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Original Article

Effect of Propolis on Experimental Allergic Conjunctivitis 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 propolis as a therapeutic agent for allergic conjunctivitis. **Methods:** The effect of propolis eye drops on experimental allergic conjunctivitis in rats was investigated. The allergic conjunctivitis in rats was occurred by sensitization and challenge of egg albumin and the number of eye scratching, hyperemia, chemosis, vascular permeability and histamine content in conjunctival tissue and tears were assayed. **Results:** Propolis significantly inhibited allergic symptoms including eye scratching, hyperemia and chemosis observed in conjunctivitis compared with the control group. Furthermore, this agent significantly inhibited increased vascular permeability induced by antigen and histamine release from the rat conjunctiva in correlation with a decrease in histamine content in tears. **Conclusion:** The efficacy of the propolis on allergic conjunctivitis in rats may be attributable to the antiallergic and anti-inflammatory action.

Key words: Allergic conjunctivitis, Histamine release, Propolis, Vascular permeability, Experimental model.

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INTRODUCTION

Allergic conjunctivitis is one of the most common ocular conditions encountered by clinicians. The pathogenesis of ocular allergy involves various mechanisms that result in mast cell degranulation and the release of mediators. These mediators lead to itching, conjunctival vasodilatation, increased vascular permeability, leukocyte chemotaxis, and ocular surface alterations.[1,2]

Propolis is a resinous, strongly adhesive natural substance, collected by honeybees from buds and leaves of trees and plants, mixed with products of their salivary glands and wax. Chemical composition of propolis is very complex and more than 200 compounds have been identified. Its biological activity depends on compounds from the polyphenolic fractions, mainly flavonoids, followed by aromatic acids, phenolic acids esters, triterpenes, lignans etc. flavonoids isolated from propolis reported to have bactericidal and antiprotozoal antiviral, antioxidant, anti-inflammatory and immunomodulatory activities.[3-5]

The anti-allergic action of propolis for allergic conjunctivitis is unknown. In the present study, we investigated the anti-allergic effects of propolis in egg albumin- induced allergic conjunctivitis in rats.

MATERIAL AND METHODS

Animals

Six weeks-old male Wistar rats were provided by Laboratory Animal Centre of Pyongyang University of Medical Sciences. All rats were housed with regular diet for two weeks before the experiment in institute of pathophysiology, Pyongyang University of Medical Sciences. They were treated as recommended in the Guide for the Care and Use of Laboratory Animals issued by the DPRK Association of Laboratory Animal Care. All animal experiments approved from the institutional Animal Ethics Committee.

Preparation of Propolis Eye Drops

Propolis collected from the suburban area of Pyongyang, DPR of Korea was authenticated by National botanical institute of DPR of Korea and propolis eye drops prepared by Traditional Medicine Centre of Pyongyang University of Medical Sciences. This preparation using propolis was certificated by National Medicine authentication of DPR of Korea.

Experimental Design

Animals were divided into the following groups as Control, conjunctivitis model and Experiment, Propolis eye drops. Each group consisted of 12 and 10 rats respectively.

Reagents

Egg albumin (Sigma, St. Louis, MO, USA), aluminum hydroxide hydrate gel (LSL, Tokyo, Japan), Evans blue (Wako, Tokyo, Japan), Bordetella pertussis inactive microorganism suspension (Kitasato Institute Research Center for Biology, Saitama, Japan)

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Sensitization

The rats were sensitized by injection of 0.6 ml of physiological saline containing egg albumin (1 mg), alum (2 mg) and 10^{10} *B. pertussis* into the four footpads on the first day. Five days later, they were boosted by subcutaneous injection of 1 ml of physiological saline containing egg albumin (0.5 mg) at 10 sites on the back. Then, local sensitization was performed every day from day 14 to day 20 by instilling egg albumin in physiological saline (10 mg/ml, 5 ml/site) into the bilateral eyes using a micropipette.

Instillation of Propolis

Propolis eye drops was instilled at 15 min before antigen challenge into the bilateral eyes at 5ml/site.

Evaluation of Conjunctival Symptoms

Before the experiment, the animals were placed in an observation cage (33×22×10 cm) for about 10 min for acclimatization. After the instillation of 5 ml/site of egg albumin dissolved in physiological saline solution (10 mg/ml) into the bilateral eyes (Antigen challenge), they were placed into the observation cage (one animal/cage), and the number of eye scratches was counted for 20 min. Eye scratching behavior was defined as fore-limb movements over 2 times directed to the ocular surface, and the number of instances. Allergic symptoms (hyperemia and edema of the conjunctiva) were observed using the scoring system shown in Table 1. Hyperemia and edema were evaluated at 5 and 20 min, respectively, after topical antigen challenge.

Vascular Permeability of the Conjunctiva

On the day following antigen challenge, 2% Evans blue solution was intravenously injected. Fifteen minutes later, the rats were anesthetized with diethylether, bled, and the conjunctiva was removed. Then, Evans blue was extracted with 0.5 ml of 1 N KOH solution for 12 hr at 37°C, and 4.5 ml of H_3PO_4-acetone (0.6 N H_3PO_4 : acetone5 : 13) was added and mixed well. After centrifugation at 400 *g* for 20 min, the amount of extracted dye was determined using a spectrophotometer at 620 nm.[6]

Histamine Release from the Conjunctiva

Thirty minutes after antigen challenge, the conjunctiva was carefully excised, weighed and washed twice with saline. The tissues were homogenized with 0.4 N perchloric acid and placed in an ice-bath for 1 h. After centrifugation at 1000 *g* for 10 min at 4°C, histamine content in the supernatant was determined by an automated fluorometric assay. The percentage of histamine release was calculated by the following equation:

$$c=(a-b)/a\times100$$

c: % histamine release

Table 1: The scoring system used for estimating the severity of conjunctivitis.

Score	Symptoms	
	Hyperemia	Edema
0	No symptoms	No symptoms
1	Slight hyperemia in one-eye	Slight edema in one-eye
2	Slight hyperemia in bilateral eyes	Slight edema in bilateral eyes
3	Severe hyperemia in one-eye and Slight hyperemia in the other eye	Severe edema in one-eye and Slight edema in the other eye
4	Severe hyperemia in bilateral eyes	Severe edema in bilateral eyes

Hyperemia and edema were evaluated 5min and 20min after antigen challenge, respectively.

a: histamine content of the conjunctiva in immunized animals

b: histamine content of the conjunctiva (antigen challenged) in immunized animals

Histamine Content in Tears

Fifteen minutes after antigen challenge, 50 ml saline was applied to both eyes. This procedure was repeated 3 times and a 200 ml sample was carefully collected from both eyes. The sample and the same quantity of 0.8N perchloric acid were then mixed together. After centrifugation at 10000 ***g*** for 10 min at 4°C, the histamine content of the supernatant was determined by high performance liquid chromatography using a fluorometric detector.[7]

Statistical Analysis of Data

All data were represented as the mean±SEM. Statistical analysis was performed using SPSS Statistics 14. Parameters were analyzed by student *t* test. P<0.05 was considered to be statistically significant.

RESULTS

Table 2 shows the effect of propolis eye drops on eye scratching behavior and allergic symptoms induced by antigen. Propolis eye drops significantly inhibited eye scratching behavior and allergic symptoms than the control group. Table 3 shows the effect of propolis eye drops on vascular permeability increase induced by antigen in rats. Propolis eye drops significantly inhibited the vascular permeability increase in the conjunctiva than the control group. Table 4 shows the effect of propolis eye drops on histamine release from the conjunctiva. Propolis eye drops significantly inhibited histamine release in the conjunctival tissue and histamine content in tears.

Table 2: The Effect of propolis eye drops on eye scratching behavior and allergic symptoms induced by antigen.

Group	Eye scratching behavior (counting)	Hyperemia (score)	Chemosis (score)
Control (n=12)	14.5±1.2	5.2±0.6	4.9±0.4
Experiment (n=10)	4.8±0.3*	2.5±0.1*	2.5±0.2*

Data was expressed as mean±SE.

* Significantly different from the control group, P<0.05

Table 3: The effect of propolis eye drops on vascular permeability increase induced by antigen.

Group	Conjunctival dye content (ng/ mg tissue)
Control (n=12)	340.6±25.4
Experiment (n=10)	80.4±13.2**

Data was expressed as mean±SE.

**Significantly different from the control group, P<0.01

Table 4: The effect of propolis eye drops on histamine release from the conjunctiva.

Group	Histamine release (%)	Histamine content in tears(ng/ml)
Control (n=12)	75.1±12.4	5.4±2.7
Experiment (n=10)	45.6±8.3*	1.5±1.0*

Data was expressed as mean±SE.

* Significantly different from the control group, P<0.05

DISCUSSION

In this study, we attempted to examine the effect of newly prepared propolis eye drops on allergic conjunctivitis induced in rats so as to provide data available in the ophthalmic area. Based on the various chemical composition and immense medicinal and therapeutic values of propolis, the present study was aimed to provide a basis for the use of propolis eye drops as a therapeutic agent for allergic conjunctivitis. Allergic conjunctivitis is one of the major allergic diseases in the ophthalmic clinic that cause allergens in the air exposed to the surface of the eyeball, resulting in the degranulation of mast cells by immunoglobulin E (IgE) and the development of allergic inflammatory symptoms, which is recognized as a major disease affecting quality of life. Type 1 hypersensitivity has early and late periods. Vasodilatation and increased vascular permeability are the hallmarks of the early period and present themselves as severe itching, conjunctival injection, chemosis, and tearing. The selective H1 receptor is mainly responsible for itching, while the stimulation of the H_2 receptor results in redness. The early period reactions are elicited within 5-30 min and then disappear slowly. Late period reactions initiate within 2-6 h without exposure to additional antigens, and inflammation is elevated by secondary mediators.[8]

We were sure that the anti-allergic action of propolis is to be mainly related to its composition, Flavonoids. They are a broadly distributed class of plant pigments, universally present in plants. Among them Chrysin (5,7-dihydroxyflavone) is a natural and biologically active compound extracted from many plants, honey, and propolis. It possesses potent anti-inflammatory, anti-oxidant properties, promotes cell death, and perturbing cell cycle progression.[9]

However, the mechanism by which chrysin inhibits allergic expression remains poorly understood. Therefore, we examined anti-allergic action of propolis on experimental allergic conjunctivitis in rats. In present study, we demonstrated experimentally that propolis have a significant inhibitory effect on eye scratching, allergic symptoms, vascular permeability, and histamine release in egg albumin- induced allergic conjunctivitis in rats. These results show that flavonoids of propolis including chrysin exert anti-allergic action. Furthermore, no obvious side effects of propolis were detected during the experimental period time. We suggest that propolis may be useful for the treatment of allergic conjunctivitis as herbal medicine.

CONCLUSION

Our results demonstrate that propolis inhibits eye scratching, allergic symptoms, vascular permeability, and histamine release in egg albumin-induced allergic conjunctivitis in rats. Furthermore, our results could open new avenues for the treatment of allergic conjunctivitis by propolis.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

KOH: Potassium Hydroxide; **H_3PO_4:** Phosphoric Acid; **IgE:** Immunoglobulin E.

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