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Influence of artificial luminous environment and TCM intervention on development of myopia rabbits

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ABSTRACT

Objective: To explore the influence of artificial luminous environment and preventive function of traditional Chinese medicine (TCM) intervention based on "Theory of yin-yang clock" on myopia. **Methods:** A total of 45 New Zealand young rabbits were randomly divided into 5 groups, 9 for each group. Control group was exposed in natural light. Fluorescent group and full spectrum group were exposed in fluorescent light and full spectrum light, on which basis fluorescent TCM group and full spectrum TCM group were added with "Rizhong Yinyang Formulas", respectively. Optical parameters were measured and the influence of different lights on the serum and retinal dopamine (DA) levels as well as the retinal histopathological tissues was observed. **Results:** The spectrum of fluorescent light mainly focused at 420-490 nm with the peak value of wavelength near 450 nm, whereas that of full spectrum was wider (400-800 nm) with the peak value near 600 nm. After 4 and 12 weeks, fluorescent group was evidently lower in serum and retinal DA levels ($P<0.01$), and there was no significant difference among full spectrum group, fluorescent TCM group and full spectrum TCM group ($P>0.05$). Histopathological observation showed that there was significant difference in pigment epithelium layer, photoreceptor and nerve fiber layer between fluorescent group and control group, but the difference among the test groups was not significant. **Conclusions:** Fluorescent light has certain influence on retinal histological construction and visual performance. However, TCM intervention may have some degree of protective function on retina.

1. Introduction

Human had lived primarily on natural light and followed the natural day-night rhythm of getting up at sunrise and sleeping at sunset before the industrial revolution. Moreover, the circadian rhythm of living organisms has been formed according to the long-term evolution of rhythm changes of the natural illumination. However, with the social development, the appearance of artificial light sources has made a great difference between modern and previous luminous environment where human lives. The invention of electric lamps has greatly changed the natural luminous environment and the indigenous biological rhythm with normal alternation of days and nights, which may lead to the changes of partial disease spectrums.

Epidemiological survey has demonstrated that the morbidity of myopia increases annually while the ages are showing a younger trend[1]. Reports of recent studies have indicated that in some developed Asian regions, like China, Singapore, India, Japan and Korea, the ratio of myopia in adolescent students increases evidently

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than that of their previous generation[2–4]. Though genetic gene makes it more easier for Asian people to have myopia problems, the ratio of patients with myopia in European and America also increases to some extent[5]. Nevertheless, environmental factor, as a potential cause of myopia, has received increasingly more attentions from researchers. A research found that long-term study under indoor artificial light could lengthen the eyeballs, thus inducing the myopia[6].

The traditional Chinese medicine (TCM), which emphasizes the relevant adaptation of human body to natural environment and pays more attention to the autologous integrity of human body as well as the unity and correlation among human, nature and social environment, has irreplaceable advantages in regulating the biological rhythm than western medicine. In order to further explore the influence of artificial luminous environment and the preventive function of TCM intervention based on “Theory of yin-yang clock” on myopia, this study selected New Zealand young rabbits as the study modals to investigate the interventional function and action points of “Rizhong Yinyang formulas” under the guidance of “Yin-yang Rizhong regulation method” on the development of their eyes at different time points.

2. Materials and methods

2.1. Animals

A total of 45 healthy New Zealand young male and female rabbits without oculopathy, aged 1 month with weight of 400-600 g were provided by the Laboratory Animal Center of Qinglong Mountain.

2.2. Experimental Chinese medicines

Rizhong yin formula: Danggui (*Radix angelicae sinensis*) 10 g, Chishao (*Radix paeoniae rubra*) 10 g, Baishao (*Radix paeoniae alba*) 10 g, Shanyao (*Rhizoma dioscoreae*) 10 g, Shanzhuyu (*Fructus corni*) 10 g, fried Danpi (*Cortex moutan*) 10 g, Fuling (*Wolfiporia extensa*) 10 g, Chuanduan (*Radix dipsaci*) 10 g, Shengdi (Rhizome of rehmannia) 10 g and Gancao (*Glycyrrhiza uralensis*) 3 g.

Rizhong yang formula: Dangshen (*Codonopsis pilosula*) 15 g, fried Baizhu (*Rhizoma atractylodis*) 10 g, Fuling 10 g, fried Danpi 10 g, Chuanduan 10 g, Duzhong (*Eucommia ulmoides*) 12 g, Ziheche (*Human placentophagy*) 10g, Lujiaoshuang (*Cervus nippon temminck*) 10 g and Gancao 3 g.

“Rizhong Yinyang formulas” provided by TCM Dispensary of Jiangsu Province Hospital of TCM were lavaged to the New Zealand young rabbits in commuted dosage of that for adults in clinic, in which the Rizhong yang formula was taken at 12:00 and the other at 24:00 daily.

2.2. Methods

2.3.1. Detection of optical parameters

American Photo Research PR 655 Colorimeter was applied to detect all optical parameters of fluorescent light, full spectrum light and natural light. Specific operations: in darkroom with standard radiation, the distance between light and detector was set as 1.5 m. The detector was powered on for >1 h, and then the samples were collected 1 time/30 min for totally 10 times. 3 groups of effective data were selected and normalized procession was performed with origin 8.0 software.

2.3.2. Grouping and molding

All rabbits were randomly divided into 5 groups, 9 for each group, namely control group, fluorescent group, fluorescent TCM group, full spectrum group and full spectrum TCM group. Control group was fed in grid box with natural light. The box was put to the south-facing window to receive natural illumination. Fluorescent group was put into the box with several low-power fluorescent lights on the top and photosensory self-adjusting lighting device outside in order to automatically adjust the intensity of illumination according to the outside light intensity so as to ensure that the luminous rhythm in the box was similar to the natural light. Fluorescent TCM group was added with “Rizhong Yinyang formulas” on the basis of fluorescent group. Full spectrum group was put into the box with full spectrum lights on the top and photosensory self-adjusting lighting device outside, on which basis full spectrum TCM group was added with “Rizhong Yinyang formulas”.

2.3.3. Sample collection and detection

After 4 and 12 weeks, 2 mL venous blood from rabbit ear margin was collected and centrifuged at 3 000 rpm for 15 min. 1 mL serum was retained and stored at -4 ℃ for utilization. Intramuscular injection of 120 mg/kg 0.05% ketamine hydrochloride was conducted for the anesthesia of rabbits. Eyeballs on one side were randomly removed from each rabbit, from which the retina was excised and frozen at -70 ℃ liquid nitrogen immediately. Samples were collected at low temperature and under low light. DA level was detected according to the instructions of enzyme-linked immunosorbent assay determination kits (ELISA, German IBL company). High performance liquid chromatography (HPLC) was used to determine the DA level in retinal samples.

2.3.4. Retinal histopathological observation

16 weeks after the experiment, intramuscular injection of 120 mg/kg 0.05% ketamine hydrochloride was conducted for the anesthesia of rabbits. Eyeball on one side was randomly removed from each rabbit, from which the sclera and posterior outer retina

was excised under microscope. The sclera and retina were put into stationary liquid of electric speculum, embedded, sliced and stained. The patterns of retinal ultra-structure, pigment epithelium layer, photoreceptor and nerve fiber layer were observed under electric microscope.

2.4. Statistical analysis

SAS 9.3 software package was adopted for all data analysis. Measurement data was expressed by mean±standard deviation (mean ±SD), and comparison between two groups was analyzed by t-test. P<0.05 was considered to be statistically significant.

3. Results

3.1. Detection of optical parameters

The spectrum of fluorescent light mainly focused at 420-490 nm with the peak value of wavelength near 450 nm. Moreover, it had a secondary peak value at 500-700 nm with the values of wavelength and the normalized intensity being about 550 nm and 0.5, respectively. The range of full spectrum was wider (400-800 nm) with the peak value near 600 nm, which was similar to the light spectrum of natural light (Figure 1-3).

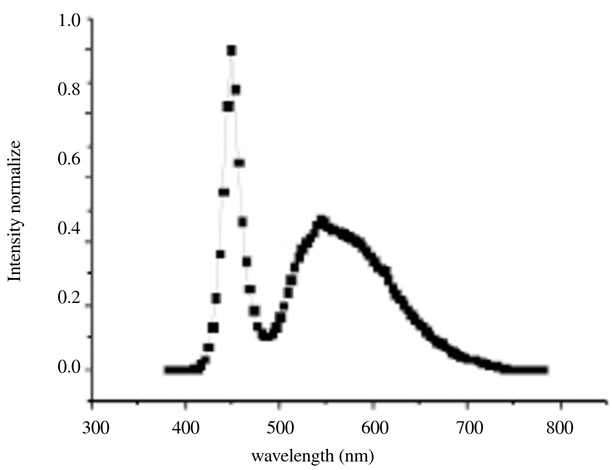

Figure 1. Distribution diagram of fluorescent light spectrum.

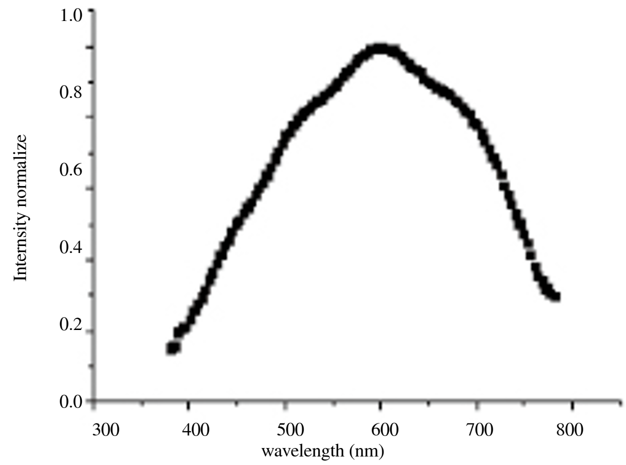

Figure 2. Distribution diagram of full spectrum.

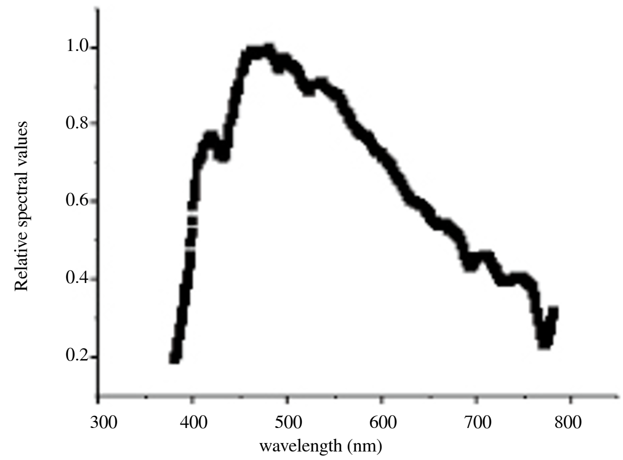

Figure 3. Distribution diagram of natural light.

3.2. Detection of retinal and serum DA levels

After 4 and 12 weeks, retinal and serum DA levels were apparently lower in fluorescent group than in control group, fluorescent TCM group and full spectrum group (*P*<0.01), but there was no significance in fluorescent TCM group, full spectrum group and full spectrum TCM group when compared with control group (*P*>0.05) (Table 1).

3.3. Retinal histopathological observation

No significant abnormality was observed in each retinal layer

Table 1
Comparison of retinal and serum DA levels among the groups (mean±SD).

DA level	*n*	Control group	Fluorescent group	Fluorescent TCM group	Full spectrum group	Full spectrum TCM group
4 weeks						
Retina (ng/mg)	3	0.118±0.013	0.065±0.009**	0.130±0.011##	0.123±0.028##	0.133±0.015
Serum (ng/mL)	3	1.175±0.450	0.460±0.148**	1.046±0.311##	1.138±0.268##	1.043±0.440
12 weeks						
Retina (ng/mg)	3	0.121±0.014	0.059±0.003**	0.137±0.016##	0.136±0.014##	0.132±0.015
Serum (ng/mL)	3	0.840±0.085	0.572±0.090**	0.759±0.040##	0.787±0.082##	0.790±0.079

Compared with control group in corresponding period, ***P*<0.01; Comparison of fluorescent TCM group and full spectrum group with fluorescent group in corresponding period, ##*P*<0.01.

in control group. Electric microscope showed obvious difference between fluorescent group and control group in retinal tissues, which was mainly reflected in pigment epithelium layer, photoreceptor and nerve fiber layer. Full spectrum group showed lipid granules located in the cytoplasm of pigment epithelium cells, whose counts and size were less significant than those in control group. Therefore, they had less damage on cell membrane and microvillus. In addition, the granules also had clear interior and exterior structures and orderly arrangement in photoreceptors and little vacuolization phenomenon in nerve fiber layer, which was less severe than those in fluorescent group. Fluorescent TCM group and full spectrum TCM group had relatively clear interior and exterior structures and orderly arrangement in photoreceptors with cells of outer nuclear layer in regular shape and even staining and nerve fiber layer in closer arrangement, but without vacuolization phenomenon. However, compared with full spectrum group, the nucleus of ganglion cells was less regular in shape in fluorescent TCM group (Figure 4-6).

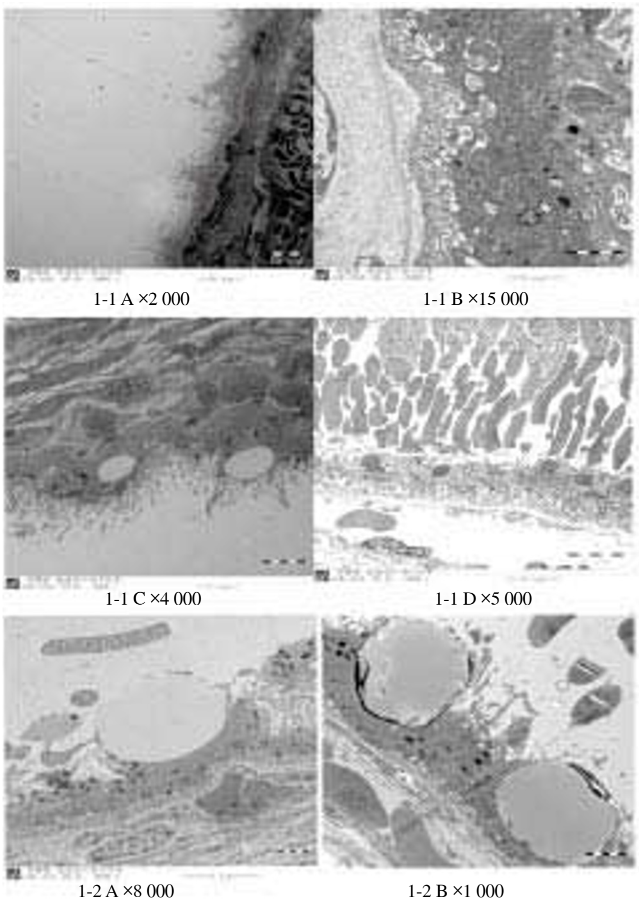

Figure 4. Pigment epithelial cell.
Note: Control group showed single-layer structure in retinal pigment epithelial cells with amounts of detached membranous discs of microvillus absorbing photoreceptor towards the photoreceptor; slightly saturated cytoplasm, in which there was membranous discs of photoreceptor in mitochondria and during the swallowing and digestion process (1-1 A, 1-1B), and small lipid granules occasionally (1-1 C); and larger nucleus in flat round shape (1-1 D). Fluorescent group showed normal nucleus and organelles in retinal pigment epithelium cells, but with large amounts of lipid granules in cytoplasm over the medial surface of epithelial cells (1-2 A), which damaged the cell membrane and superficial microvillus structure in medial surface (1-2 B).

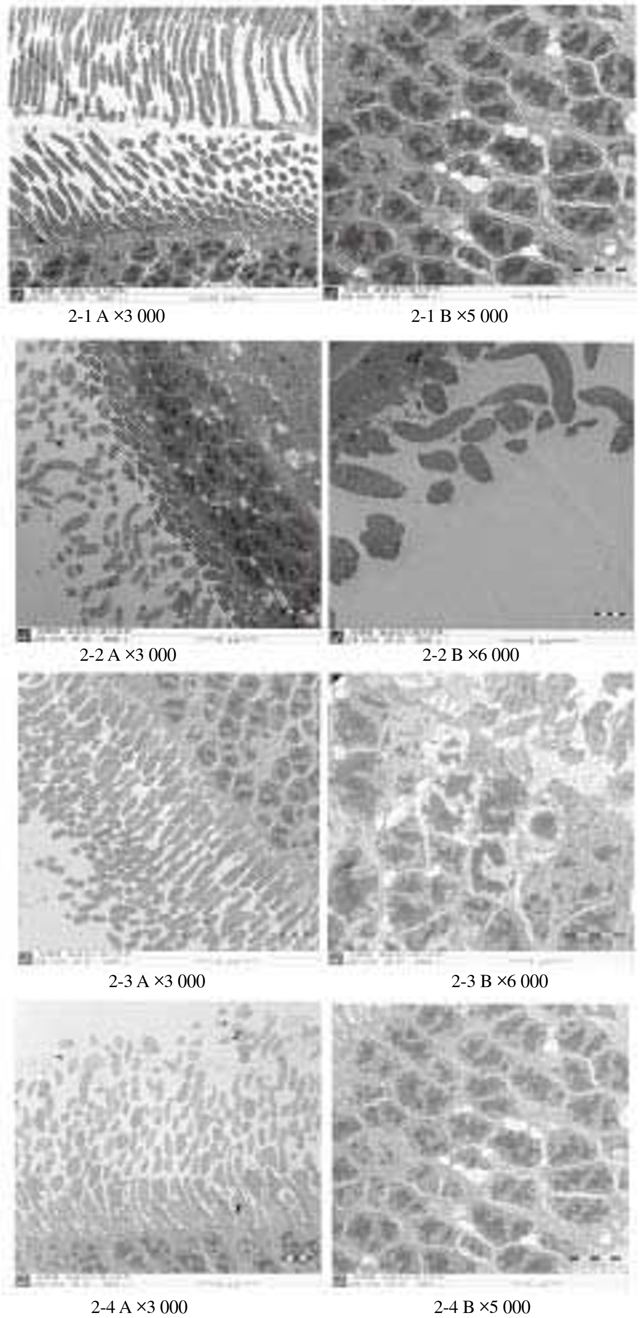

Figure 5. Photoreceptor cell.
Note: In control group (2-1 A), fluorescent TCM group (2-3 A) and full spectrum TCM group (2-4 A), the interior and exterior segments of retinal photoreceptor in rabbits were clear in construction and orderly in arrangement. In control group (2-1 B) and full spectrum TCM group (2-4 B)the nucleus of outer nuclear layer regular in shape and even in staining, whereas in fluorescent TCM group (2-3 B), the nucleus of outer layer was concentrated in chromatin and shrinking in nucleus membrane. In fluorescent group, the exterior segment of retinal photoreceptor was disorderly in arrangement and large in gap (2-2 A) with limited rupture and detachment, ad partial external membranous disc showed “insect-biting phenomenon” (2-2 B).

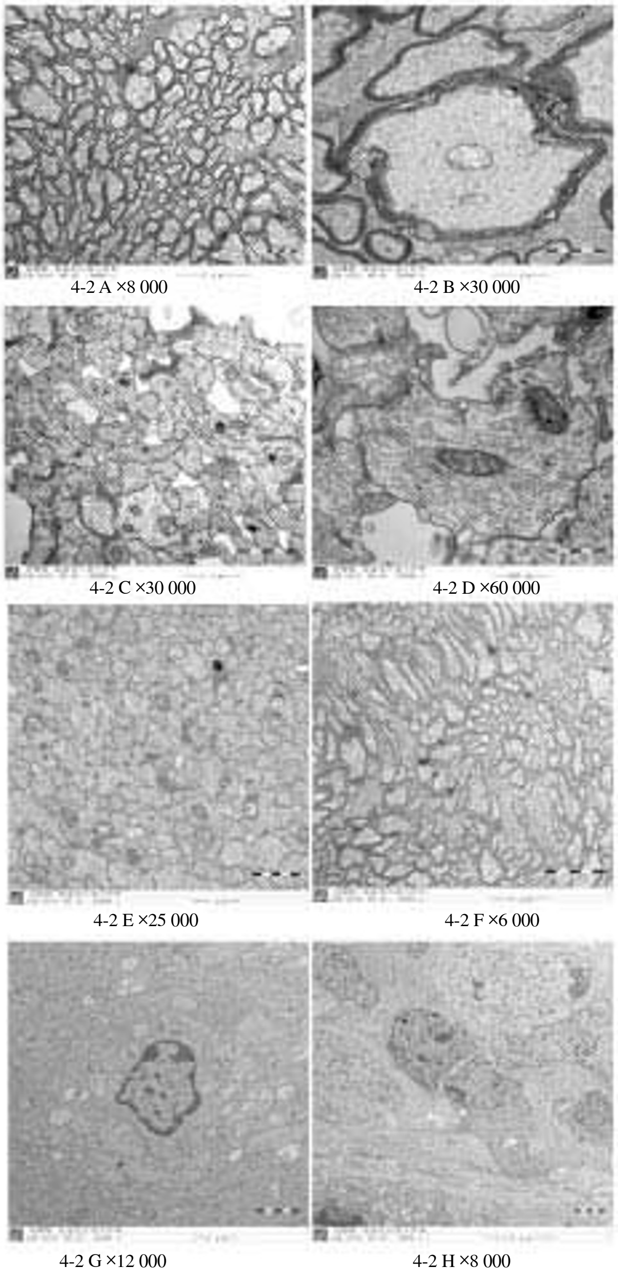

Figure 6. Nerve fiber layer.

Note: The nerve fiber layer arranged closely without vacuole in control group (4-2 A), fluorescent TCM group (4-2 E) and full spectrum TCM group (4-2 F). The nucleus showed regular elliptical shape in control group (4-2 B) and full spectrum TCM group (4-2 G), whereas the nucleus was less regular in shape in fluorescent TCM group (4-2 H). In fluorescent group, nerve fiber layer was surrounded by extensive vacuole (4-2 C) with irregular-shape nucleus (4-2 D).

4. Discussion

Epidemiological studies in multiple regions (America, Singapore, Australia and China, etc) revealed that the outdoor activity time was shorter in infants with myopia than those with normal eyesight, predicating that there was significant connection between spectrum environment and the morbidity of myopia[7]. Researchers[8,9] also discovered that people with long-term outdoor activity had obviously lower rate of myopia, indicating that the natural light might have a protective function on eyes instead of physical sports, and 2-3 h outdoor activity daily combined with exposure of natural light could inhibit the progression of axial lengths.

As early as 2006, this research group had conducted the investigation on the light pollution harm as well as the prevention and treatment in municipal environment, which verified through animal experiments that long-term exposure in artificial luminous environment had certain influence on the visual performance of rats, and the results demonstrated that compared with control group, artificial light group had lower electro-physiological amplitude of visual function and the retina was atrophied and thickened with unclear layers. Meanwhile, this research group surveyed the people in Ili Kazakh in Sinkiang, showing that though the diet and national habit of residents were similar in the city and grazing district of Ili Kazakh, the number of people with uncorrected visual acuity (UCVA) >1.0 was less in city than in the grazing district due to the long-term activity in artificial light in the city of Ili Kazakh[10]. In 2008, this group performed a study on the influence of artificial luminous environment and the intervention of TCM on the biological rhythm, proving that the artificial illumination could reduce the retinal function, change the microstructure of retinal cells and decrease the Cry2 protein expression that could be recovered to normal level by regulating day rhythm with TCM intervention in the retina of rats[11]. In 2011, a study on the influence of circadian rhythm and TCM intervention on the eyeball growth of rats was performed, and the results suggested that biological rhythm change due to luminous environment change could lengthen the eye axis in developmental period, for which the TCM intervention based on “Theory of yin-yang clock” had certain inhibition on the abnormal growth of eye axis[12]. Accordingly, Professor Yuliang Wang proposed the conception of “Yinyang Rizhong regulation method” to prevent myopia, which theory was derived from the “TCM endocrinic rhythm regulation method” under the guidance of the “Theory of yin-yang clock” created by Professor Guicheng Xia and Professor Yong Tan. The waxing and waning of yin and yang in human body has synchronous day rhythm with the alternation of day and night, which will alternate the waxing and waning following the alternation of twelve hours, such as disorder of day rhythm and imbalance of Yinyang , thus triggering various diseases. Based on the theory of “relevant adaptation of the human body to natural environment”, Professor Yuliang Wang put forward the “Yin-yang day rhythm regulation method” aiming to administrate the corresponding formula at corresponding time in accordance with the difference of yin-yang property in day rhythm and complying with the time phase rule of midnight-midday ebb and flow, in which the theory of “tonifying yin to invigorate yang, which means to nourish blood at yin time and supplement qi at yang time so as to smooth qi and blood and harmonize the activities of Yinyang ” proposed by professor Guicheng Xia was applied to correct the disorders caused by arrhythmia, then providing new thoughts for the prevention and treatment of myopia in clinic.

Multiple works pointed out that the development of eye axis was in close association with DA level, and biochemical and pharmacological research results indicated that retinal DA level of experimental myopia was lower than that of normal eyes, showing that there was osculating correlation between DA and the progression of axial lengths of eyes[13]. Studies discovered that exogenous melatonine could change the anterior chamber depth of deprivation myopia and control eyes, volume of vitreum cavity and thickness of choroid via acting on corresponding receptors, thus participating in the diurnal rhythm of eyeball growth[14]. However, DA is the mediator of the expression of light in the integral function of retinal melatonine, whose system can regulate the biological rhythm along with melatonine in eyes[15].

This study deeply observed the influence of different spectrums on the eyeball growth of rabbits to explore the action points of “Yinyang rizhong regulation method” proposed by Professor Yuliang Wang on the axial length of rabbit eyes in the developmental period, to observe the growth of rabbit eyes in different luminous environments and to explore its mechanism using retinal and serum DA levels as the core index. The results of this study demonstrated that the retinal and serum DA levels in fluorescent group were markedly lower in control group and full spectrum group, which had no significant decrease after TCM intervention; and the histopathological observation showed that there was significant difference between fluorescent group and control group in pigment epithelium layer, photoreceptor and nerve fiber layer, but no significant difference was found among the rest groups, indicating that the fluorescent light had certain influence on retinal microstructure and visual performance while TCM intervention might have some protective function on retina, which still need large-sample data to be further studied and confirmed.

Conflict of interest statement

We declare that we have no conflict of interest.

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