Chinese Medicine, 2021, 12, 10-18
https://www.scirp.org/journal/cm
ISSN Online: 2151-1926
ISSN Print: 2151-1918

Cytotoxicity of Mongolian Medicine *Aconitum kusnezoffii*

Xiong Su[1#], Xiaoyu Gao[2#], Lijuan Shang[3#], Zhiqiang Li[2], Wancheng Zhao[2], Baofeng Chi[1], Shiqi Wang[1], Hairong Zhang[1], Dejun Sun[2*], Juan Sun[2*]

[1]Inner Mongolia Medical University, Hohhot, Inner Mongolia Autonomous Region, China
[2]Inner Mongolia People's Hospital, Hohhot, Inner Mongolia Autonomous Region, China
[3]Hohhot Mongolian Traditional Medicine and Chinese Medicine Hospital, Hohhot, Inner Mongolia Autonomous Region, China
Email: *dejunsun123456@163.com, *sj6840@163.com

How to cite this paper: Su, X., Gao, X.Y., Shang, L.J., Li, Z.Q., Zhao, W.C., Chi, B.F., Wang, S.Q., Zhang, H.R., Sun, D.J. and Sun, J. (2021) Cytotoxicity of Mongolian Medicine *Aconitum kusnezoffii*. *Chinese Medicine*, **12**, 10-18.
https://doi.org/10.4236/cm.2021.121002

Received: December 10, 2020
Accepted: March 22, 2021
Published: March 25, 2021

Abstract

Aconitum kusnezoffii used as an analgesic and anti-inflammatory. It is often used not only in Mongolian medicine but also in traditional Chinese medicine. However, there are few studies on its impact of inflammatory cytokines. This study explored the effects of *Aconitum kusnezoffii in vitro* used human colon cancer (Caco-2) cells. Cytotoxicity was assessed by measuring effects on expression of IL-1β, IL-6, IL-8, and TNF-α used enzyme-linked immunosorbent assay (ELISA). *In vitro* study demonstrated that incubation of Caco-2 cells with some kinds of *Aconitum kusnezoffii* at some concentrations inhibit secretion of TNF-α and FAXIU *ACONITUM KUSNEZOFFII* could inhibit the secretion of IL-1β. While IL-6 and IL-8 levels were unaffected after 24 h of treatment with *Aconitum kusnezoffii*, qujian *Aconitum kusnezoffii*, faxiu *Aconitum kusnezoffii*.

Keywords

Aconitum kusnezoffii, Cytotoxicity, Proinflammatory Cytokines

1. Introduction

Aconitum kusnezoffii grows in the forest edge meadow, valley meadow and broad-leaved forest. It is a perennial herb. It is the dry tuberous root of *Aconitum carmichaeli*, a Ranunculaceae plant. About 350 species grow in temperate zone of the northern hemisphere, mainly in Asia, then in North America and Europe. Except Hainan Island, they can grow in Taiwan and other provinces and

[#]Xiong Su, Xiaoyu Gao and Lijuan Shang contributed equally to this paper.
*Corresponding authors.

regions of mainland China. They are mainly distributed in Inner Mongolia, Shanxi, Beijing, Heilongjiang, Jilin and Liaoning. Up to now, 167 species have been found in China. As a medicinal material, it is mainly produced in northern Inner Mongolia, Jilin and Heilongjiang provinces. It grows on the slopes or meadows of 200 - 450 meters above sea level, and in grass slopes or sparse forests in Shanxi and Hebei provinces [1] [2].

Aconitum kusnezoffii, as a component of Chinese patent medicine, is included in Chinese Pharmacopoeia, which is a national medicinal material developed vigorously in China. According to the theory of Mongolian medicine, *Aconitum kusnezoffii* has the function of killing bacteria and relieving pain. It is used for pestilence, tingling, sore throat, gout, rheumatism, toothache, etc. [1] [2], it is also widely used in the clinical practice of traditional Chinese medicine.

The research on *Aconitum kusnezoffii* is mainly about the active component alkaloids and the toxicity or property of *Aconitum kusnezoffii* extract, and then the determination method of alkaloids in *Aconitum kusnezoffii* and its different processing methods [3]. The researchers of General Hospital of Beijing military region found that alkaloids cause cardiovascular toxicity and lead to arrhythmia [4], and in Heilongjiang Mongolian hospital etc. found that alkaloids can cause nervous system, respiratory system, digestive system toxicity [4] [5]. Researchers in Inner Mongolia University and Jilin University studied alkaloid properties and found that it can enhance humoral immune function of mice [6]. Tingting Gao *et al.* studied the immune mechanism of water-soluble polysaccharides from *Aconitum kusnezoffii* [7].

Inflammation-associated cytokines including IL-6, IL-1β, TNF-α, and IL-8, are produced by various cell types and play a major role in communication within the immune system regulating many physiological and pathological functions [8]. Alterations in cytokine expression may be critical in toxin-induced disease and tissue injury [9]. Previous studies demonstrated that proinflammatory cytokines play a role in inflammatory responses induced by various chemicals [10] [11]. Therefore we investigated the change of proinflammatory cytokines (TNF-α, IL-1β, IL-6, and IL-8) from Caco-2 cells induced with raw *Aconitum kusnezoffii*, faxiu *Aconitum Kusnezoffii*, qujian *Aconitum kusnezoffii* to evaluate the medicinal value of *Aconitum kusnezoffii* and provide evidence for the development of more efficacy and clinical application of *Aconitum kusnezoffii*. It will accumulate data for pharmacological action of *Aconitum kusnezoffii* and provide a scientific basis for the rational and safe use of the medicinal material, especially its use method.

2. Materials and Methods

2.1. ELISA Kit

Tumor necrosis factor-α, TNF-α ELISA Kit (E-EL-H0109c, Irelite Biotechnology Co., Ltd.). interleukin-1β, IL-1β ELISA Kit (E-EL-H0149c, Irelite Biotechnology Co., Ltd.). interleukin-8, IL-8 ELISA Kit (E-EL-H0148c, Irelite Biotechnology Co., Ltd.). interleukin-6, IL-6ELISA Kit (E-EL-H0102c, Irelite Biotechnology Co., Ltd.).

2.2. Treatment of Crude *Aconitum kusnezoffii*

Cut off the stems and fibrous roots of crude *Aconitum kusnezoffii*, wash the sediment with water, cut them into 1 - 2 cm thick slices, place them in a beaker, cover them with tin foil, and dry them in Boxun drying oven at 65˚C.

2.3. Processing Qujian *Aconitum kusnezoffii*

Weigh appropriate amount of crude *Aconitum kusnezoffii*, scrape off its skin, and remove the tip from the root tip 0.5 cm, and reserve it.

2.4. Processing Faxiu *Aconitum kusnezoffii*

Put the coarse powder of *Aconitum kusnezoffii* into pig iron container, add appropriate amount of child urine, stir several times a day, and take it out (3 days) when it turns black (3 days). Dry it at a low temperature and reserve it.

2.5. Preparation of *Aconitum kusnezoffii* Decoction

Crude *Aconitum kusnezoffii*, qujian *Aconitum kusnezoffii*, faxiu *Aconitum kusnezoffii* add 200 ml distilled water to each group, soak for 30 min, pour it into the casserole and boil it until it boils. Then boil it for another 20 minutes at low heat. Collect the decoction. Add 100 ml of distilled water to the boiled *Aconitum kusnezoffii*. The same method was used for the second decocting, and the two decoctions were combined in the beaker. Filter with medium speed qualitative filter paper and filter with 0.22 um filter in the ultra clean workbench. The filtrate is kept at 4˚C for standby.

2.6. Human Colon Cancer Cell Line Caco-2 Cells Were Subcultured

Since the most commonly used way of *Aconitum kusnezoffii* is to decoct it into soup and then take it orally, we chose Caco-2 cells for the experiment. When the cells adhere to the wall, discard the original culture medium and add fresh complete medium. change the fluid every 2 - 3 days. When the cells reached 70% - 80% fusion, the cells were passaged. Cell passage: discard the old culture medium, wash with PBS three times, add 0.25% trypsin solution for digestion. When the cells were nearly round and there was a clear gap between the cells under the inverted microscope, 1 ml of complete medium was added to terminate digestion. Cells were collected by centrifuging and the supernatant was discarded, adding complete medium and mixing well. The waste product accumulated medium was changed to fresh purification medium every 2 days.

2.7. Caco-2 Cells Were Induced

Caco-2 cells in logarithmic growth phase were added with three kinds of decoction of *Aconitum kusnezoffii* diluted with DMEM, and the control group was added with equal volume of DMEM medium. According to the different induction time of each experiment, the infected cells were placed in the cell incubator at 37˚C, 5% CO_2, saturated humidity for static culture until the detection time.

2.8. The Contents of TNF-α, IL-1β, IL-8 and IL-6 Were Determin

The Caco-2 cells were incubated with different concentrations of raw *Aconitum kusnezoffii*, faxiu *Aconitum kusnezoffii*, qujian *Aconitum kusnezoffii* (0 to 80 mg/ml) for 24 h. After incubation, the culture media were collected for interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α) assays. The protein content was determined by enzyme-linked immunosorbent assay (ELISA).

2.9. Analysis of Experimental Data

Statistical analysis was performed using SPSS version 16.0 (SPSS, Inc., Chicago, IL, USA). Data from three parallel experiments are expressed as the means ± standard deviation (SD). Students t-test was used to perform comparisons between two groups. Multi-group comparisons of the means were performed by one-way analysis of variance (ANOVA) with the post hoc Student-Newman-Keuls test. P values equal to or less than 0.05 were considered statistically significant. This experiment will use Excel 2000 software and SPSS 10.0 statistical software for analysis.

3. Result

3.1. Images of Various *Aconitum kusnezoffii*

Raw *Aconitum kusnezoffii*, processed faxiu *Aconitum kusnezoffii*, and processed qujian *Aconitum kusnezoffiiis* shown in Figure 1.

Figure 1(a) Treatment of crude *Aconitum kusnezoffii*: Cut off the stems and fibrous roots of crude *Aconitum kusnezoffii*, wash the sediment with water, cut them into 1 - 2 cm thick slices, place them in a beaker, cover them with tin foil, and dry them in Boxun drying oven at 65˚C.

Figure 1(b) Processing qujian *Aconitum kusnezoffii*: Weigh appropriate amount of crude *Aconitum kusnezoffii*, scrape off its skin, and remove the tip from the root tip 0.5 cm, and reserve it.

Figure 1(c) Processing faxiu *Aconitum kusnezoffii*: put the coarse powder of *Aconitum kusnezoffii* into pig iron container, add appropriate amount of child urine, stir several times a day, and take it out (3 days) when it turns black (3 days). Dry it at low temperature and reserve it.

Figure 1. Preparation of *Aconitum kusnezoffii*. (a) raw *aconitum*; (b) faxiu *aconitum*; (c) qujian *aconitum*.

3.2. Analysis of Cytokine TNF-α Levels

TNF-α was significantly inhibited at each concentration although dose-dependent response could not be demonstrated in raw *Aconitum kusnezoffii*. TNF-α secretion was inhibited in the presence of faxiu *Aconitum kusnezoffiifor* various concentrations while it is weaker than that in raw *Aconitum kusnezoffii*. In qujian *Aconitum kusnezoffii* induced Caco-2 cells, TNF-α was inhibited at low concentrations and the inhibition disappeared at high concentration. (See **Figure 2**)

Figure 2 Effects of *aconitum* exposure on the expression of TNF-α. Caco-2 cells were treated with raw *aconitum*, rust concocted *aconitum* and removed tip *aconitum*, respectively.

3.3. Analysis of Cytokine IL-1β Levels

Caco-2 cells secrete IL-1β with a large variation ranging from 4 pg/ml to 6 pg/ml in the presence or absence of raw *Aconitum kusnezoffii*. We couldn't find raw *Aconitum kusnezoffii* affect secretion IL-1β on Caco-2 Cell. Caco-2 cells secretion IL-1β is inhibited at the lowest concentration (10 mg/ml) of faxiu *Aconitum kusnezoffii* with a negative dose-dependent relationship and the inhibition became weakest at the highest concentration of faxiu *Aconitum* kusnezoffii compared with those in control. When exposure of Caco-2 cells to 10 pg/ml concentration of qujian *Aconitum kusnezoffii*, IL-1β secretion is minimal and the secretion of other concentration groups was similar to that of control group. (See **Figure 3**)

Figure 3 Effects of *aconitum* exposure on the expression of IL-1β. Caco-2 cells were treated with raw *aconitum*, rust concocted *aconitum* and removed tip *aconitum* respectively.

3.4. Analysis of Cytokine IL-6 Levels

Raw *Aconitum kusnezoffii* had no effect on the induction of IL-6 with the variation range was 4.5 pg/ml - 5 pg/ml, which was in the designed *Aconitum kusnezoffii*

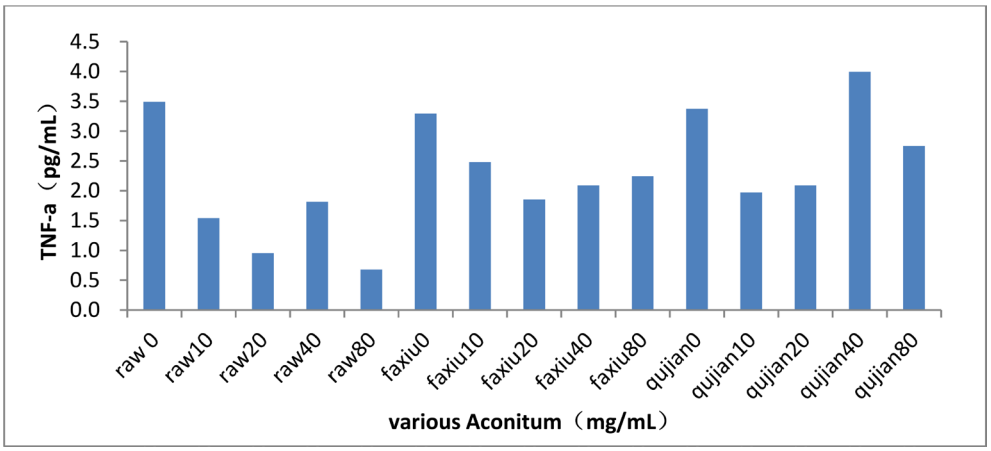

Figure 2. TNF-α in cultured Caco-2 cells induced by various *aconitum*.

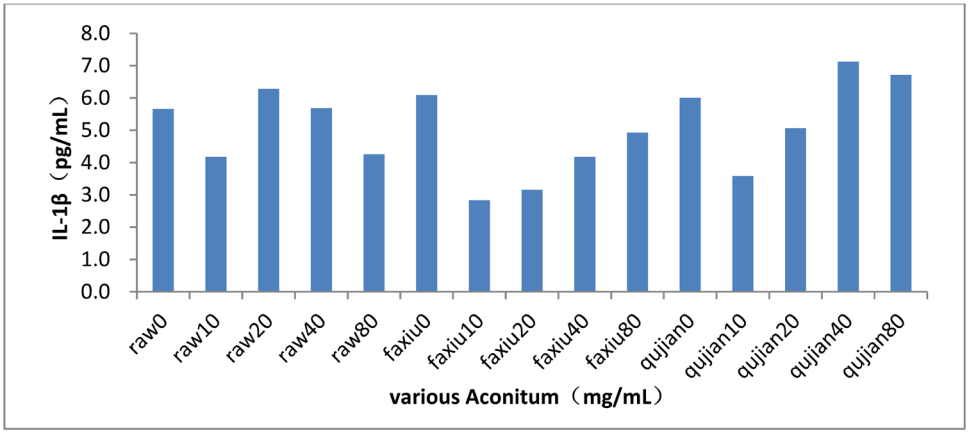

Figure 3. IL-1β in cultured Caco-2 cells induced by various *aconitum*.

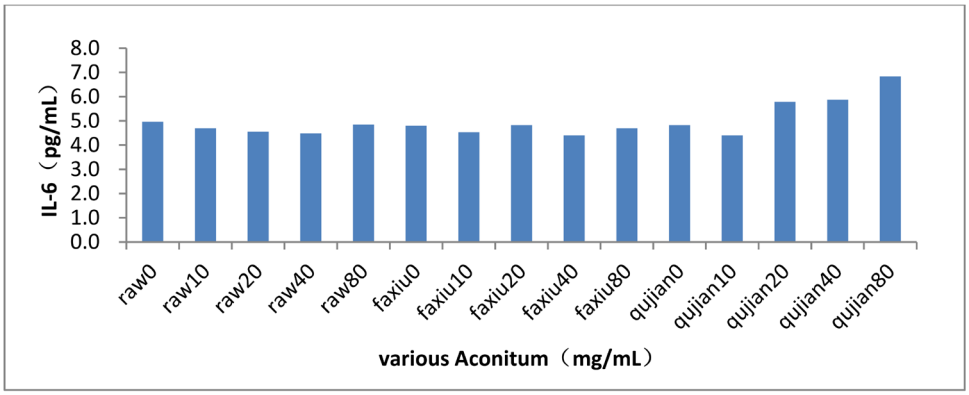

Figure 4. IL-6 in cultured Caco-2 cells induced by various *aconitum*.

concentration range. As far as faxiu *Aconitum kusnezoffii* is concerned, it had no obvious effects on expression of IL-6 in Caco-2 cells and it is similar to those of raw *Aconitum kusnezoffii*. IL-6 secretion is minimal (4.8 pg/ml) in non-induced cells, while can be induced IL-6 reached 5.8 pg/ml - 6.8 pg/ml by a range of 20 mg/ml - 80 mg/ml of qujian *Aconitum kusnezoffii*. (See **Figure 4**)

Figure 4 Effects of *aconitum* exposure on the expression of IL-6. Caco-2cells were treated with raw *aconitum*, rust concocted *aconitum* and removed tip *aconitum*, respectively.

3.5. Analysis of Cytokine IL-8 Levels

Raw *Aconitum kusnezoffii* had no effect on the induction of IL-8 Regardless of the presence or absence of raw *Aconitum kusnezoffii* with a narrow variation range 33 pg/ml - 36 pg/ml. IL-8 secretion ranges from 36 pg/ml - 40 pg/ml induced by Faxiu *Aconitum kusnezoffii* which including the control group (0 mg/ml). Obviously faxiu *Aconitum kusnezoffii* has no effect of inducing IL-8 in Caco-2 cell. Similarly, no induction IL-8 secretion of qujian *Aconitum kusnezoffii* was observed. (See **Figure 5**)

Figure 5 Effects of *aconitum* exposure on the expression of IL-8. Caco-2 cells were treated with raw *aconitum*, rust concocted *aconitum* and removed tip

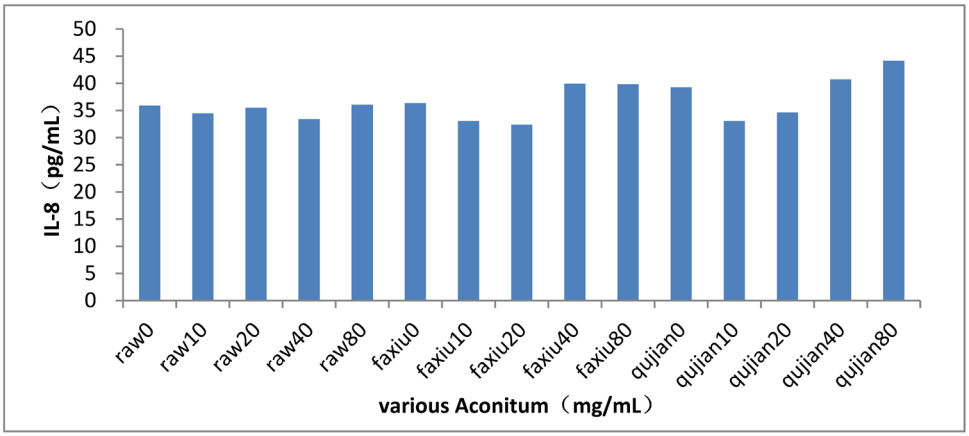

Figure 5. IL-8 in cultured Caco-2 cells induced by various *aconitum*.

aconitum, respectively.

4. Discussion

Aconitum kusnezoffii for clinical use has toxic effect. Previous studies have shown that the decoction of *Aconitum kusnezoffii* may inhibit the proliferation of A549 cells by down regulating the activities of MMP2 and MMP9 [12]. The effective component of *Aconitum kusnezoffii* causing toxicity is alkaloid, which has been shown to cause cardiovascular toxicity and arrhythmia [4]. In addition, some studies have found that alkaloids can cause toxicity to nervous system, respiratory system and digestive system [5]. In order to reduce the toxicity of alkaloids, many processing methods for *Aconitum kusnezoffii* have been developed [13], No matter what kind of processing method, the aim is to reduce the alkaloids to achieve the purpose of toxicity reduction. After the *Aconitum kusnezoffii* was treated with a series of methods of soaking, boiling and extraction, the acute toxicity test was carried out in rats. It was found that the median lethal dose was still not reached at 1.77 ml/kg, indicating that the toxicity of *Aconitum kusnezoffii* after treatment was greatly reduced [14]. However, the processing of *Aconitum kusnezoffii* not only reduced the alkaloid content, but also changed the properties of other components in the plant, and then the function of *Aconitum kusnezoffii* also changed.

According to the results of this study, the effects of the two processing methods on cytokines were different compared with raw *Aconitum kusnezoffii*. The results showed that the crude *Aconitum kusnezoffii* had obvious inhibitory effect on TNF-α in Caco-2 cell. After processing, the inhibitory effect was significantly reduced, and this effect only appeared in the low concentration group. It can be seen that the inhibitory effect of processed *Aconitum kusnezoffii* is different from that of raw *Aconitum kusnezoffii*. No effect of raw *Aconitum kusnezoffii* on IL-1β was detected in Caco-2 cell. However, faxiu *Aconitum kusnezoffii* significantly inhibited the secretion of IL-1β in the low-dose group, and this effect gradually weakened with the increase of concentration, showing a negative dose-response relationship. At the same concentration, No significant

effect of qujian *Aconitum kusnezoffii* on IL-1β secretion was observed. It can be seen that not only the effect of processing and no processing on IL-1β is different, but also there are qualitative differences among various processing methods. Whether processed or not, *Aconitum kusnezoffii* had no effect on the secretion of IL-8 in Caco-2 cell. It means that their effects on IL-8 are consistent. The three kinds of *Aconitum kusnezoffii* also had no obvious effect on the secretion of IL-6.

The effect of *Aconitum kusnezoffii* before and after processing and different processing methods on several common inflammatory factors is either no effect or inhibition *in vitro* experiment for Caco-2 cells, which is consistent with one of the main functions of *Aconitum kusnezoffii* of anti-inflammatory effect in clinical practice.

So far, we have not found any reports about the effect of raw or processed *Aconitum kusnezoffii* on inflammatory factors. Our experiments can provide some preliminary and original information for the related research in this field. More research is need into the relationship between inflammatory cytokine production and *Aconitum kusnezoffii* in Caco-2 cells to clearly the act mechanism of *Aconitum kusnezoffii*.

5. Conclusion

Aconitum kusnezoffii, processing qujian *Aconitum kusnezoffii* and processing faxiu *Aconitum kusnezoffii* in Caco-2 cells have different degrees of inhibition on four proinflammatory cytokines (TNF-α, IL-1β, IL-8 and IL-6). Compared with raw *Aconitum kusnezoffii*, the regulatory effects of two processed *Aconitum kusnezoffii* on inflammatory cytokines of Caco-2 cells are very different. And the two kinds of *Aconitum kusnezoffii* processed also have their own way of action.

6. Funding

This research was financially supported by the science plan project of Inner Mongolia Autonomous Region (Grant No. 201505016) and non-profit central research institute fund of Chinese academy of medical sciences, (2019PT350001).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Zhang, T.Y., Wang, G.J., Liu, S.M. and Zhi, Q. (2008) Application of *Aconitum kusnezoffii* in Traditional Chinese Medicine and Mongolian Medicine. *Modernization of Traditional Chinese Medicine and Materia Medica-World Science and Technology*, **1**, 113-116.

[2] Ling, S. and Gong, Q.F. (2011) Research Progress of *Aconitum kusnezoffii*. *Journal of Jiangxi University of Traditional Chinese Medicine*, **3**, 90-94.

[3] Han, Z.Q. and Na, S.S. (2008) Study on the Processing Method of Mongolian Medicine *Aconitum kusnezoffii*. *Modernization of Traditional Chinese Medicine and Materia Medica-World Science and Technology*, **1**, 150-154.

[4] Tong, H.F., Wang, H.L., Han, C.B. and Zhang, Z.L. (2013) Report of 3 Cases of Refractory Arrhythmia Caused by Acute Aconitine Poisoning. *Chinese Journal of Evidence-Bases Cardiovascular Medicine*, **4**, 422.

[5] Yang, S.J. (2011) Rescue and Nursing Experience of Aconitine Poisoning. *Chinese Journal of Disability Medicine*, **5**, 96.

[6] Ulijitgus, Liu, Z.B., Wang, H.S., Ding, Y.X. and Su, Y.F. (2008) Effect of Total Alkaloids from *Aconitum kusnezoffii* Leaves on Immune Function in Mice. *Chinese Herbal Medicine*, **7**, 1064-1065.

[7] Gao, T.T., Ma, S., Song, J.Y., Bi, H.T. and Tao, Y.D. (2011) Antioxidant and Immunological Activities of Water-Soluble Polysaccharides from *Aconitum kusnezoffii reichb*. *International Journal of Biological Macromolecules*, **49**, 580-586. https://doi.org/10.1016/j.ijbiomac.2011.06.017

[8] Gabay, C. (2006) Interleukin-6 and Chronic Inflammation. *Arthritis Research and Therapy*, **8**, Article No. S3. https://doi.org/10.1186/ar1917

[9] Kakar, S. (2015) Cytokines Evolution: Role in Various Diseases. *Current Medicine Research and Practice*, **5**, 176-182. https://doi.org/10.1016/j.cmrp.2015.07.002

[10] Van Och, F. M. M., Loveren, H. V., De Jong, W.H., Vandebriel, R. J. (2002) Cytokine Production Induced by Low-Molecular-Weight Chemicals as a Function of the Stimulation Index in a Modified Local Lymph Node Assay: An Approach to Discriminate Contact Sensitizers from Respiratory Sensitizers. *Toxicology and Applied Pharmacology*, **184**, 46-56. https://doi.org/10.1006/taap.2002.9473

[11] Watanabe, H., Unger, M., Tuvel, B., Wang, B. and Sauder, D. N. (2002) Contact Hypersensitivity: The Mechanism of Immune Responses and T Cell Balance. *Journal of Interferon and Cytokine Research*, **22**, 407-412. https://doi.org/10.1089/10799900252952181

[12] Xu, X.F., Chen, Y.H., Wang, J., Liao, J.J. and Wu, J.H. (2010) Effects of *aconitum scabra* on Proliferation, Invasion and Metastasis of Human Lung Cancer A549 Cells and Its Mechanism. *Chinese Materia Medica*, **12**, 1909-1912.

[13] Han, Z.Q. and batudligan. (2015) Comparative Study on the Processing Effect of Different Texture Mongolian Medicine *Aconitum kusnezoffii* by Baking Method. *Chinese Journal of Clinical Pharmacology*, **1**, 67-69.

[14] Kim, J., Kim, S., Lee, S., Jeong, H., Park, M., Kim, D., Song, B., Lee, J. and Kim, S. (2012) Study of Single-Dose Toxicity of *Aconitum kusnezoffii Reichb*. Pharmacopuncture in Rats. *Journal of Pharmacopuncture*, **15**, 48-52. https://doi.org/10.3831/KPI.2012.15.007