, Forsythia have broad antimicrobial spectrum and anti-virus effect.

This research selected the phagocytosis of peritoneal macrophages as immune function indexes of nonspecific, hemolysin and hemolytic plaque as specific humoral immune indices, lymphocyte transformation as cell immune index [7], to observed the effects of KOL on immune function in normal mice. The experimental results shown that the large, middle and small dosage KOL group and Hornsey brand KOL could improve the mouse peritoneal macrophage phagocytic percentage and phagocytic index, promoted serum hemolysin and hemolytic plaque formation, promoted lymphocyte transformation, proved that KOL on immune function of normal mice with

enhanced effect.

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