

Effects of astragaloside IV on inflammation and immunity in rats with experimental periodontitis

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Abstract: This study aimed to investigate the effects of astragaloside IV (AsIV) on inflammation and immunity in rats with experimental periodontitis. Periodontitis was established in 48 Wistar rats, which were then randomly divided into model and 10, 20 and 40 mg/kg AsIV groups, with 12 rats in each group. The latter 3 groups were treated with AsIV at doses of 10, 20 and 40 mg/kg, respectively. The control group (12 rats, without periodontitis) and model group were given the same amount of 5% sodium carboxymethyl cellulose. The treatment was performed once per day for 8 weeks. Before and after treatment, the tooth mobility scores of the rats were determined. After treatment, the salivary occult blood index (SOBI), plaque index (PLI), peripheral blood T lymphocyte subsets, and serum inflammatory factor and immunoglobulin levels were determined. The results showed that, after treatment, compared with that in model group, in 40 mg/kg AsIV group, the general state of rats was improved, while the tooth mobility score, SOBI and PLI were significantly decreased ($p < 0.05$); the peripheral blood CD_4^+ T cell percentage and CD_4^+/CD_8^+ ratio were significantly increased ($p < 0.05$), while the CD_8^+ T cell percentage was significantly decreased ($p < 0.05$); the serum tumor necrosis factor- α , interleukin-1 β and interleukin-2 levels were significantly decreased ($p < 0.05$); the serum immunoglobulin A and immunoglobulin G levels were significantly decreased ($p < 0.05$). In conclusion, AsIV can alleviate inflammation and enhance immunity in rats with experimental periodontitis.

Declaration of Interests: The authors certify that they have no commercial or associative interest that represents a conflict of interest in connection with the manuscript.

Keywords: Periodontitis; Inflammation; Immunity.

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Introduction

Periodontitis is a chronic and destructive disease that invades the gingiva and periodontal support tissues. It is one of the major oral diseases and has a high prevalence. The main characteristics of periodontitis are periodontal pocket formation, pocket wall inflammation, alveolar bone resorption and tooth loosening.¹ Periodontitis is one of the main causes of tooth loss in adults, and it can cause or induce a variety of diseases, seriously endangering human health. It has been recognized that bacteria and their products in the periodontal tissues and gingival crevicular fluid are important factors in the pathogenesis of periodontitis.²

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The inflammatory factors tumor necrosis factor- α (TNF- α), interleukin-1 β (IL-1 β) and interleukin-2 (IL-2) play important roles in the occurrence and development of periodontitis.^{3,4} The key to the successful prevention and treatment of periodontitis is controlling the bacteria and their products in the periodontal tissues and gingival crevicular fluid to maintain the microecological balance of the local periodontal environment.⁵ The mechanical removal of plaque is usually applied to treat periodontitis, but this approach will cause some mechanical damage to the tooth surface.⁶ In addition, antibiotic therapy is often used to treat periodontitis, but it is easy to cause drug resistance, toxic effects, bacterial imbalance and other side effects.⁷ Astragaloside IV (AsIV) is one of the active ingredients in the medical plant *Astragalus membranaceus*, and it is also the quality index for the content determination of *Astragalus membranaceus*. According to the literature, AsIV has pharmacological activities that include immunity enhancing,⁸ anti-inflammatory,⁹ antiviral,¹⁰ antiapoptotic,¹¹ antihypertensive effects¹² among other aspects. To date, there has been no reports on the effect of AsIV on periodontitis. Thus, the purpose of this study was to investigate the effects of AsIV on inflammation and immunity in rats with experimental periodontitis.

Methodology

Animals

This study used Wistar rats (7 weeks of age; 222 \pm 10 g; half male and half female) at the beginning of the study. The rats were acclimatized for 10 days before the experiment, and they were kept in temperature-controlled cages that were exposed to a 24-h light-dark cycle with equal light and dark time. The experimental procedure was approved by the Institutional Animal Care and Use Committee of Tongji Medical College, Huazhong University of Science and Technology.

Construction of a periodontitis model

A periodontitis model was constructed in rats. The rats were anaesthetized with 0.3% pentobarbital sodium at a dose of 10 ml/kg via intraperitoneal

injection. The gingiva was peeled open using a tooth probe, and then the maxillary first molar was ligated using 0.2 mm-diameter stainless steel wire with knotting at the palatal side. Then, the rats were fed daily with soft feed and high-sugar water (200 g/L). The rats in the control group only received the abdominal anesthesia and were fed normal feed and water. The feeding of the rats was continued for 4 weeks. The periodontitis model was confirmed to be successfully established in 48 rats by digital X-ray imaging and pathological section observation.

Treatment of rats

Forty-eight model rats were fed normally for 2 weeks and then divided into a model group and 10, 20 and 40 mg/kg AsIV groups, with 12 rats in each group. The ligature wire in these rats was untied. The rats in the 10, 20 and 40 mg/kg AsIV groups were intragastrically administered AsIV (HPLC purity \geq 98%; Chengdu Mansite Biotechnology Co., Ltd., Chengdu, China); the suspension was prepared using 5% sodium carboxymethyl cellulose, and the doses were 10, 20 and 40 mg/kg, respectively. The rats in the control group (12 rats) and the model group were intragastrically administered the same amount of 5% sodium carboxymethyl cellulose. The administration was conducted once per day for 8 weeks.

Determination of tooth mobility

According to the reported method,¹³ before and after treatment, rat tooth mobility was scored as follows: 1 point, only buccal-lingual loosening; 2 points, both buccal-lingual and mesiodistal loosening; and 3 points, buccal-lingua, mesiodistal and vertical loosening.

Detection of the salivary occult blood index and plaque index

After treatment, the salivary occult blood index (SOBI) was detected using saliva test paper. The detection results are presented as scores (1–3 points). A higher score indicated a higher SOBI. The experimental procedures were performed in accordance with the instructions of the manufacturer. The plaque index (PLI) was detected using the

erythrosine method.¹⁴ The detection results are presented as scores (1–5 points). A higher score indicated a higher PLI.

Determination of peripheral blood T lymphocyte subsets

After treatment, rats were anesthetized using 5% chloral hydrate. Five milliliters of venous blood was taken by the retro-orbital puncturing. Some of the blood was used to prepare serum. The rest of the blood was used to assess T lymphocyte subsets using flow cytometry.¹⁵ The experimental procedures were performed in accordance with the instructions of the manufacturer (Becton, Dickinson and Company, NJ, USA).

Determination of serum inflammatory factor and immunoglobulin levels

The venous blood of rats was centrifuged at 1000 r/min for 15 min, and the supernatant was obtained. The serum levels of inflammatory factors, including TNF- α , IL-1 β and IL-2, and immunoglobulins, including immunoglobulin A (IgA), immunoglobulin G (IgG) and immunoglobulin M (IgM), were determined using enzyme-linked immunosorbent assays. The process was performed according to the instructions of the kits (Sigma-Aldrich Corp., MO, USA).

Statistical analysis

SPSS 18.0 software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. The data are presented as the mean \pm SD. The differences among different groups were analyzed using one-way analysis of variance with the *q* test. $p < 0.05$ indicated a statistically significant difference.

Results

General state of the rats after treatment

During the treatment period, no rats died in any of the groups. After treatment, the rats in the control group had bright and clean fur and normal activity levels, with no obvious differences compared with the same parameters before treatment. In the model group, the rats exhibited rough and less glossy fur to different degrees and decreased activity. Compared with that in the model group, the state in the other three groups was improved, especially in the 40 mg/kg AsIV group, as the state of rats in that group was close to that of the rats in the control group.

Tooth mobility scores before and after treatment

Before treatment, the tooth mobility scores were not significantly different among the model and 10, 20 and 40 mg/kg AsIV groups ($p > 0.05$). After treatment, the tooth mobility scores in the 20 and 40 mg/kg AsIV groups were significantly lower than the corresponding scores before treatment ($p < 0.05$). In addition, after treatment, the tooth mobility scores in the 20 and 40 mg/kg AsIV groups were significantly lower than those in the model and 10 mg/kg AsIV groups ($p < 0.05$) (Table 1).

SOBI and PLI after treatment

After treatment, the SOBI and PLI in the model group were significantly higher than those of the control group ($p < 0.05$). In the 40 mg/kg AsIV group, the SOBI and PLI were significantly lower than those in the model group and the 20 and 40 mg/kg AsIV groups ($p < 0.05$) (Table 2).

Table 1. Tooth mobility scores in different groups before and after treatment.

Group	Before treatment (points)	After treatment (points)
Model	2.83 \pm 0.32	2.79 \pm 0.38
Low-dose AsIV	2.81 \pm 0.39	2.76 \pm 0.33
Middle-dose AsIV	2.78 \pm 0.35	1.77 \pm 0.51***
High-dose AsIV	2.77 \pm 0.41	1.71 \pm 0.33***

* $p < 0.05$ compared with model group; ** $p < 0.05$ compared with low-dose AsIV group; and *** $p < 0.05$ compared with before treatment. AsIV, astragaloside IV.

Peripheral blood T lymphocyte subsets after treatment

After treatment, the peripheral blood CD₄⁺ and CD₈⁺ T cell percentages and their ratios in the control group were 37.56 ± 8.46%, 11.56 ± 3.56% and 3.25 ± 0.55, respectively, and those in the model group were 32.26 ± 6.43%, 15.26 ± 4.33% and 2.11 ± 0.43, respectively; significant differences were observed for each index between the two groups (p < 0.05). The CD₄⁺ T cell percentage and CD₄⁺/CD₈⁺ ratio in the 40 mg/kg AsIV group were 37.26 ± 5.66% and 3.04 ± 0.58, respectively,

and these values were significantly higher than those in the model group (p < 0.05). The CD₈⁺ T cell percentage in the 40 mg/kg AsIV group was 12.26 ± 2.71%, which was significantly lower than that in the model group (p < 0.05) (Figure 1).

Serum TNF-α, IL-1β and IL-2 levels after treatment

After treatment, the serum TNF-α, IL-1β and IL-2 levels in the model group were 4.68 ± 0.92, 299.33 ± 79.52 and 23.33 ± 8.84 pg/ml, respectively,

Table 2. SOBI and PLI in different groups after treatment.

Group	SOBI (points)	PLI (points)
Control	0.65 ± 0.26	2.43 ± 0.63
Model	2.12 ± 0.55*	4.08 ± 0.84*
Low-dose AsIV	1.98 ± 0.48*	4.01 ± 0.88*
Middle-dose AsIV	1.47 ± 0.41***	3.89 ± 0.79*
High-dose AsIV	1.12 ± 0.33****	2.95 ± 0.75****

*p < 0.05 compared with the control group; **p < 0.05 compared with the model group; ***p < 0.05 compared with the low-dose AsIV group; and ****p < 0.05 compared with the middle-dose AsIV group. AsIV, astragaloside IV; SOBI, salivary occult blood index; and PLI, plaque index.

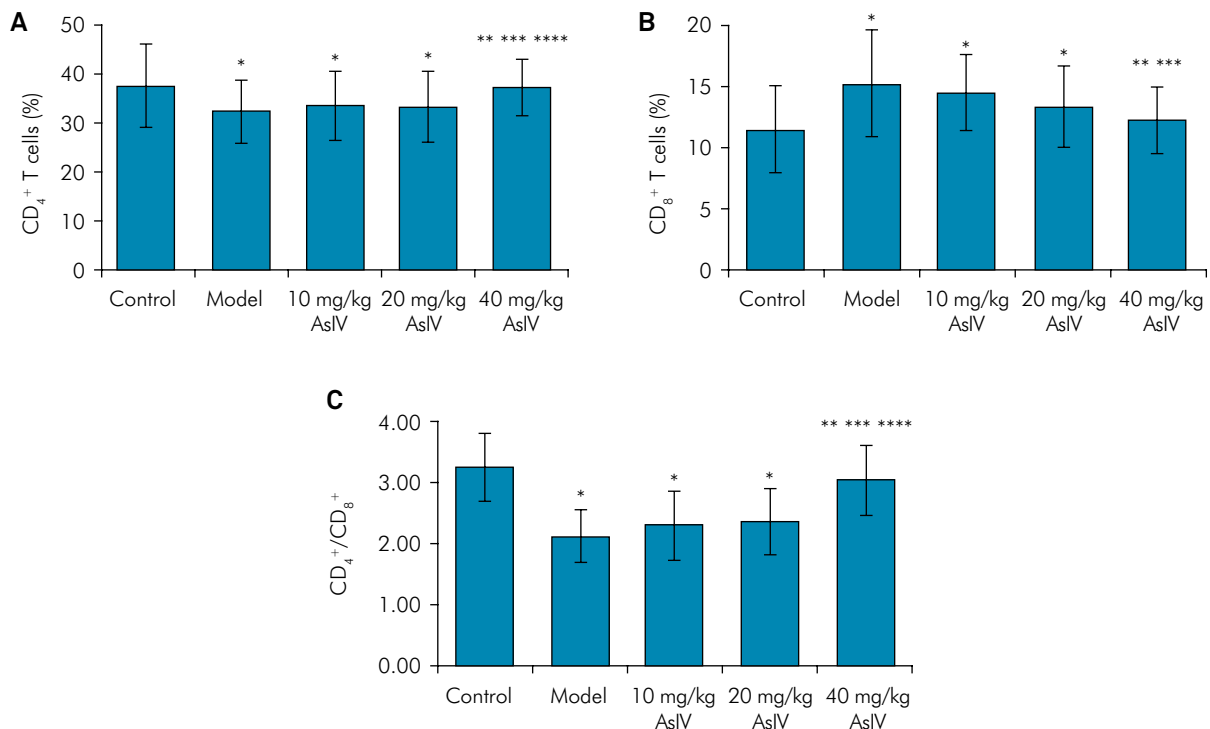


Figure 1. Peripheral blood T lymphocyte subsets in different groups after treatment. A: CD₄⁺; B: CD₈⁺; and C: CD₄⁺/CD₈⁺ ratio. *p < 0.05 compared with the control group; **p < 0.05 compared with the model group; ***p < 0.05 compared with the 10 mg/kg AsIV group; and ****p < 0.05 compared with the 20 mg/kg AsIV group. AsIV, astragaloside IV.

which were significantly higher than the levels in the control group, which were 3.62 ± 0.84 , 179.67 ± 39.56 and 36.67 ± 9.67 pg/ml, respectively ($p < 0.05$). In the 40 mg/kg AsIV group, the serum TNF- α , IL-1 β and IL-2 levels were 3.72 ± 0.75 , 187.12 ± 58.17 and 33.12 ± 7.65 pg/ml, respectively, and these levels were significantly lower than those in the model group ($p < 0.05$) (Figure 2).

Serum IgA, IgG and IgM levels after treatment

After treatment, the serum IgA and IgG levels in the model group were 1.12 ± 0.37 and 4.33 ± 0.92 mg/ml, respectively, which were significantly higher than the levels of 0.36 ± 0.05 and 0.66 ± 0.25 mg/ml, respectively, in the control group ($p < 0.05$). In the 20 mg/kg AsIV group, the serum IgA and IgG levels were 0.83 ± 0.45 and 1.55 ± 0.54 mg/ml, respectively. In the 40 mg/kg AsIV group, the serum IgA and IgG levels were 0.61 ± 0.34 and 1.19 ± 0.24 mg/ml, respectively. Each

index in the 20 and 40 mg/kg AsIV groups was significantly lower than the corresponding index in the model group ($p < 0.05$). There was no significant difference in the serum IgM levels among the five groups ($p > 0.05$) (Figure 3).

Discussion

This study investigated the effects of AsIV on inflammation and immunity in rats with experimental periodontitis. The results indicate that AsIV can mitigate periodontitis in rats. T lymphocyte subsets are important indicators of cellular immune function. CD₄⁺ and CD₈⁺ T cells are two kinds of important immune regulatory cells in the immune system and are the central links that determine the stability of the internal immune environment. Under normal circumstances, the ratio of CD₄⁺/CD₈⁺ remains stable.^{16,17} Studies have shown that the ratio of CD₄⁺/CD₈⁺ in the peripheral blood of animals or patients with periodontitis is

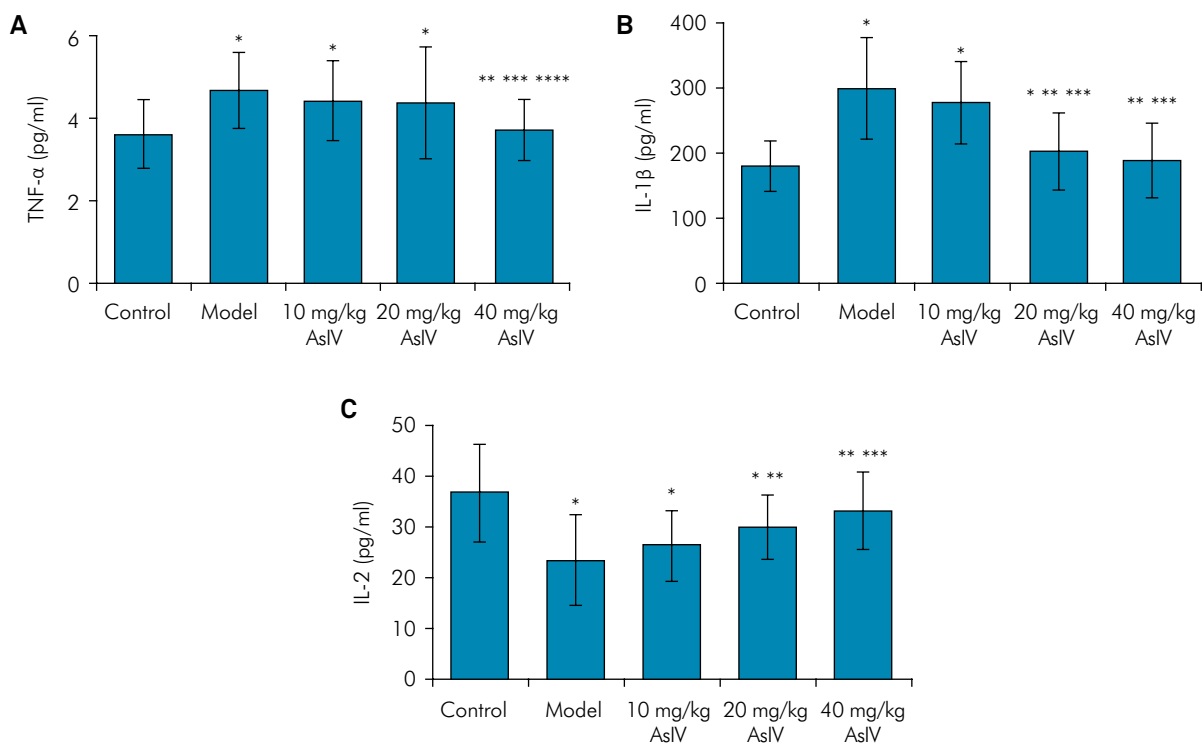


Figure 2. Serum TNF- α , IL-1 β and IL-2 levels in different groups after treatment. A: TNF- α ; B: IL-1 β ; and C: IL-2. * $p < 0.05$ compared with the control group; ** $p < 0.05$ compared with the model group; *** $p < 0.05$ compared with the 10 mg/kg AsIV group; and **** $p < 0.05$ compared with the 20 mg/kg AsIV group. AsIV, astragaloside IV; TNF- α , tumor necrosis factor- α ; IL-1 β , interleukin-1 β ; and IL-2, interleukin-2.

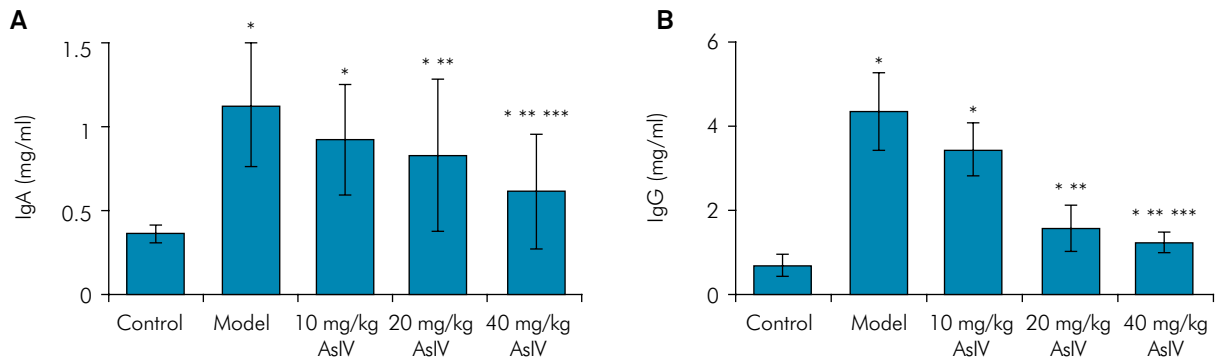


Figure 3. Serum IgA, IgG and IgM levels in different groups after treatment. A: IgA; B: IgG; and C: IgM. * $p < 0.05$ compared with the control group; ** $p < 0.05$ compared with the model group; and *** $p < 0.05$ compared with the 10 mg/kg AsIV group. AsIV, astragaloside IV; IgA, immunoglobulin A; IgG, immunoglobulin G; and IgM, immunoglobulin M.

significantly reduced.¹⁸ In this study, after treatment, the CD₄⁺ T cell percentage and CD₄⁺/CD₈⁺ ratio in the model group were significantly lower than those in the control group, and the CD₈⁺ T cell percentage in the model group was significantly higher than that in the control group. Compared with the model group, the 40 mg/kg AsIV group exhibited significant increases in the CD₄⁺ T cell percentage and CD₄⁺/CD₈⁺ ratio and a significant decrease in the CD₈⁺ T cell percentage. These findings indicate that AsIV can improve the distribution of T lymphocyte subsets in the peripheral blood.

The occurrence of periodontal disease involves a series of immune responses.¹⁹ IL-1 is a cytokine produced mainly by monocytes/ macrophages. There are two types of IL-1 (α and β 2), and IL-1 β plays a key role in the process of bone destruction in periodontitis.²⁰ In animals with experimental periodontitis, blocking IL-1 receptors can significantly reduce the aggregation of inflammatory cells and formation of osteoclasts, which ultimately reduces the loss of alveolar bone.²¹ IL-2 is the major cytokine responsible for the proliferation of T cells. In addition to promoting the proliferation and activation of T cells, IL-2 can mediate the immune process in the body by inhibiting apoptosis in T cells, stimulating the growth of NK cells, enhancing the killing function of NK cells, and stimulating the growth of B cells and antibody production.²² TNF- α also plays an important role in periodontal tissue destruction. It can stimulate collagenase-mediated tissue destruction,

induce osteoclast precursor cell proliferation and differentiation, and indirectly act on mature osteoclasts to stimulate bone resorption.²³ In this study, compared with those in the model group, the serum TNF- α , IL-1 β and IL-2 levels in the 40 mg/kg AsIV group were significantly decreased. These observations indicate that AsIV can alleviate the inflammatory reaction in periodontitis rats.

Poor immune function can lead to infection, tumor formation or immunodeficiency-related diseases. Immunoglobulins are a group of proteins with antibody activities. They combine with antigens to eliminate pathogens and neutralize toxins. Immunoglobulins are an important part of the immune system, and their content, to a certain extent, reflects the strength of the body's immune function.²⁴ A previous study showed that the serum IgA, IgG and IgM levels in patients with periodontitis are increased, especially in the early stage of disease.²⁵ In this study, after treatment, the serum IgA and IgG levels in the 20 and 40 mg/kg AsIV groups were significantly lower than those in the model group. This result indicates that AsIV can alleviate immunological stress in periodontitis rats. However, there was no significant difference in the serum IgM levels among the five groups. The reason needs to be further investigated.

In conclusion, AsIV can alleviate inflammation and enhance immunity in rats with experimental periodontitis. This study has provided a theoretical basis for the clinical application of AsIV for the

treatment of periodontitis. There are still limitations to this study. First, the correlations between inflammation and immunity indexes have not been investigated.

Second, there may be other mechanisms underlying how AsIV alleviates periodontitis in rats. These issues need to be addressed in further studies.

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