Annals of Clinical Case Reports

Research Article
Published: 25 Nov, 2021

To Study the Effect of Indigo Naturalis on Systemic Lupus Erythematosus and Its Mechanism of Action

*Yane Tan and Weidong Xu**

Department of Rheumatology, Jiangxi University of Traditional Chinese Medicine, China

Abstract

Background: As a traditional Chinese medicine, Indigo Naturalis mainly has the following functions: Clearing away heat and detoxification, treating sores and promoting muscle growth, cooling blood to stop bleeding, clearing lungs and relieving coughs, calming liver and relieving convulsions. Modern and widely used in infectious diseases, skin diseases, digestive tract diseases, tumors and other diseases.

Objective: To study the effect of Indigo Naturalis on Systemic Lupus Erythematosus (SLE) and its mechanism of action.

Methods: Except for mice in the normal model group, mice of ten weeks old were taken orally every day for eight consecutive weeks. Measure the serum levels of anti-dsDNA antibodies IgG, IgM, IL-6, TNF-α, Creatinine (Cr) and Blood Urea Nitrogen (BUN), and measure 24-h urine protein. Pathological analysis of nephritis was evaluated by Hematoxylin-Eosin (H&E) staining. Western blot method was used to detect mouse PD-L1 and APRLL protein expression.

Results: In the high-dose treatment group (Indigo QHD, 0.4 g/kg/d), the mice's serum levels of anti-dsDNA, IgG, IgM, BUN, Cr and inflammatory cytokines IL-6, TNF-α and proteinuria are significantly reduced which treated with Indigo Naturalis. High-dose Indigo Naturalis can significantly inhibit renal pathological changes. Indigo Naturalis can reduce the expression of PD-L1 and APRLL protein.

Conclusion: Studies have shown that Indigo Naturalis may be a potential drug for the treatment of SLE; it may be through inhibiting the expression of PD-L1 and APRLL protein to play a role in the treatment of SLE.

Keywords: Systemic lupus erythematosus; MRL/lpr Mice; SLE; Indigo naturalis

OPEN ACCESS

Correspondence:
Weidong Xu, Department of Rheumatology, Affiliated Hospital of Jiangxi University of Traditional Chinese Medicine, Nanchang City, Jiangxi Province, 330004, China,
E-mail: jindadong169@163.com

Received Date: *04 Oct 2021*
Accepted Date: *10 Nov 2021*
Published Date: *25 Nov 2021*

Citation:
Tan Y, Xu W. To Study the Effect of Indigo Naturalis on Systemic Lupus Erythematosus and Its Mechanism of Action. Ann Clin Case Rep. 2021; 6: 2049.

ISSN: 2474-1655

Introduction

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune disease characterized by chronic inflammation of a variety of tissues, including kidneys, skin, heart, joints, lungs, saliva/lacrimal glands, and brain [1]. Its prevalence is manifested as a significant ethnic difference. A large-scale epidemiological study in my country shows that the prevalence of SLE is 70 per 100,000 people, and that of women is as high as 1.13 per 100,000 people [2]. Current research shows that its etiology and pathogenesis may be related to environmental factors, genes, innate immunity and acquired immunity, and specific organ damage [3]. Traditional treatment is effective for some patients with SLE, but its toxic and side effects are obvious. Therefore, the search for safer and effective treatment is still the current research hotspot of SLE. Indigo Naturalis is a dark blue powder, light in body and easy to fly. It is a dry granular material obtained by processing the leaves or stems of horse blue, indigo blue or Isatis Indica. Mainly produced in Fujian, Yunnan, Jiangsu, Anhui and other places. Slightly grassy smell, light taste, salty and cold in nature, has the effects of clearing away heat and detoxification, cooling blood and reducing spots, purging fire and relieving shock, anti-cancer, anti-pathogenic microorganisms, anti-inflammatory and analgesic, and inhibiting leukemia, etc [4]. Indigo Naturalis has various ingredients, and there are 2 main active ingredients: Indirubin and indigo. Yan Sufang et al. [5] found that indirubin has the effect of inhibiting tumor cell proliferation. Xie Xiangjiang's [6] research found that indirubin can inhibit the secretion and expression of CCL20, interfere with the secretion of Interleukin (IL)-17 by γ&T cells, and further clarify the target and mechanism of action. Systemic lupus erythematosus is characterized by chronic inflammation of various tissues. Indigo Naturalis has anti-inflammatory and analgesic effects and inhibits tumor cell proliferation. Therefore, the scientific question of whether Indigo Naturalis is effective in the

treatment of systemic lupus erythematosus is raised. This article aims to explore whether Indigo Naturalis has an effect on the treatment of systemic lupus erythematosus and its mechanism of action.

Materials and Methods

Animals

Female MRL/lpr mice (age: 10 weeks; body weight: 23.5 ± 2.0 g) and BABL/C mice matched by sex and age (body weight: 21.5 ± 1.0 g) were provided by Shanghai Slack Laboratory Animal Co., Ltd. (Shanghai, China). Place the test animals in a sterile environment (24 ± 1°C, relative humidity 50 ± 5%, normal 12-h light/12-h dark cycle) in the Animal Experiment Center of Jiangxi University of Traditional Chinese Medicine. The mice ate standard food voluntarily, and disinfectants were provided by the factory. All mice were kept alive under controlled conditions for one week before the test. All research experiments were conducted under the approval of the Animal Experiment Ethics Committee of Jiangxi University of Traditional Chinese Medicine, and were in compliance with the guidelines of the National Institutes of Health (National Research Council, 1996). Our test procedures meet the approved standards.

Preparation method and experimental design of Indigo Naturalis

Indigo Naturalis powder with an average particle size of 40 μm was prepared by high-energy ball milling and dissolved in distilled water. Experimental design: Twelve BABL/C mice were used as a control group (10 ml/kg, 0.9% normal saline) after 1 week of adaptation. 48 MRL/lpr mice were randomly divided into 4 groups, of which group A: Model (10 ml/kg, 0.9% saline), group B: Treated with prednisone (Beijing United Pharmaceutical Company, China) (5 $mg.kg)^{-1}$/d, positive control), group C: Treated with high-dose Indigo Naturalis (Indigo HQD, 0.4 g/kg/d), group D: Treated with low-dose Indigo Naturalis (Indigo LQD, 0.02 g/kg/d), each test group consisted of 12 mice. The experimental protocol used in this study was approved by the Animal Ethics Committee of Jiangxi University of Traditional Chinese Medicine. All mice were continuously administered by gavage in the same volume (0.2 ml) for 8 weeks. All mice were killed by de-neck method, the kidney and spleen of fresh mice were aseptically separated on the second day after the last intragastric administration. A metabolic cage was used to obtain 24 h proteinuria from the mice, and they were evaluated at 0, 4, and 8 weeks. Serum was obtained by centrifugation at 3000 rpm for 10 min at 4°C, and stored at -20°C before use. The kidney tissue was fixed in 10% formaldehyde and embedded in paraffin for histopathological examination. Put the spleen into a cryotube, put it into a liquid nitrogen tank, and then transfer it to a refrigerator at -80°C for subsequent Westen blot detection.

Measurement of renal function

A commercially available ELISA kit (Jiancheng Institute of Biological Engineering, Nanjing City, Jiangsu Province) was used to measure the serum BUN and Cr levels in the 8th week. Test according to reagent specifications, and strictly implement relevant operating steps. The content is calculated in mg/dL.

Measurement of urine protein

In order to study the protective effect of Indigo Naturalis on the progression of kidney disease, the mice were transferred to steel grids inserted into sterile plastic mouse cages, without feed or water, for two consecutive times under the conditions of minimal interference to animals. During the process, a few drops of urine were voluntarily released onto the cage floor. In order to have enough time to urinate, urine collection starts in the early morning (100 μl to 200 μl, then frozen at -20°C). The collected urine samples were centrifuged at 3000 rpm for 5 min to remove impurities, and then the supernatant was collected and stored at -20°C before use. The 24-h urine protein was measured by BCA protein determination kit (Zhongshan Institute of Biotechnology, Beijing).

Determination of inflammatory cytokines in peripheral blood

In the 8th week, serum Interleukin-6 (IL-6) and Tumor Necrosis Factor-α (TNF-α) were detected using a commercially available ELISA kit (Jiancheng Institute of Bioengineering, Nanjing City, Jiangsu Province). Strictly implement relevant operating steps during testing.

Measurement of serum levels of anti-dsDNA antibodies, IgG, IgM and complement C3

The levels of anti-dsDNA antibodies, IgG and IgM (Oumeng Company, Germany) were detected according to the reagent specifications using spot immunoassay. Its normal mouse level is considered as a negative control. At the 8th week, the level of complement C3 in the serum was detected using an appropriate commercially available ELISA kit (Diaclone, France).

Histopathological observation

The HE staining procedure is as follows. 1. Fixation: fix the above appropriate amount of kidney tissue for 24 h, and then rinse with running water for 24 h. 2. Dehydration and embedding: 95% ALC I 4 h, II 2 h; anhydrous ALC. Xylene + absolute ethanol (1/1) for 20 min, xylene: I 10 min, II 10 min; soft wax (52°C): I 30 min, II 1 h, xylene + absolute ethanol (1/1) for 20 min, Xylene: I 10 min/10 min (52°C): I 30 min/min). Immerse in hard wax (60°C): I 30 min, II 30 min. 3. Slicing, repairing, cutting, expanding, pasting and baking (baking at 55°C and 60°C) for 5 h. 4. HE staining, xylene deparaffinization, 5 min each time; absolute ethanol 10 min: I 5 min, II 5 min; 95% ethanol: I 5 min, II 5 min >80% ethanol 5 min; 70% ethanol 5 min; DW 5 min; wash the Harris Hematoxylin solution for 5 min; separate with water, 0.5% hydrochloric acid and ethanol for 10 sec, and observe under a microscope. Wash and bluish for 30 min; 70% ethanol for 5 min; 80% ethanol for 5 min; Eosin solution (95% ethanol solution) for 30s; 95% ethanol: I 1 min, II 5 min; Absolute ethanol: I 5 min, II 5 min; Xylene + ethanol (1/1) for 5 min; Xylene: I 5 minutes, II 5 min, III 5 min; Seal with neutral gum, then observe under ordinary optical microscope.

Western blot method to detect PD-L1 and APRLL protein expression

Extract protein, determine protein concentration, add sample, electrophoresis, transfer membrane, incubate primary antibody and secondary antibody, use ECL luminescent solution to expose in gel imaging analysis system, collect images, and analyze each band with gel graphics analysis software Image Lab 3.0 grayscale value.

Statistical analysis

The results of the measurement data are expressed as mean ± Standard Deviation (SD). SPSS 24.0 software is used to compare the differences between the two groups, and multiple independent measurement data that meets normality and homogeneity of variance are used single-factor analysis of variance to further group The LSD method is used for inter-comparison. $p<0.05$ is statistically significant.

Results

Indigo Naturalis can reduce 24-hour proteinuria

Proteinuria is the main symptom indicating the development of kidney disease in MRL/lpr mice. Compared with the BABL/C control group, the 24-h proteinuria of the model group showed a gradual increase trend. Compared with the model group, the 24-h proteinuria content of the Qingdai treatment group was significantly lower (Figure 1). At the 8th week, the 24-h proteinuria of the mice treated with Indigo Naturalis was significantly less than that of the model group mice (Figure 1). Obviously, the 24-h proteinuria content in mice treated with Indigo LQD group decreased from 0.88 ± 0.07 g/24 h (week 0) to 0.36 ± 0.05 g/24 h (week 8) (Figure 1). In the Indigo HQD group, the 24-h proteinuria content on day 0 was 0.86 ± 0.08 g/24 h. At 4 weeks, this number dropped significantly to 0.43 ± 0.07 g/24 h, and continued to decrease at week 8. To 0.29 ± 0.09 g/24 h (Figure 1). Overall, Indigo Naturalis demonstrated a significant proteinuria reduction in the progression of SLE in MRL/ lpr mice with time dependence (Figure 1).

The effect of Indigo Naturalis on the renal function of MRL/lpr mice

In MRL/lpr mice treated with Indigo Naturalis, renal function was significantly reduced (Figure 2); especially in the Indigo Naturalis HQD group (Figure 2). Compared with the model group, the prednisone group also had a significant decrease (Figure 2). The results show that Indigo Naturalis can improve the renal function of MRL/lpr mice (Figure 2).

The influence of Indigo Naturalis on serum inflammatory cytokines

Compared with the control group, the expression levels of IL-6 and TNF-α in the serum of the model group were significantly higher than those of the control group (Figure 3). Indigo Naturalis

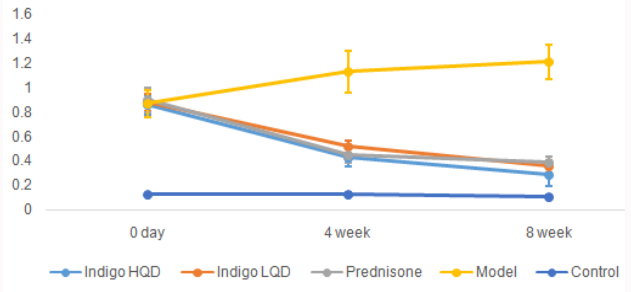

Figure 1: Indigo Natural is reducing 24-hour proteinuria in the serum of MRL/lpr mice (n=12, Mean ± SD).

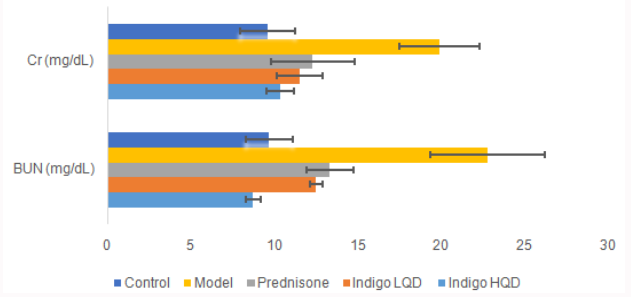

Figure 2: Effect of indigo natural is in renal function in MRL/lpr mice (n=12, Mean ± SD).

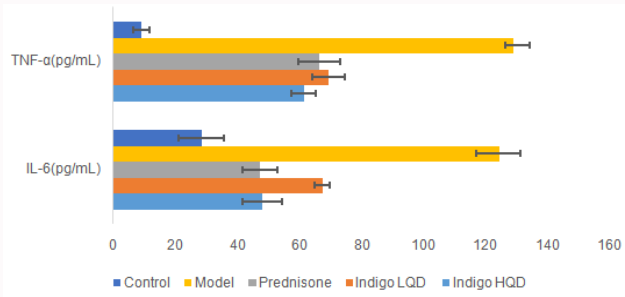

Figure 3: Effect of Indigo Natural is on the serum inflammatory cytokine (n=12, Mean ± SD).

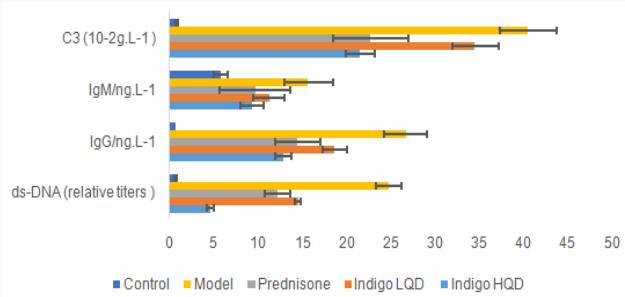

Figure 4: Effect of Indigo Natural is on the level of anti-ds-DNA, IgG, IgM, and C3 in serum (n=12, Mean ± SD).

can significantly reduce the expression of IL-6 and TNF-α (Figure 3). Similarly, there was a significant reduction in the prednisone group (Figure 3). The level of inflammatory cytokines in the Indigo HQD group was significantly lower than that of the model group Figure 3. In addition, the prednisone treatment group also had a significant decrease (Figure 3). However, compared with the prednisone group, the inflammatory cytokines of the Indigo HQD group did not significantly decrease (Figure 3).

The effect of Indigo Naturalis on the levels of anti-ds-DNA, IgG, IgM and complement C3 in serum

No anti-dsDNA antibodies in the serum were detected in the control group. In other words, there is no change in LN. On the contrary, the level of anti-dsDNA antibodies in the model group of mice is a significant evidence of LN. In addition, the Indigo Naturalis group significantly inhibited the production of anti-dsDNA, IgG and IgM, especially in the HQD group (Figure 4). In addition, the serum complement C3 level of the model group was significantly higher than that of the control group (Figure 4). The complement C3 level of the Indigo Naturalis HQD group was significantly lower than that of the model group (Figure 4).

Histopathological determination

At the end of the study, HE staining was used to assess the severity of kidney inflammation in lupus mice. Compared with the model group, the mesangial hyperplasia of the mice in the high-dose and low-dose groups of Indigo Naturalis gradually reduced. At the same time, the tissue necrosis of renal tubular epithelial cells gradually decreased, and the infiltration of inflammatory cells around small blood vessels in the renal interstitium gradually decreased (Figures 5A-5C), similar to the prednisone group (Figure 5D).

Comparison of Western blot detection results of PD-L1 and APRLL proteins in each group of mice

Figure 6 shows that, compared with Model, the expression of

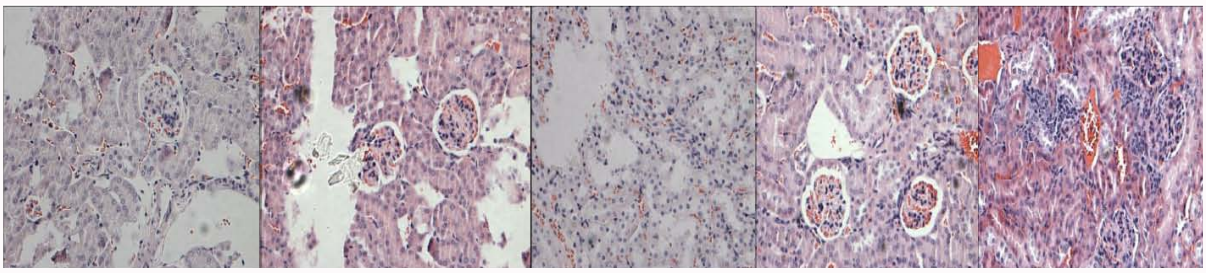

Figure 5: The H&E (x400) of the kidney sections in MRL/lpr mice (The order of the pictures is A, B, C, D, E). A. Model group; B. Indigo HQD group; C. Indigo LQD group; D. prednisone group; E. Control group.

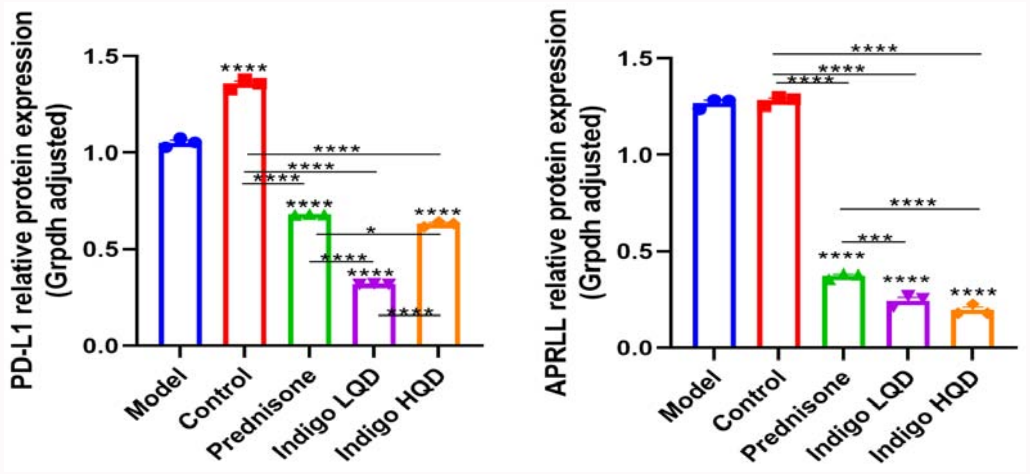

Figure 6: Comparison of Western blot detection results of PD-L1 and APRLL proteins in each group of mice (x ± s, n=3).
Note: PD-L1: Programmed Death Protein Ligand 1; APRLL: A Proliferation-Inducing Ligand; ****: p<0.0001; ***: p=0.0008; *: p=0.0303

Prednisone, Indigo LQD, Indigo HQD PD-L1 protein decreased, the expression of Control PD-L1 protein increased, and the protein expression of Prednisone, Indigo LQD, Indigo HQD APRLL decreased (p<0.01). There was no statistically significant difference in APRLL protein expression between Model and Control (p>0.05). The protein expressions of Prednisone, Indigo LQD, Indigo HQD PD-L1 and APRLL were lower than those of Control (p<0.01). The protein expression of Indigo LQD, Indigo HQD PD-L1, and APRLL was lower than that of Prednisone (p<0.01 or p<0.05). Indigo HQD PD-L1 protein expression was higher than Indigo LQD (p<0.01). There was no statistically significant difference in protein expression between Indigo LQD and Indigo HQD APRLL (p>0.05). Figure 7 shows the electrophoresis diagram of PD-L1 and APRLL protein expression in each group.

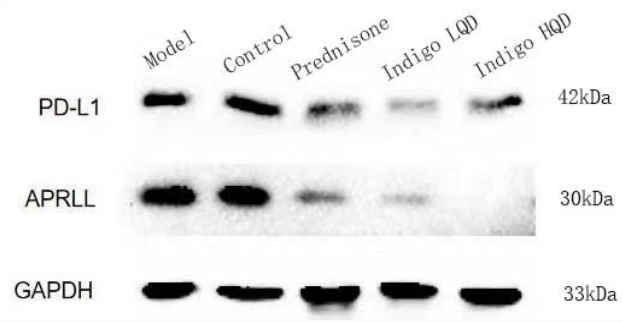

Figure 7: Electrophoresis of PD-L1 and APRLL protein expression in each group of mice.

Discussion

In this study, we selected MRL/lpr mice to study the potential therapeutic effect of Indigo Naturalis in SLE. The production of auto antibodies is closely related to LN activity [7,8], so the serum levels of anti-dsDNA antibodies and complement C3 were determined. The study was designed to treat 24-week-old MRL/lpr mice, because at this time all mice had positive anti-dsDNA antibody titers, and more than 90% of the mice had detectable proteinuria, indicating autoimmune nephritis it exists before the start of treatment. The results of the study showed that the anti-dsDNA, IgG, IgM, BUN, Cr and proteinuria levels in the serum of the Qingdai group were significantly reduced. In addition, compared with untreated MRL/lpr mice, Indigo Naturalis suppressed the levels of inflammatory cytokines IL-6 and TNF-α, indicating that Indigo Naturalis has a therapeutic effect on MRL/lpr mice with lupus nephritis. Therapeutic trials in MRL/lpr mice usually involve starting treatment before the onset of the disease [9]. Few drugs show benefits after the onset of the disease [10]. The discovery that Indigo Naturalis can effectively treat mice with confirmed MRL/lpr lupus suggests that its efficacy may be more easily translated into the treatment of human lupus. As a traditional Chinese medicine, Indigo Naturalis was mostly used as an external medicine or as a pill or powder because of its insoluble nature. According to the literature, its functions mainly include: Clearing away heat and detoxification, treating sores and promoting muscle growth, cooling blood to stop bleeding, clearing lungs and relieving cough, calming liver and relieving convulsions [11]. It is widely used in infectious diseases, skin diseases, digestive tract diseases, tumors and other diseases in modern

times. At present, Indigo Naturalis preparations with evidence-based clinical effects are mainly concentrated in the three major diseases of psoriasis, leukemia and ulcerative colitis [12]. SLE is the name of a modern medical disease. There is no record of this disease in ancient Chinese medicine books. The description of its symptoms is scattered in "yin and yang poison", "blood wind sores" and other diseases. In recent years, some scholars believe that in the entire disease process of SLE, the signs of flaming fever can occur repeatedly, or even febrile toxins are invaded, and the fever is volatile [13,14]. Through long-term clinical observation, it is believed that "toxin" is one of the main etiology and pathogenesis of SLE. Studies have shown that Indigo Naturalis has certain immunomodulatory effects, anti-inflammatory, anti-pathogenic microorganisms and anti-tumor effects [15]. There are two main active ingredients in Indigo Naturalis: Indirubin and indigo. Indirubin has been confirmed to have a killing effect on tumor cells and drug-resistant tumor cells [16]. Liu Lijuan et al. [17,18] found that the concentration of Interleukin-6 (IL-6)/Tumor Necrosis Factor-α (TNF-α) in the indirubin group was significantly lower than that in the normal group. Indigo can reduce the expression of IL-6 through direct intervention, so as to achieve the *in vitro* anti-inflammatory effect. It can be seen that Indigo Naturalis does have an effect on the treatment of SLE. Programmed Death protein 1 (PD-1) and its ligand PD-L1 can maintain the homeostasis of immune cells and the health of the body through negative regulation [19]. Recently, PD-1/PD-L1 has been proven to be a negative immunomodulatory molecule of SLE [20,21]. In recent years, studies have suggested that blocking PD-1/PD-L1 may be related to the pathogenesis and progression of SLE [22,23]. A Proliferation-Inducing Ligand (APRIL) is a new member of the tumor necrosis factor ligand superfamily. It has the functions of promoting tumor cell proliferation, regulating humoral immunity, and participating in the maturation and activation of lymphocytes. The occurrence and development of immune diseases are closely related [24-26].

Conclusion

"Toxic evil" is one of the main causes of SLE, so detoxification is one of the basic methods to treat SLE. At the same time, there are clinical reports showing that detoxification therapy has a certain effect on SLE. Therefore, finding new medicines for SLE from Chinese medicines with detoxification function is one of the important topics worthy of study. Indigo Naturalis is one of the traditional Chinese medicines with detoxification function and has no toxic side effects. This study found that Indigo Naturalis can inhibit the level of anti-dsDNA, IgG, IgM, BUN, Cr, proteinuria, complement C3 and inflammatory cytokine IL-6 and TNF-α. The research results show that Indigo Naturalis can reduce the expression of PD-L1 and APRLL protein. Therefore, we believe that Indigo Naturalis can treat SLE by inhibiting the expression of PD-L1 and APRLL. The findings of this study are of great significance to the treatment of SLE, but there is still a lack of more experimental data to support the discussion of its effectiveness and mechanism of action.

References

1. JA L, J M, WA R, FJ S, AH S, VR K. Programmed death ligand 1 regulates a critical checkpoint for autoimmune myocarditis and pneumonitis in MRL mice. J Immunol. 2008;181(4).2513-21.
2. Zhaoxia L, Shan Z, Huixia W, Jie R, Lihua Z, Yi Z. The role of targeted regulatory T cell immunosuppressive receptors in the treatment of systemic lupus erythematosus. Guangdong Med J. 2019;40(9):1225-30.
3. Lengmei B, Chuanmei X. The expression levels and significance of co-signal molecules CD28, ICOS, PD-1, and CTLA-4 in peripheral blood of patients with systemic lupus erythematosus. J North Sichuan Med College. 2018;33(6):832-5,9.
4. Jing X. Recent research on the pharmacology of Indigo Naturalis. Inner Mongolia Traditional Chinese Med. 2012;31(15):100.
5. Sufang Y. Clinical observation of 43 cases of psoriasis treated with indirubin. Yunnan J Traditional Chinese Med. 1982(02):21-2.
6. Xiangjiang X. The effect of Liangxue Jiedu Decoction and Indirubin on KC/γδT activation involving CCL20 in psoriasis models *in vivo* and *in vitro* [Master]: Beijing University Traditional Chinese Med. 2017.
7. Furukawa F, Yoshimasu T. Animal models of spontaneous and drug-induced cutaneous lupus erythematosus. Autoimmun Rev. 2005;4(6):345-50.
8. G RI, M RC, P BZ, A KM. Clinical efficacy and side effects of antimalarials in systemic lupus erythematosus: A systematic review. Ann Rheum Dis. 2010;69(1):20-8.
9. Y B, M LR, J A-S, D SB, E D. Treatment of lupus nephritis in adult (NZB + NZW)F1 mice by cortisone-facilitated tolerance to nucleic acid antigens. J Clin Invest . 1978;61(2):276-86.
10. H HB, L K, M N, R HT. Influence of cyclophosphamide and other immunosuppressive drugs on immune disorders and neoplasia in NZB/NZW mice. Arthritis Rheum. 1975;18(2):145-52.
11. Xiliang S, Guanhua L, Chenghui Z, Jinling J, Tong F. Applied research on Qingdai in the past. Chinese J Chinese Med. 2020;35(08):1653-5.
12. Sizhen G, Danbo D. Thoughts on oral treatment of ulcerative colitis with single-flavored Qingdai. Chinese J Integrated Traditional Chinese Western Medicine Digestion. 2020;28(03):238-42.
13. Yuanyuan W. Peripheral blood T lymphocyte PD-1 molecule and mononuclear cell PD-L1 molecule expression in patients with systemic lupus erythematosus [Master]: Anhui Medical University; 2011.
14. Yuanyuan W, Xiaorong C, Tong L, Jie T, Changhao X. Expression analysis of PD-1 molecule on CD4+CD25 high Treg in peripheral blood of patients with systemic lupus erythematosus. J Bengbu Med College. 2011;36(11):1184-6.
15. Siyu G, Caihong B, Xiaobo W, Xu Z. Research on the quality standard of Indigo Naturalis. Chinese Med Guide. 2020;18(12):35-6.
16. Hongwei P, Fei L, Xuelian Z, Yanni L, Xiaochun S, Zhouping D, et al. Indirubin derivative PH II-7 promotes the mechanism of sTRAIL killing breast cancer cells and drug-resistant strains. Chinese Pharmacological Bulletin. 2015;31(05):679-85.
17. Lijuan L, Yunliang W, Junxiang L. The effect of indigo on the inflammation model of ulcerative colitis *in vitro*. Chinese J Traditional Chinese Med. 2014;29(05):1666-9.
18. Lijuan L, Yunliang W, Shuqing X, Shibing W, Junxiang L. Anti-inflammatory effects of indirubin and tryptamine on LPS-induced RAW264.7 inflammatory cell model. World J Integrated Traditional Chinese Western Med. 2015;10(08):1069-72.
19. Xiaoqun C, Xing L, Shuzhen L, Chunfei Z. Research status and prospects of PD-1/PD-L1 in systemic lupus erythematosus and rheumatoid arthritis. Chinese J Immunol. 2020;36(11):1397-402,406.
20. Jie G, Nan G, Li W, Kang L, Xing-Han L, Lin-Ting W, et al. Meta-analysis of programmed cell death 1 polymorphisms with systemic lupus erythematosus risk. Oncotarget. 2017;8(22).
21. Canto LMD, Farias TDJ, Medeiros MD, Coêlho CC, Sereia AFR, Back LKFdC, et al. Association of PDCD1 polymorphism to systemic lupus erythematosus and rheumatoid arthritis susceptibility. Revista Brasileira de Reumatologia (English Edition). 2016;56(6).
22. J WE, Jihua C, D ST, S HJ, Carol M, I NR, et al. Durable complete responses

off all treatment in patients with metastatic malignant melanoma after sequential immunotherapy followed by a finite course of BRAF inhibitor therapy. Cancer Biol Ther. 2015;16(5).

23. Kimberly S, Suzanne M, Rosalie E, Y CE. Lupus-like cutaneous reaction following pembrolizumab: An immune-related adverse event associated with anti-PD-1 therapy. J Cutan Pathol. 2018;45(1):74-7.

24. Hengli D, Zhaohua Y, Xiaoxin X, Yan W, Huixin C. Significance of APRIL mRNA expression in peripheral blood of patients with esophageal squamous cell carcinoma. Chinese J Clin Oncol Rehabil. 2008(04):345-9.

25. Yuanyuan W, Changhao X, Meiqun S, Jixia C, Yanmei Z, Yong C, et al. Expression and significance of CD4+ and CD8+ T cell PD-1 in peripheral blood of patients with systemic lupus erythematosus. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi. 2013;29(9):962-5.

26. Min X, Haiyan X, Lanfang C. Expression of PD-1 molecule in children with systemic lupus erythematosus. J Clin Pediatr. 2008;26(11):959-61.