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Original Paper

The Effect of Polyvalent Staphylococcus Vaccine on the Gene Expression of IL-8, IFN-γ, IL-1R and Phagocytes in Mice Spleen

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Abstract

Objectives: To study the effect of Polyvalent staphylococcus vaccine on cellular immune function in mice. Methods: Forty SD kunming species of mice were randomly divided into low dose group (500 million/ml bacteria), middle dose group (1 billion/ml bacteria), high dose group (2 billion/ml bacteria) and control group, Mice were immunized with different doses of Polyvalent staphylococcus vaccine. The expression of IL-8, IFN - γ and IL-1R genes in spleen tissues of mice in different doses of experimental group and control group were detected by RT-PCR, and the phagocytic effect of phagocytes in abdominal cavity of mice was detected by abdominal smear Results: Compared with the normal control group, the expression level of IL-8, IFN - γ, IL-1R gene and phagocytic function of spleen in the experimental group were significantly higher than those in the control group ($P < 0.01$), the effect in the middle dose group were the best. Conclusion: Polyvalent staphylococcus vaccine can significantly improve the expression level of IL-8, IFN - γ, IL-1R gene in mouse spleen, and enhance the phagocytic function of phagocytes, and its effect showed dose-dependent manner.

Keywords

Polyvalent Staphylococcus Vaccine, IFN-γ, IL-8, IL-1R, Phagocyte

1. Introduction

Intractable bacterial infection of face and neck skin is a common disease in dermatology, Antibiotics and single vaccine treatment are used in clinical, the treatment effect is not good, the recurrence rate is high, and there is no ideal treatment method. Our research group separated different types of Staphylococcus from the infected parts of patients with refractory bacterial infection, and formed a multivalent Polyvalent staphylococcus vaccine according to the proportion. On the basis of a series of basic studies, therapeutic multivalent staphylococcal vaccine injection was made for volunteers with

intractable bacterial infection in face and neck. In the preliminary experiment, 1195 patients were treated clinically, and achieved good curative effect, with the effective rate of 97% and the cure rate of 56% - 77% (Yue, Zhou, Feng, Liang, Zhang, Zhang, Liu, You, & Guan, 2016). It has no obvious side effects and is very popular with patients. In order to investigate the effect of multivalent staphylococcal vaccine on cellular immunity, in this study, the effect of Polyvalent staphylococcus vaccine on the gene expression of IL-8, IFN-γ, IL-1R and phagocytes in mice spleen were observed and the mechanism of Polyvalent staphylococcus vaccine in the treatment of intractable bacterial infection was discussed. It is reported as follows:

2. Materials and Methods

2.1 Materials

2.1.1 Development of Multivalent Staphylococcus Vaccine

The screening and identification of multivalent staphylococcal vaccine (Yue, Zhou, Feng, Liang, Zhang, Zhang, Liu, You, & Guan, 2016; Yue, Zhao, Chen, Gong, Yang, Qian, You, & Guan, 2007; Yue, Zhao, You, Zhao, Chen, Yu, & Guan, 2000; Yue, Zhao, You, Zhao, Chen, Yu, & Guan, 1999) widely adopts the focus of intractable infection patients with recurrent upper neck for bacterial isolation and identification. Different types of Staphylococcus aureus, Staphylococcus epidermidis and other Staphylococcus are screened and combined into multivalent staphylococcal bacterial solution according to a certain proportion and concentration.

2.1.2 Preparation and Standard Control of Multivalent Staphylococcal Vaccine

When preparing multivalent staphylococcus vaccine, first increase the bacteria in the broth culture tube, then culture in a Kirschner flask, finally wash the bacteria on the surface of the culture medium with 0.4% formaldehyde sterile normal saline, place it in a 37 ℃ water bath for 48h, centrifuge at 2500 rpm for 20min, wash with sterile normal saline for 3 times, and dilute different types of Staphylococcus to 2 billion bacteria / ml with sterile normal saline, Polyvalent Staphylococcus bacterial solution was prepared according to a certain proportion. After sterilization at 100 ℃ for 30 min, it was packaged in ampoules for use.

2.1.3 Product Inspection

(1) The amount of bacteria is controlled. After the multivalent staphylococcus vaccine preparation is combined, the concentration is measured according to the Chinese bacterial turbidity standard tube produced by the China Institute for the control of pharmaceutical and biological products, so that the concentration of bacterial solution can reach 2 billion bacteria / ml. (2) Sterility test: after sterilization, multivalent Staphylococcus bacterial solution shall be sampled on the ultra clean workbench and cultured on three blood plates, broth tubes, blister meat medium and Sabouraud medium respectively. When it grows aseptically for 48 hours, the culture shall be extended for 24 hours. When there is still no bacterial growth, it can be separately packed in ampoules. After repacking, randomly sample 10 sealed ampoules of multivalent Staphylococcus bacterial solution, open them and carry out aseptic

culture. Only when it is confirmed again that there is no growth of viable bacteria can they be regarded as qualified products. (3) Pyrogen test: according to the test requirements of the Pharmacopoeia, three healthy white rabbits weighing 1.5 ~ 1.6kg were selected. Fasting was started the afternoon before the test, only drinking water was supplied, and the basal body temperature was measured repeatedly. During the test, a disposable sterile injection needle and needle were used to inject 20 ~ 30 times the adult amount of multivalent Staphylococcus bacterial solution through the rabbit ear vein. The anal temperature was measured once every 1H for 4 consecutive times. (4) Acute toxicity test: 30 mice aged 6 ~ 8 weeks and weighing 18 ~ 22G were randomly divided into 3 groups, half male and half female. Before the test, fasting for 14h, only drinking water was supplied. The injection doses of multivalent Staphylococcus were divided into three groups: 0.25ml/animal, 0.5ml/animal and 0.75ml/animal. The animals were injected intraperitoneally for 7 days. There was no abnormality, that is, LD50 was not measured. Then the maximum tolerance dose (MTD) was measured. Another 20 white mice, half male and half female, were injected into the hip muscle twice a day, 0.5ml/mouse each time. After continuous observation for 7 days, the animals did not appear abnormal phenomenon and death. This dosage is equivalent to 1250 times that of adults, which again shows that the preparation has low toxicity and is a safe biological preparation. (5) Allergy test: guinea pigs weighing 250g were injected with 0.5ml of multivalent Staphylococcus solution intraperitoneally, and 1ml of multivalent Staphylococcus solution was injected into the heart after 2 weeks. The animals had no allergy.

SD kunming species of mice were purchased from Qingdao institute for drug control; PCR diagnostic reagent was purchased from Shanghai Sangon Biotech; Wright's-Giemsa Staining Solution was purchased from Jinan Baibo Biological Technology Co., Ltd.

2.2 Animal Experiments in Mice

2.2.1 Preparation of Mice Tissue Specimen

Forty healthy adult Kunming mice weighing 20-30 g, half male and half female, were reared in separate cages in a clean environment with a temperature of 22 ℃ to 28 ℃ and a relative humidity of 50% - 60%. The mice were randomly divided into 4 groups with 10 mice in each group. The experimental group was divided into normal control group, polyvalent staphylococcus vaccine dosage was divided into low dose group (500 million ml), medium dose group (1 billion ml), high dose group (2 billion ml), each intraperitoneal injection of 1 ml, the normal control group with the same amount of normal saline instead. Immunization programme: the first, the third, the fifth and the ninth day were injected four times at intervals; On the 14th day, 1 hour before the experiment, each group of mice was intraperitoneally injected with 1 ml sterile broth. Half an hour later, the mice were intraperitoneally injected with 1 ml polyvalent staphylococcus vaccine, and the control group was intraperitoneally injected with 1 ml normal saline. And then put the animals into a glass stained with ether cotton balls and cover the glass plate cover. It was observed that the mice lost their reaction. When they were in deep anesthesia, the mice were killed by carotid bloodletting, and the samples were taken for experiments. The abdomen was cut open with small scissors, and the small intestine was fully exposed. The peritoneal fluid was stained

with slide pressing method, dried naturally and stained before microscopic examination. Then the spleen and liver of mice were taken out by aseptic technique and weighed.

2.2.2 RNA Extraction from Spleen Tissue

The spleen tissue of test mice was taken aseptically and ground with high-pressure sterilized tissue homogenizer. Then Trizol was added to form homogenate. After low-temperature and high-speed centrifugation, the supernatant was taken and added with chloroform, isopropanol and ethanol respectively. After reaction, the supernatant was discarded by low-temperature and high-speed centrifugation, and RNA was dissolved with DEPC water (stored in refrigerator at - 80 ℃).

2.2.3 RT-PCR Reaction

The RNA was mixed with primers, and then heated at a certain temperature bath, then ice bath, and then 5 × Buffer, dNTP, rnasin, reverse transcriptase, deionized water. The reaction system was incubated at 42 ℃ to synthesize cDNA. After the synthesis of cDNA, the reverse transcriptase was inactivated and stored in the refrigerator.

Table 1. IL-8, IFN-γ, IL-1R, β-actin Primer Sequence

Primer name	Primer sequence
IL-8	5'-TCCCGTTTGAGGGTCGTA-3'
	5'-CTGTGGAGGAAGCCAAGAAT-3'
IFN-γ	5'-AATGGAGGAGAGCCAGGGAAC-3'
	5'-AAGAGGAGCAACCACCAGAA-3'
IL-1R	FTGCAAAGTGTTTCTGGGAAC
	RATATTGCCCCCACAACCAAG
β-actin	FCGT GTT CCT ACC CCC AAT GT
	RTGT CAT CAT ACT TGG CAG GTT TCT

cDNA template 2 μl add 10 × buffer 5 μl, 1～4 μl dNTP（2.5 mM, 3 μl MgCl2 (25 mm), upstream primer 0.5 μl. Downstream primer 0.5 μl. Taq enzyme 0.2μl (5μl) Add deionized H_2O to make up to 50 μl. Add 30 ~ 50% paraffin oil into the PCR system of 50μl. Place the reaction tube in the PCR instrument and conduct PCR reaction according to the following conditions: Pre denaturation at 94 ℃ for 5 min, then denaturation at 94 ℃ for 30s, annealing at 54 ℃ for 30s and extension at 72 ℃ for 30s, a total of 42 cycles were carried out. After amplification, the amplified products of PCR were detected by electrophoresis, and the bands and products of electrophoresis products were determined by gel imaging system.

2.3 Statistical Analysis

Data analysis with SPSS16.0 software, the experimental data are represented by ($x \pm s$), T test was used to compare two groups of samples.

3. Results

3.1 Effect of Polyvalent Staphylococcus Vaccine on Phagocytic Function of Mice Phagocytes

After the small intestinal mucus was lightly pressed with glass slide in abdominal cavity, it was naturally dried, and then stained with Wright's-GiemsaStaining Solution for microscopic examination. The experimental results showed that there were a large number of neutrophils in the pictures of the medium dose group, and bacteria were engulfed in most neutrophils. A large number of neutrophils also appeared in the low-dose group, but the number was significantly lower than that in the medium dose group. In the high-dose group, the number of neutrophils decreased significantly and the number of bacteria increased significantly. The normal control group had more bacteria, less neutrophils and more lymphocytes. The experimental results were statistically analyzed and compared with the control group by t-test ($P < 0.01$). The comparison between the experimental group and the control group was also statistically significant ($P < 0.01$). The middle dose group had the best effect on the phagocytic function of mouse peritoneal phagocytes. The results are shown in Table 2.

The expression level of IL-8 gene in spleen tissue of mice in each experimental group was significantly higher than that in the normal control group. By t-test, there were significant differences between (low / medium / high) dose group and normal control group ($P<0.01$), and there were also significant differences between each dose group ($P<0.01$), The expression level of IL-8 gene in spleen tissue of mice in middle dose group increased most obviously, and the results are shown in Table 3.

T-test was used to compare the IFN in spleen tissue of mice in the low / medium / high dose group- γ The expression level of IFN in spleen tissue of mice in middle dose group was significantly higher than that in normal control group ($P < 0.01$)- γ The results are shown in Table 4.

T-test was used to compare the expression of IL-1R gene in spleen tissue of mice with polyvalent staphylococcus vaccine. The results showed that there were significant differences between the experimental groups and the normal control group ($P < 0.01$), and there were also significant differences between the groups with different doses ($P < 0.01$). The effect of medium dose group was the best, and the results were shown in Table 5.

Table 2. Effect of Polyvalent Staphylococcus Vaccine on Phagocytic Function of Mice Phagocytes ($\bar{x} \pm s$)

Group	No.(n)	phagocytic percent	phagocytic index	P
Normal control group	10	25.50±1.58	1.808±0.08	
Low dose group	10	30.30±2.11	2.772±0.08	P < 0.01
middle dose group	10	38.30±2.06	3.872±0.12	P < 0.01
High dose group	10	31.60±2.32	2.840±0.13	P < 0.01

*: (low / medium / high): normal, $P < 0.01$; Medium: (low / high) $P < 0.01$; Low: high $P > 0.05$

Table 3. Effect of Polyvalent Staphylococcus Vaccine on IL-8 Gene Expression in Spleen Tissue of Mice * ($\bar{x} \pm s$)

Group	No.(n)	Grey value	t	P
Normal control group	10	4.28±0.10		
Low dose group	10	27.13±0.10	718.85	P < 0.01
middle dose group	10	32.48±0.17	513.76	P < 0.01
High dose group	10	15.91±0.12	241.14	P < 0.01

*: (low / medium / high) dose group: control group, P < 0.01; Medium: (low / high) P < 0.01; Low: high P > 0.05

Table 4. Effect of Polyvalent Staphylococcus Vaccine on IFN-γ Gene Expression in Spleen Tissue of Mice* ($\bar{x} \pm s$)

Group	No.(n)	Grey value	t	P
Normal control group	10	3.25±0.18		
Low dose group	10	13.83±0.13	167.16	P < 0.01
middle dose group	10	22.36±0.23	216.49	P < 0.01
High dose group	10	16.54±0.15	253.84	P < 0.01

*: (low / medium / high) dose group: control group, P < 0.01; Medium: (low / high) P < 0.01; Low: high P > 0.05

Table 5. Effect of Polyvalent Staphylococcus Vaccine on IL-1R Gene Expression in Spleen Tissue of Mice* ($\bar{x} \pm s$)

Group	No.(n)	Grey value	t	P
Normal control group	10	4.21±0.13		
Low dose group	10	26.97±0.15	508.23	P < 0.01
middle dose group	10	31.99±0.19	349.71	P < 0.01
High dose group	10	15.95±0.22	113.92	P < 0.01

*: (low / medium / high) dose group: control group, P < 0.01; Medium: (low / high) P < 0.01; Low: high P > 0.05

4. Discussion

Intractable bacterial infection of skin and face is a skin disease caused by mixed infection of many bacteria. The incidence rate is high. At present, there is no effective cure for this disease. This study mainly discussed the mechanism of action of multivalent staphylococcus vaccine. Our research group applied the multivalent staphylococcus vaccine to the patients with intractable bacterial infection of face and neck skin (Yue, Zhou, Feng, Liang, Zhang, Zhang, Liu, You, & Guan, 2016; Yue, Zhao, Chen, Gong,

Yang, Qian, You, & Guan, 2007; Yue, Zhao, You, Zhao, Chen, Yu, & Guan, 2000; Yue, Zhao, You, Zhao, Chen, Yu, & Guan, 1999; Zhou, Liang, Qu, Zhai, Zhang, Qi, Zhang, & Yue, 2019). The treatment effect and follow-up showed that the multivalent staphylococcus vaccine had significant effect on intractable bacterial infection of face and neck, high cure rate and low recurrence rate (< 5%).

In order to further explore the immune mechanism of multivalent staphylococcus vaccine, the effects of multivalent staphylococcus vaccine on IL-8 and IFN in mouse spleen were studied- γ、 Ideal experimental results were obtained by studying the expression of IL-1R gene and the effect of IL-1R on phagocytes.

IL-8 is a neutrophil chemotactic factor. Its main biological activity is to chemotactic and activate neutrophils. It has been named as neutrophil activating peptide (NAF), neutrophil activating factor (NAF), neutrophil chemotactic peptide (GCP) and so on (Hussain, Iqbal, Sadiq, Feroz, Shafique Satti, 2015; Yang, Guan, Zhang, Pan, Wu, Wang, & Sun, 2014). In the inflammatory reaction, neutrophils can migrate to the reaction site, release active products and phagocytize bacteria to achieve the function of sterilization and cellular immunity (Marzano, Fanoni, Antiga, Quaglino, Caproni, Crosti, Meroni, & Cugno, 2014). The results showed that multivalent staphylococcus vaccine could significantly increase the expression of IL-8 gene in mouse spleen, and the effect of medium dose group was the most obvious. IFN-r is a major cytokine. On the one hand, it can activate T cells and natural killer cells (NK) to affect the immune response and play a regulatory role. On the other hand, it can induce the expression of chemokines, chemotactic immune effector cells and regulate the specific immune response, which plays an important role in the fight against bacterial infection (Feingold, 2014; Langan, Vidali, Pigat, Funk, Lisztes, B ŕó, Goffin, Griffiths, & Paus, 2013; Varol & Sagi, 2018; Foote, Patel, Yona, & Segal, 2019). Multivalent staphylococcus vaccine could significantly increase IFN in mouse spleen- γ The effect of gene expression was the most obvious in the medium dose group. IL-1 is a cytokine that mediates the inflammatory response (Palomo, Dietrich, Martin, Palmer, & Gabay, 2015; Villegas, Poza, Talayero, Teller, Zafra, Garcia, Vera, Hidalgo, Lopez, Cuellar, Zamanillo, Íñiguez, Paz-Artal, & Aguado, 2020). It can accelerate the clearance of pathogenic bacteria by attracting phagocytes to the local inflammation and play an anti infection role. Multivalent staphylococcus vaccine could significantly increase IFN in mouse spleen- γ The effect of gene expression was the most obvious in the medium dose group (Yoshida, Maeda, Tashiro-Yamaji, Yasuda, Shibayama, Hirose, & Kubota, 2020; Palomo, Dietrich, Martin, Palmer, & Gabay, 2015; Villegas, Poza, Talayero, Teller, Zafra, Garcia, Vera, Hidalgo, Lopez, Cuellar, Zamanillo, Íñiguez, Paz-Artal, & Aguado, 2020). The results showed that the gene levels of IL-8, IFN-γ、IL-1and the phagocytic function of peritoneal phagocytes in the spleen tissue of mice injected with polyvalent staphylococcus vaccine were significantly increased, and there was statistical significance between each dose group and the control group ($P < 0.01$), The most obvious increase was in the middle dose group. This indicates that the treatment of refractory bacterial infection with polyvalent staphylococcus vaccine may be related to the improvement of autoimmune function by increasing the levels of IL-8, IFN-γ and IL-1cytokines (Taniguchi, Yamamoto, Hitomi, Inada, Suyama, Sugioka, &

Hamasaki, 2013; Zhou, Liang, Qu, Zhai, Zhang, Qi, Zhang, & Yue, 2019; Baggiolini & Clark-Lewis, 1992; Kondo, Kono, Sauder, & McKenzie, 1993; Kak, Raza, & Tiwari, 2018; Lin, Lin, Lee, Wu, Feng, Chen, Chuang, Chen, Wang, Tseng, & Tsai, 2017; Yoshida, Maeda, Tashiro-Yamaji, Yasuda, Shibayama, Hirose, & Kubota, 2020). The experimental results showed that multivalent staphylococcus vaccine had significant effects on IL-8 and IFN in mouse spleen- γ, There is a significant dose-dependent relationship between the expression level of IL-1R gene and the phagocytic function of peritoneal phagocytes. The effect of medium dose group is the best, and the effect of low or high dose group is slightly worse. This study provides a new research direction for the mechanism of polyvalent staphylococcal vaccine in the treatment of intractable bacterial infection.

In conclusion, polyvalent staphylococcus vaccine is effective, safe and reliable in the treatment of intractable bacterial infection, and its treatment mechanism needs to be further studied, so as to provide a new theoretical basis for the clinical treatment of intractable bacterial infection of face and neck skin.

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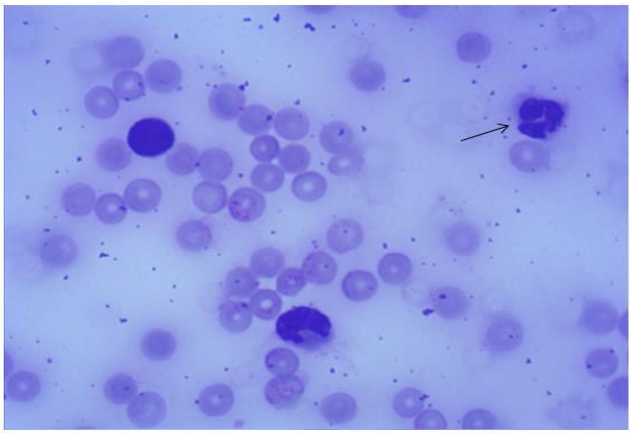


Figure A1. *Normal dose group.*

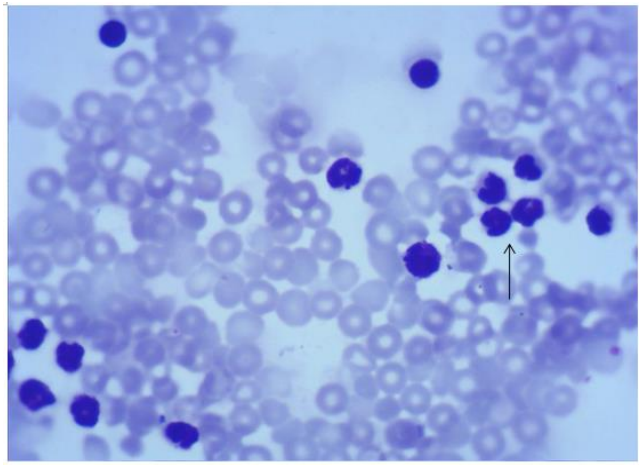


Figure A2. *Low dose group.*

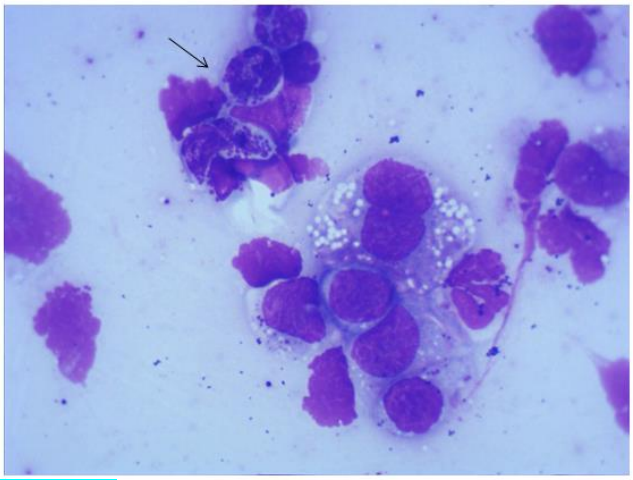


Figure A3. *Middle dose group.*

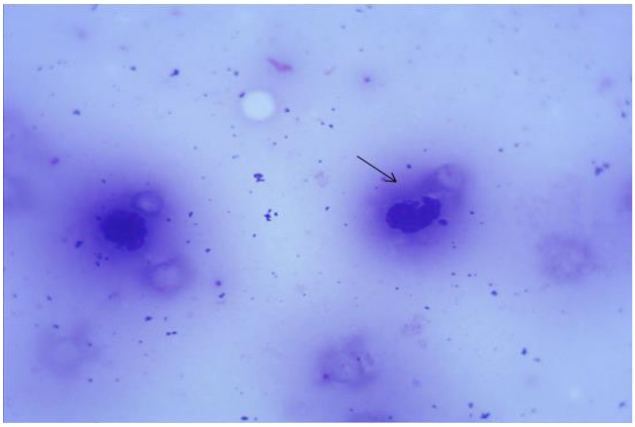
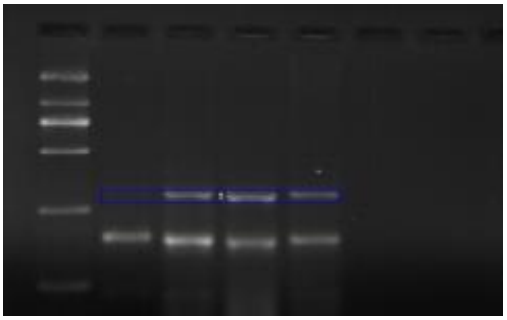
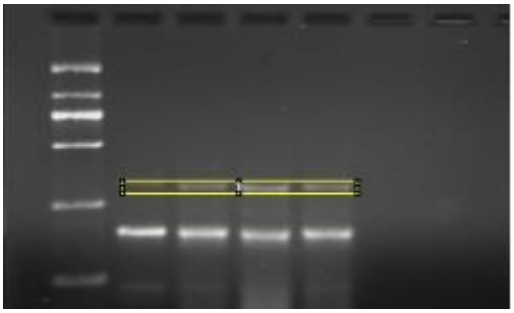


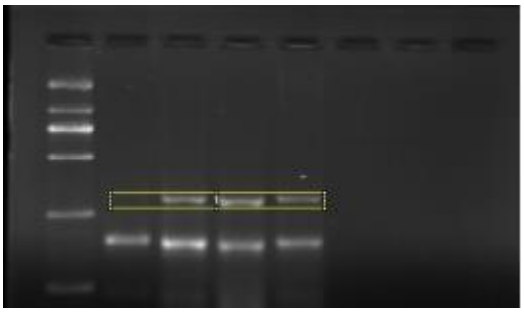
Figure A4. *High dose group.*



IL-8R



IFN-γ



Il-1R

Figure A5. *IL-8R/ IFN-γ/ Il-1R Electrophoretic map of gene expression.*

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