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Original Article

Effects of Goji Berries Extract on Acute Anterior Uveitis in Rats

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ABSTRACT

Background and Aim: The purpose of the present study is to provide a basis for the use of Goji berries as a therapeutic agent for acute uveitis. **Methods:** The rat's model of acute uveitis was induced by endotoxin as previously described and the effect of Goji Berries Extract (GBE) was evaluated through the measurement of clinical inflammatory scores, histological changes in anterior chamber tissues and inflammatory status in the aqueous humor. **Results:** In rats treated with GBE (400 mg/kg, IP), clinical inflammatory score at 24 hr after LPS injection was significantly reduced. Histological changes in anterior chamber tissues were significantly decreased in GBE treatment group, while in DXM treatment group nearly returned to normal. The number of infiltrating cells and the levels of protein leakage in aqueous humor were significantly elevated in EIU model group, but the administration of GBE improved these parameters. **Conclusion:** Our results suggest that GBE used in the present study has potential for the treatment of AAU.

Keywords: Goji berries extract, Acute anterior uveitis, Endotoxin-induced uveitis, Experimental.

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INTRODUCTION

Uveitis is one of the intraocular conditions describing the inflammation of the uveal tract of the eye, including the iris, Ciliary Body (CB), and choroid.[1] It represents a major cause of visual impairment, ultimately resulting in vision loss, accounting for 10% of all cases of legal blindness worldwide.[2,3] Uveitis models in rodents are important in the investigation of pathogenesis in human uveitis and the development of appropriate therapeutic strategies for treatment. Over the decades, the experimental rodent models of uveitis have been extensively used as an effective preclinical model to understand the pathogenesis of human uveitis and evaluate the therapeutic efficacy of new medications because of their resemblance in immunopathogenic mechanisms to the human uveitis. In Endotoxin-Induced Uveitis (EIU), a well-established animal model of Acute Anterior Uveitis (AAU), an injection of LPS into certain susceptible strains of rodents induces an acute and preferential inflammation of the iris and ciliary body that closely resembles AAU in humans.[4] Goji berries, the fruits derived from *Lycicum barbarum* L., are a local food in DPR of Korea, China and other Asian countries. In nature, there are about 70 different species of Lycicum, growing in the Mediterranean area and in temperate regions throughout the world. The *Lycicum chinense* species is more common but less valuable both for palatability and chemical composition, in particular having a lower content of bioactive molecules.[5] Goji berries are traditionally employed in Korea medicine from ancient times, and their use is now extending to many countries where they are consumed, above all, as food supplements. In particular, the concentrated juice or extracts from this fruit are added to beverages with the aim to improve the hepatic function or alleviate the fatigue and oxidative stress in DPR of Korea. However, following the interest of the scientific community on Goji berry extracts, other biological activities are emerging and include effects on aging, cancer, irradiation- or chemotherapy-induced organ toxicities, cardiovascular and reproductive apparatus injuries, colitis, stroke, diabetes, Alzheimer's disease, glaucoma, and immune system functionality.[6,7] A few studies have reported biological components of Goji berry for the treatment of many diseases but there has been no research to use its extract as herbal medicine for the treatment of ocular inflammation.

MATERIALS AND METHODS

Reagents

Lipopolysaccharide (V. cholera, classical Biotype, serotype Ogawa) was provided by the Biological institute of Medicine, DPR of Korea. Goji berry extract was kindly provided by Traditional Medicine Centre of Pyongyang University of Medical Sciences.

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The level of main compounds of Goji berry was certificated by National Medicine authentication of DPR of Korea.

Animals

Adult male pathogen-free Wistar rats (8–10 weeks old, weighing 180–200 g) and mice (purebred, weighing 20–22 g) were obtained from the Laboratory Animal Centre of the Pyongyang University of Medical Sciences. 60 rats were randomly divided into four groups (n=15 per groups): Normal control group, EIU model group, GBE treatment group and Dexamethasone (DXM) treatment group. 8 rats respectively in each group were used for the test of histological change and remaining rats in each group for the test of aqueous humour. 80 mice of either sex were randomly assigned to 4 groups (n=20; 10 males and 10 females per group) and employed in the study of sub-chronic toxicity. During the experiment, feed and water were available to rats at any time. The temperature was maintained at 20±2°C and the humidity was 55%. The study was approved by the Ethics Committee for Animal Experimentation, Faculty of Basic Medicine, Pyongyang University of Medical Sciences.

Subchronic Toxicity Study

Different doses of GBE (300, 600, or 1200 mg/kg) were dissolved in distilled water and administered daily by lavage to different groups of mice, while the control group received only distilled water. General activity, toxic manifestations and mortality were monitored daily for 60 days.

EIU Model

Endotoxin-induced uveitis was induced as previously described.[8] Rats received a single injection of 200 μg of LPS dissolved in 100μl of sterile saline (Normal Saline) intraperitoneally. The rats in the GBE treatment group and DXM treatment group received an intraperitoneal injection of GBE (400 mg/kg) or DXM (1 mg/kg), respectively, 1 hr before the LPS induction. The EIU model group received an equivalent volume of normal saline.

Inflammatory Signs

The intensity of the anterior segment inflammation was examined by slit microscopy before the injection and at 2 hr intervals after the injection. Inflammatory signs were recorded in detail, and photographs were taken. The severity of uveitis was graded using the scoring system in Table 1 by a blinded investigator.[9]

Histopathology

24 hr after being injected with LPS, the animals were deeply anesthetized using 17.5% chloral hydrate (2 mL/kg) by intraperitoneal injection and flushed through the left ventricle of the heart with 250–350 mL of cold (4°C) Phosphate-Buffered Saline (PBS), 1 IU heparin per ml of PBS, and 250 mL of cold (4°C) 4% paraformaldehyde. One eye of each rat was enucleated and placed in 10% neutral buffered formalin solution for 24 hr. After the stationary liquid was washed out, the tissue sample was immersed in 50%, 75%, 80%, 90%, and 100% alcohol for 1 hr, respectively, for dehydration. Fixed and dehydrated tissue was embedded in paraffin for 1 hr for three times after being treated with xylene for 30 min. Sagittal sections (4 μm thick), cut near the optic nerve head, were stained with hematoxylin and eosin. As previously described,[10] anterior chamber tissues were scored for severity of inflammation as follows: 0 = normal tissue; 1 = dilated iris vessels and thickened iris stroma with exudate, protein, and/or a few scattered inflammatory cells in the anterior chamber; 2= infiltration of inflammatory cells into the stroma of the iris and/or ciliary body, with a moderate number of inflammatory cells within the anterior chamber; 3 =heavy infiltration of inflammatory cells within the iris stroma and ciliary body and a heavy infiltration of inflammatory cells within the anterior chamber; and 4 =heavy exudation of cells in dense protein aggregation in the anterior chamber and inflammatory cell deposits on the corneal endothelium. Both eyes were scored separately in a blinded fashion, and then, the results were averaged.

Table 1: Scoring system for clinical evaluation of uveitis.

Clinical signs	Grade of uveitis (score)
Iris hyperemia	
Absent	0
Mild	1
Moderate	2
Severe	3
Pupil	
Normal	0
Miosis	1
Exudate in anterior chamber	
Absent	0
Small	1
Large	2
Hypopyon	
Absent	0
Present	1
Maximum possible score	7

Quantification of Infiltrating Cells and Protein Concentration in Aqueous Humour

The rats were euthanized 24 hr after LPS injection, and the aqueous humour (20-25 μL/rat) was collected from both eyes immediately by an anterior chamber puncture using a 30-gauge needle under a surgical microscope. For cell counting, the aqueous humour

was suspended in an equal amount of Türk stain solution, and the cells were counted using a haemocytometer under light microscopy. The number of cells per field (an equivalent of 0.1 µL) was obtained by averaging the results of four fields from each sample. The aqueous humour samples were then centrifuged at 2,500 rpm for 5 min at 4°C to obtain the supernatant. The total protein concentration in the aqueous humour samples was measured using a Bio-Rad Protein Assay Kit.

Statistical Analysis of Data

Results were expressed as the mean and SEM. Data were analyzed by one-way Analysis of Variance (ANOVA) using SPSS 16.0 and the differences between the means assessed using Dunnet's multiple range test. A P value of < 0.01 was taken as the level of statistical significance.

RESULTS

Subchronic Toxicity of GBE

In the study of the subchronic toxicity on GBE provided by manufacturer, the control as well as the treated mice of both sexes appeared generally healthy throughout the experimental period. No mortality was recorded and no toxicity signs were detected in any of the treated mice. The subchronic toxicity of GBE indicated that a single oral administration of GBE up to 1200 mg/kg dose caused neither visible sign of toxicity nor mortality to experimental animals. Determined the LD_{50} of GBE is more than 1200 mg/kg.

Clinical Score of Inflammation

The clinical inflammatory scores in the 4 groups at 24 hr after LPS injection were shown in Table 2.

The inflammatory scores of 3 groups (EIU, GBE and DXM) were significantly increased compared to that of the normal group (P <0.01). The GBE treatment significantly reduced the inflammatory degree of the rats compared to the EIU group and in particular, the score of the GBE group displayed no significant difference compared with the DXM group.

Effect of GBE on Histological Changes

H–E staining results were in accordance with the inflammatory manifestations as described above at 24 hr after LPS injection. The inflammatory reaction degree was presented in Table 3.

The inflammation levels in the GBE and DXM group both significantly decreased compared to the EIU model group (P <0.01). The inflammation degrees of the two treatment groups were not significantly different.

Table 2: Degree of Inflammatory score at 24 hr after LPS injection.

Group	N (eye)	Inflammatory score
Normal	30	0.21± 0.1
EIU	30	6.47 ± 0.6**
GBE	30	2.89 ± 0.4**ΔΔ
DXM	30	2.13± 0.3**ΔΔ

Each value represents the mean ± SEM of 30 eyes per group. **P<0.01 as compared with normal group. ΔΔ P<0.01 as compared with EIU group.

Table 3: Histological changes in anterior chamber tissues at 24 hr after LPS injection.

Group	N (eye)	Score
Normal	16	0.32± 0.1
EIU	16	3.84 ± 0.7**
GBE	16	1.55 ± 0.5**ΔΔ
DXM	16	0.96± 0.3**ΔΔ

Each value represents the mean ± SEM of 16 eyes per group. **P<0.01 as compared with normal group. ΔΔ P<0.01 as compared with EIU group.

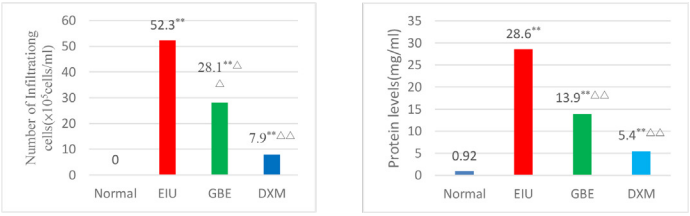

Figure 1: Effect of GBE on cell migration or protein levels in the aqueous humor.

Each value represents the mean ± SEM of 14 eyes per group. **P<0.01 as compared with normal group. ΔΔ P<0.01 as compared with EIU group.

Effect of GBE on Cellular Infiltration and Protein Concentration in Aqueous Humour

No infiltrating cells were detected in the aqueous humour in normal rats. In contrast, the number of inflammatory cells that infiltrated the aqueous humour 24 hr after LPS injection was significantly higher. On the other hand, in rats treated with GBE and DXM, the number of inflammatory cells in the aqueous humour was significantly reduced compared to EIU group. The protein concentration in the aqueous humour was 28.6 mg/mL in rats 24 hr after LPS injection (P <0.01 compared to normal group), and the treatment with GBE and DXM significantly reduced the protein leakage (Figure 1).

DISCUSSION

Uveitis is a common intraocular inflammatory disease and a leading cause of visual impairment or blindness, which mainly affects the iris, ciliary body, and choroid.[11] At present, the pathogenic mechanisms of uveitis are not clear. The majority of

uveitis may be caused by immune-mediated factors, and only a small part of the infectious uveitis is due to pathogen invasion.[12] Acute anterior uveitis, especially HLA-B27-associated AAU, is a common form of non-infectious uveitis, but clinical and laboratory studies have proven that Gram-negative bacteria such as Klebsiella, Salmonella, Yersinia, and Shigella species may be involved in triggering it.[13] Endotoxin-induced uveitis is a well-established animal model of AAU. Injection of LPS from Gram-negative bacteria at sites remote from the eye induces AAU without significantly affecting other tissues.[14] Due to the high health-benefit potential of Goji berries, a careful investigation of phytocomplex has been carried out. In the last decade, more than 200 different components, comprising carotenoids, phenylpropanoids, flavonoids, other polyphenols and polysaccharides, have been identified, characterized and analyzed. All of them showed some interesting biological properties.[15,16] Herein, we investigated the protective effects of GBE on EIU in rats. In this study, similar to previous study, the inflammatory response of the Wistar rats in the EIU model group reached the maximum at 24 hr after LPS injection. However, the intensity of the anterior segment inflammation of the rats significantly decreased in the GBE treatment group and there were no significant differences compared to the DXM treatment group. These results demonstrate that GBE could successfully suppress the ocular inflammation in the EIU model as measured by slit lamp assessment and histopathology. Namely, except normal group, ocular inflammatory signs were observed in rats of each of the remaining three groups after LPS injection. Conjunctival edema, ciliary congestion, and blood vessel dilatation in the iris appeared within 6 hr after the LPS injection in all three groups, but cell and flare in the anterior chamber could only be observed in the EIU group. The intensity of the anterior segment inflammation reached a maximum at 22–24 hr. Pupil occlusion and fibrinous membrane were observed in the EIU group. Small amounts of exudate on the pupillary margin could be found in both of the GBE and DXM groups occasionally. In the EIU group, the iris stroma was thickened, and vasodilatation was observed. Fibrin exudations and a large number of inflammatory cell infiltrations of massive neutrophils and small amounts of mononuclear cells and lymphocytes could be observed in the anterior chamber. In GBE and DXM groups, only a small amount of inflammatory cell infiltrations was found in the iris-ciliary body complex and a small amount of fibrin exudation could be observed at the anterior chamber angle. Finally, in GBE group, the histological changes in anterior chamber were similar to that in DXM group. Also, the treatment with GBE strikingly reduced both cellular infiltration and protein leakage in the aqueous humor, but the positive effect in DXM group was higher than that in GBE group on the test of the aqueous humor. Our results show that GBE administration significantly suppress the development of ocular inflammation in rats with EIU.

CONCLUSION

In conclusion, this study suggests that the anti-inflammatory potency of 400 mg/kg GBE intraperitoneal injection was as strong as that observed with 1 mg/kg DXM. The results suggest that GBE might pave the way for a novel therapeutic agent for the management of ocular inflammation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

GBE: Goji Berries Extract; **AAU:** Acute Anterior Uveitis; **EIU:** Endotoxin-Induced Uveitis; **CB:** Ciliary Body; **DXM:** Dexamethasone.

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