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Effects of *Evodia rutaecarpa* Acupoint Sticking Therapy on Rats with Insomnia Induced by Para-Chlorophenylalanine in 5-$HT_{1A}$and 5-HT_{2A} Gene Expressions

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HIGHLIGHTS

- *E. rutaecarpa* acupoint sticking therapy can improve insomnia.
- Significantly upregulated 5-HT_{1A} mRNA expression and downregulated 5-HT_{2A} mRNA expression.

Abstract: The main feature of insomnia is difficulty in starting or maintaining sleep. 5-HT_{1A} receptor and 5-HT_{2A} receptor are two subtypes of the classic central 5-HT neurotransmitter closely related to sleep and wakefulness. To observe the effects of *Evodia rutaecarpa* acupoint sticking therapy on the mRNA expressions of 5-HT_{1A} and 5-HT_{2A} in the hypothalamus, brainstem, and hippocampus of insomnia rats induced by para-chlorophenylalanine (PCPA). Ten rats were randomly selected as the normal group, and 80 PCPA insomnia model rats were randomly divided into eight groups, including a model group, a positive control group (diazepam group), a low-dose *E. rutaecarpa* acupoint sticking therapy group (Yongquan acupoint group and Shenque acupoint group; the same applied below), a middle-dose *E. rutaecarpa* acupoint sticking therapy group, and a high-dose *E. rutaecarpa* acupoint sticking therapy group. The normal group and the model group did not receive treatment. The positive control group was given diazepam through intragastric administration, and the three-dose *E. rutaecarpa* acupoint sticking therapy groups were divided into the Yongquan acupoint group and the Shenque acupoint group. After seven days of administration, the rat hypothalamus, brainstem, and hippocampus tissues were taken, and a real-time polymerase chain

reaction method was used to detect the mRNA expressions of 5-HT_{1A} and 5-HT_{2A}. *E.rutaecarpa* acupoint sticking therapy significantly upregulated 5-HT_{1A} mRNA expression and downregulated 5-HT_{2A} mRNA expression in rats with insomnia caused by PCPA. No significant differences were found in the expression between the two acupoints and the expression among the three brain tissues. *E. rutaecarpa* acupoint sticking therapy can improve insomnia. The mechanism may be related to the upregulation of 5-HT_{1A} mRNA expression and the downregulation of 5-HT_{2A} mRNA expression in brain tissue.

Keywords: Evodia rutaecarpa; acupoint sticking; anti-insomnia; serotonin 1 A gene; serotonin 2A gene.

INTRODUCTION

Insomnia is one of the diseases with a high incidence in today's society, and it is also one of the clinical symptoms that often coexist with others. Good sleep can play an important role in the recovery of brain and body functions. The structures related to the control of arousal in the central nervous system are mainly located in the hypothalamus, brainstem, and hippocampus, among others [1]. Serotonin (5-HT, 5-hydroxytryptamine) is important in the regulation of sleep and is one of the recognized mechanisms of insomnia.

However, the specific conditions of the neurotransmitter receptor subtypes and related signal transduction in the 5-HT sleep regulation mechanism are not completely clear, and relatively few studies have been conducted about them. The most notable of these receptors are $5HT_{1A}$ and $5HT_{2A}$. Owing to their high density in the brain and important role in the nervous system, these receptors have been widely used to explain the pathogenesis of neurological diseases and the development of antidepressants and anti-anxiety drugs [2].

Studies have shown that 5-HT_{1A} receptors are distributed in high density in the hippocampus and that the activation of this receptor can relieve anxiety symptoms. Generally, low-dose 5-HT_{1A} receptor agonists can increase deep sleep and light sleep, and stimulated 5-HT_{2A} receptors can inhibit diffuse wave sleep and cause increased wakefulness [2-4].

The pathogenesis of insomnia is very complicated. Currently, the treatment for insomnia is still based on drug intervention. The most widely used drugs in clinical practice are benzodiazepine sedatives and hypnotics, which can effectively relieve insomnia symptoms and have sedative, hypnotic, and anti-anxiety effects. However, long-term use of these drugs can cause serious adverse reactions, such as central nervous system depression, drug resistance, withdrawal symptoms, addiction, and even chronic liver damage.

Traditional Chinese medicine has the good effects of nourishing the mind and soothing the nerves, with slight side effects and stable curative effects. Therefore, its use is recognized by the majority of insomnia patients [5–7]. Traditional Chinese medicine *Evodia rutaecarpa* is a commonly used acupoint drug in clinical practice. *E. rutaecarpa* can promote communication between the heart and kidneys and conduct a downward flow of heat. In clinical practice, vinegar is often used to prepare *E.rutaecarpa* powder and is applied to Yongquan acupoints, Shenque acupoints, and other acupoints to treat insomnia. It has achieved good results [8–9].

In this study, we observed the effects of vinegar blended with *E.rutaecarpa* powder on the Yongquan acupoints and Shenque acupoints in related neurotransmitters in insomnia rat models induced by PCPA and explored the mechanism of *E.rutaecarpa* in the treatment of insomnia to provide a basis for optimizing the clinical medication regimen for insomnia treatment.

MATERIAL AND METHODS

Experimental animals

Male Sprague-Dawley (SD) rats (weighing 200 ± 20 g) were purchased from the Experimental Animal Center of the Air Force Military Medical University (animal production license number: SCXK (Army) 2012-0007). The animals were fed a standard diet and water and maintained under the following conditions: room temperature 20 ℃–25 ℃, indoor lighting according to daylight cycle mode, feed timing, free drinking, and adaptive feeding for 15 days.

Instruments and reagents

P-Chlorophenyl alanine (PCPA) was purchased from Alfa Aesar (USA; batch number: H30Y014), Trizol from Invitrogen, and DEPC-treated water from Wuxi Bohe Biomedical Technology Co., Ltd. Chloroform/isopropanol/absolute ethanol was obtained from Shanghai Sinopharm (Shanghai, China). The SYBR Green polymerase chain reaction (PCR) kit (Thermo F-415XL) and the Reverse Transcription Kit (Thermo #K1622) were obtained from Thermo. Diazepam tablets (National Medicine Standard H37023039) were purchased from Shandong Xinyi Pharmaceutical Co., Ltd. (Shandong, China). E. rutaecarpa was obtained from Anhui Hui Rentang Traditional Chinese Medicine Decoction Pieces Co., Ltd. (Anhui, China). Vinegar was purchased from Shaanxi Qishan Tianyuan Food Co., Ltd. (Tianyuan, China). Sterile water for injection was purchased from Huaren Pharmaceutical Co., Ltd. (Shandong, China, Batch number 161221R1). Saline was obtained from Huaren Pharmaceutical Co., Ltd. (Shandong, China, Batch number 161221R1).

Drug preparation

E. *rutaecarpa* powder was pulverized into a fine powder (80 mesh), added with rice vinegar, stirred into a paste (approximately 1.5 g : 1 mL), placed in a sealed glass bottle, and stored in a refrigerator at 4°C for application.

Modeling and grouping

After seven days of adaptive feeding, male SD insomnia rats were induced by the PCPA method [2,10–11]. PCPA powder was prepared into a suspension with weak alkaline saline (pH 7–8) and injected intraperitoneally at a dose of 0.3 g/kg (1 mL/100 g dose) once a day at 8 a.m. for four consecutive days. The rats in the normal group were injected with the same volume of weak alkaline saline. The circadian rhythm disappeared in the rats, indicating that the model was successfully replicated. However, the rats without PCPA were normal.

A total of 80 successfully modeled rats were randomly divided into a model group, a positive control group (diazepam group), a small-dose *E.rutaecarpa* acupoint sticking therapy group (Y-L for Yongquan acupoint and S-L for Shenque acupoint), a medium-dose *E. rutaecarpa* acupoint sticking therapy group (Y-M for Yongquan acupoint and S-M for Shenque acupoint), and a high-dose *E.rutaecarpa* acupoint sticking therapy group (Y-H for Yongquan acupoint and S-H for Shenque acupoint). Each group included 10 rats. The normal control group consisted of 10 rats without PCPA treatment.

Real-time PCR

Real-time PCR was used to detect 5-HT_{1A} and 5-HT_{2A} mRNA expressions in the rat hypothalamus, brainstem, and hippocampus. 5-HT_{1A} and 5-HT_{2A} gene primers and GAPDH internal reference gene primers were described in Table 1. The reaction system was carried out according to the product manual. The amplification conditions were 94℃ for 10 min (94℃ for 20 s, 55℃ for 20 s, 72℃ for 20 s) for 40 cycles. After PCR amplification, real-time fluorescent quantitative PCR automatically analyzed the cycle threshold (Ct) value. The $2^{-\Delta\Delta Ct}$ method was used to analyze the expression difference of the target gene between the control group and the different concentration groups. The calculation formula is as follows: △Ct = Ct target gene-Ct internal reference. The average value of the control group △Ct is expressed as △Ct control average. The △Ct control average is subtracted from the △Ct of each group to obtain the △△Ct value: △△Ct = △Ct sample-△Ct control average. Then, the $2^{-\Delta\Delta Ct}$ value of each group, which is the relative expression of genes in each group, was calculated.

Table 1. 5-HT_{1A}, 5-HT_{2A} and GAPDH primer list.

Primer	Upstream	Downstream	Annealing temperature	Amplification length
5-HT_{1A}	CGGCTACACCATCTACTCCACTTTC	CTGGCTGTCCGTTCAGGCTCTTC	55℃	72℃
5-HT_{2A}	CTTCCAACGGTCCATCCACAGA	GGGCACCACATTACAACAAACAGG	55℃	72℃
GAPDH	CCATCACTGCCACTCAGAAGA	ATACATTGGGGGTAGGAACAC	55℃	72℃

Statistical methods

Statistical analysis was performed using SPSS 17.0. The experimental data were expressed as the mean ± standard deviation. The comparison of measurement data between groups was performed using one-way analysis of variance. $P < 0.05$ indicates a difference and statistical significance; $P < 0.01$ indicates a significant difference.

RESULTS

Effect of E. *rutaecarpa* acupoint sticking therapy on 5-HT_{1A} mRNA expression in the hypothalamus, brainstem, and hippocampus of insomnia rats

Relative expression of 5-HT_{1A} mRNA in the hypothalamus

Unlike in the normal group, the 5-HT_{1A} mRNA expressions of rats in the model group, Y-L group, S-L group, Y-M group and S-M group decreased significantly ($P < 0.01$). The 5-HT_{1A} mRNA expression of rats in the S-H group decreased ($P < 0.05$).

Unlike in the model group, the 5-HT_{1A} mRNA expressions of rats in the diazepam group, Y-H group and S-H group increased significantly ($P < 0.01$). The 5-HT_{1A} mRNA expression of rats in the Y-M group and S-M group increased ($P < 0.05$).

Compared with the diazepam group, the Y-L group and S-L group had no significant effect on the increase in 5-HT_{1A} mRNA expression in rats ($P < 0.01$). The Y-M group and S-M group increased the expression of 5-HT_{1A} mRNA in rats ($P < 0.05$), the Y-H group and S-H group had no significant increase in the expression of 5-HT_{1A} mRNA in rats. No significant difference was found in the expression between the two acupoints.

Relative expression of brainstem 5-HT_{1A} mRNA

Unlike in the normal group, the 5-HT_{1A} mRNA expressions of rats in the model group, Y-L group, S-L group, Y-M group, and S-M group decreased significantly ($P < 0.01$). The 5-HT_{1A} mRNA expression of rats in the Y-H group and S-H group decreased ($P < 0.05$).

Unlike in the model group, the 5-HT_{1A} mRNA expressions of rats in the diazepam group, Y-M group, S-M group, Y-H group, and S-H group significantly increased ($P < 0.01$). The 5-HT_{1A} mRNA expression of rats in the S-L group increased ($P < 0.05$).

Compared with the diazepam group, Y-L group, S-L group and S-M group had no significant effect on the increase in 5-HT_{1A} mRNA expression in rats ($P < 0.01$). The Y-M group increased the expression of 5-HT_{1A} mRNA in rats ($P < 0.05$), and the Y-H group and S-H group had no significant effect on rats. No significant difference was found in the increasing effect of 5-HT_{1A} mRNA expression in rats. Moreover, no significant difference was observed in the expression between the two acupoints.

Relative expression of hippocampal 5-HT_{1A} mRNA

Unlike in the normal group, the 5-HT_{1A} mRNA expressions of rats in the model group, Y-L group, S-L group, Y-M group, and S-M group significantly decreased ($P < 0.01$). The 5-HT_{1A} mRNA expression of rats in the Y-H group and S-H group also significantly decreased ($P < 0.05$).

Unlike in the model group, the 5-HT_{1A} mRNA expressions of rats in the diazepam group, Y-M group, S-M group, Y-H group, and S-H group significantly increased ($P < 0.01$).

Compared with the diazepam group, the Y-L group, S-L group, Y-M group, and S-M group had no significant increase in 5-HT_{1A} mRNA expression in the rats ($P < 0.01$). No significant difference was found in the increase in 5-HT_{1A} mRNA expression in rats in the Y-H group and S-H group. No significant difference was observed in the expression between the two acupoints.

The above results suggest that *E.rutaecarpa* acupoint application can increase 5-HT_{1A} mRNA expression in the hypothalamus, brainstem, and hippocampus of insomnia rats and that there is no significant difference between the high-dose group and the diazepam group. This indicates that the *E.rutaecarpa* acupoint application can play a role in the treatment of insomnia. The relative expression of 5-HT_{1A} mRNA and the comparison among the groups are shown in Table 2.

Table 2. Relative expression of 5-HT1A mRNA in hypothalamus, brainstem and hippocampus (mean ± SEM, n=10).

Group	Hypothalamus 5-HT$_{1A}$ mRNA Hypothalamus	Brainstem 5-HT$_{1A}$ mRNA Brainstem	Hippocampal 5-HT$_{1A}$ mRNA Hippocampus
Normal	1.00305±0.00895	1.00276±0.00806	1.00042±0.00125
Model	0.24361±0.01495**	0.30147±0.00125**	0.20615±0.00129**
Diazepam	0.85956±0.01338$^{\#\#}$	0.85193±0.00327$^{\#\#}$	0.93938±0.00578$^{\#\#}$
WZYL-YQ	0.39538±0.00449$^{**\&\&}$	0.36389±0.01335$^{**\&\&}$	0.26593±0.00758$^{**\&\&}$
WZYL-SQ	0.34277±0.01651$^{**\&\&}$	0.41027±0.00265$^{**\#\&\&}$	0.30385±0.00550$^{**\&\&}$
WZYM-YQ	0.49440±0.00657$^{**\#\&}$	0.62073±0.00437$^{**\#\#\&}$	0.45215±0.00212$^{**\#\#\&\&}$
WZYM-SQ	0.54645±0.00093$^{**\#\&}$	0.56502±0.00830$^{**\#\#\&\&}$	0.51989±0.00337$^{**\#\#\&\&}$
WZYH-YQ	0.78779±0.01175$^{\#\#}$	0.75372±0.01021$^{*\#\#}$	0.81191±0.00382$^{*\#\#}$
WZYH-SQ	0.71350±0.00658$^{*\#\#}$	0.76542±0.00515$^{*\#\#}$	0.75196±0.01049$^{*\#\#}$

Note: Compared with the normal group, *P<0.05, **P<0.01; compared with the model group, # P<0.05, ## P<0.01; compared with the diazepam group, &P<0.05, &&P<0.01.

Effect of E.*rutaecarpa* acupoint sticking therapy on 5-HT$_{2A}$ mRNA expression in the hypothalamus, brainstem, and hippocampus of insomnia rats

Relative expression of 5-HT$_{2A}$ mRNA in the hypothalamus

Compared with the normal group, the model group, Y-L group, S-L group, Y-M group, S-M group, and S-H group had a significantly increased expression of 5-HT$_{2A}$ mRNA in rats ($P < 0.01$). The expression of 5-HT$_{2A}$ mRNA in rats in the Y-H group increased ($P < 0.05$).

Unlike in the model group, the 5-HT$_{2A}$ mRNA expression in rats in the diazepam group, the Y-M group, S-M group, Y-H group, and S-H group significantly decreased ($P < 0.01$). The expression of 5-HT$_{2A}$ mRNA in rats in the Y-L group and S-L group decreased ($P < 0.05$).

Compared with the diazepam group, the Y-L group, S-L group and Y-M group had no significant effect on reducing 5-HT$_{2A}$ mRNA expression in rats ($P < 0.01$). The S-M group had a decreased expression of 5-HT$_{2A}$ mRNA in rats ($P < 0.05$), and the Y-H group, S-H group had no significant decrease in HT$_{2A}$ mRNA expression. No significant difference was found in the expression between the two acupoints.

Relative expression of 5-HT$_{2A}$ mRNA in the brainstem

Compared with the normal group, the model group, the Y-L group, S-L group, Y-M group, S-M group, Y-H group, and S-H group had a significantly increased expression of 5-HT$_{2A}$ mRNA in rats ($P < 0.01$). Unlike in the normal group, the expression of 5-HT$_{2A}$ mRNA in rats in the diazepam group increased ($P < 0.05$).

Compared with the model group, the diazepam group, the Y-M group, S-M group, Y-H group, and S-H group had a significantly decreased 5-HT$_{2A}$ mRNA expression in rats ($P < 0.01$).

Compared with the diazepam group, the Y-L group, S-L group, and Y-M group had no significant effect on decreasing the 5-HT$_{2A}$ mRNA expression in rats ($P < 0.01$). The S-M group had a decreased expression of 5-HT$_{2A}$ mRNA in rats ($P < 0.05$), and the Y-H group and S-H group had no significant decrease in HT$_{2A}$ mRNA expression in rats. No significant difference was found in the expression between the two acupoints.

Relative expression of 5-HT$_{2A}$ mRNA in the hippocampal

Compared with the normal group, the model group, the Y-L group, S-L group, Y-M group, S-M group, Y-H group, and S-H group had a significantly increased expression of 5-HT$_{2A}$ mRNA in rats ($P < 0.01$).

Compared with the model group, the diazepam group, the Y-L group, S-L group, Y-M group, S-M group, Y-H group, and S-H group had a significantly decreased expression of 5-HT$_{2A}$ mRNA in rats ($P < 0.01$).

Compared with the diazepam group, the Y-L group, S-L group, Y-M group, and S-M group had no significant effect on the decrease in 5-HT$_{2A}$ mRNA expression in rats ($P < 0.01$). The Y-H group and S-H

group had a decreased expression of 5-HT_{2A} mRNA in rats ($P < 0.05$). No significant difference was found in the expression between the two acupoints.

The above results suggest that the acupoint application of *E. rutaecarpa* can decrease the 5-HT_{2A} mRNA expression in the hypothalamus, brainstem, and hippocampus of insomnia rats. Moreover, no significant difference was found between the high-dose group (hypothalamus and brainstem) and the diazepam group, indicating that *E. rutaecarpa* acupoint application can play a role in treating insomnia. The relative expression of 5-HT_{2A} mRNA and the comparison among the groups are shown in Table 3.

Table 3. Relative expression of 5-HT_{2A} mRNA in hypothalamus, brainstem and hippocampus (mean ± SEM, n=10).

Group	Hypothalamus 5-HT_{2A} mRNA Hypothalamus	Brainstem 5-HT_{2A} mRNA Brainstem	Hippocampus 5-HT_{2A} mRNA Hippocampus
Normal	1.00472±0.01356	1.00423±0.01296	1.00293±0.00856
Model	2.20533±0.01014**	2.19565±0.01343**	2.40645±0.00284**
Diazepam	1.19862±0.01457$^{\#\#}$	1.34710±0.01164$^{*\#\#}$	1.16622±0.00530$^{\#\#}$
WZYL-YQ	1.90702±0.01005$^{**\#\&\&}$	2.09627±0.01048$^{**\&\&}$	1.93847±0.01220$^{**\#\#\&\&}$
WZYL-SQ	1.85992±0.01386$^{**\#\&\&}$	2.03950±0.01365$^{**\&\&}$	2.07668±0.00836$^{**\#\#\&\&}$
WZYM-YQ	1.64800±0.00408$^{**\#\#\&\&}$	1.69374±0.00124$^{**\#\#\&\&}$	1.76388±0.01344$^{**\#\#\&\&}$
WZYM-SQ	1.58521±0.00692$^{**\#\#\&}$	1.69147±0.00934$^{**\#\#\&}$	1.71799±0.00444$^{**\#\#\&\&}$
WZYH-YQ	1.29353±0.00453$^{*\#\#}$	1.47376±0.01246$^{**\#\#}$	1.45224±0.00719$^{**\#\#\&}$
WZYH-SQ	1.39649±0.00719$^{**\#\#}$	1.53446±0.00513$^{**\#\#}$	1.37147±0.00961$^{**\#\#\&}$

Note: Compared with the normal group, *P<0.05, **P<0.01; compared with the model group, #P<0.05, ## P<0.01; compared with the diazepam group, &P<0.05, &&P<0.01.

DISCUSSION

As we all know, good sleep is an important sign of health. In Chinese medicine, E.*rutaecarpa* acupoint application is often used to treat insomnia. The commonly used acupoints in clinical practice are mainly Yongquan acupoint and Shenque acupoint [8-9] [12-15]. Previous research has shown that the main chemical components of E.*rutaecarpa* are alkaloids, bitters, volatile oils, flavonoids, and organic acids [16-17]. Several studies have found that evodiaeine and rutaecarpine can exert antidepressant effects by increasing the expression of neurotransmitters in the rat brain and inhibiting the apoptosis of hippocampal nerve cells in mice [18-19]

Rutaecarpine, evodiaeine, limonin, rutin, and dehydro-evodia oleifera are the main analgesic components of evodia [20-21]. The rutaecarpine exerts sedative and anti-anxiety depression effects by regulating the activity of neurotransmitter receptors [22]. Evodia can reduce the excitement caused by coffee and improve sleep disorders [23]. Flavonoids such as quercetin and rutin are active substances that improve PCPA caused insomnia [24-25].

This study used isolated rat skin as a permeation barrier, and through in vitro transdermal absorption experiments, the mass concentration of relevant components in the receiving fluid and the skin is detected by high-performance liquid chromatography. We found that evodiaeine can penetrate through the isolated skin, and the rutaecarpine is retained in the skin. The retention amount of rutaecarpine is more than that of evodiaeine, and the limonin cannot penetrate the isolated skin. According to the results of the isolated skin permeation experiment, the rutaecarpine has a good in vitro transdermal release performance, which provides a piece of evidence for further study of the mechanism of Evodia in the treatment of insomnia [26].

In this study, each rat only sticks to one acupoint, in order to observe and analyze the therapeutic effect and mechanism of the Evodia on different acupoints. Taking into account the limitation of the body surface area of rats, in actual operation, it is impossible to apply the drug to only a certain acupoint, but to apply the drug to a skin area centered on the acupoint. This approach is also the same as that of people using acupoint applications in clinics.

The serotonin synthesis inhibitor p-chlorophenylalanine (PCPA) can cause insomnia by antagonizing the serotonin precursor 5HTP. In this study, the expression of 5-HT_{1A} and 5-HT_{2A} genes in the hypothalamus,

brainstem, and hippocampus of the model group of rats showed a significant decrease or increase. After intraperitoneal injection of PCPA, rats have successively shortened their sleep time, easily awakened, irritable, the struggling amplitude becomes smaller or no longer struggling during grasping, and the amount of eating and activity is markedly reduced, indicating that the insomnia model was successfully generated. That is to say, combining the neurotransmitter expression level with the external physical signs of rats can be more accurately judged whether the PCPA insomnia model has been successfully prepared.

5-HT in the brain has a wide range of functions, mainly involved in the regulation of sleep-wake cycles, pain, and mental and emotional activities. Due to their high density and important role in brain tissue, $5HT_{1A}$ and $5HT_{2A}$ receptors are widely used in the research of anti-insomnia drugs. E.*rutaecarpa* acupoint application can increase the expression level of 5-HT_{1A} mRNA in rat brain tissue, and the expression level is positively correlated with the dosage of application. E.*rutaecarpa* acupoint application can reduce the expression level of 5-HT_{2A} mRNA in rat brain tissue, and the expression level is negatively correlated with the dosage of application. This is consistent with the current research results of the two receptor subtypes 5-HT_{1A} and 5-HT_{2A} in the treatment of insomnia.

Based on the 5-HT_{1A} mRNA and 5-HT_{2A} mRNA expression levels in the brain in this study, there is no significant difference between the two acupoints and in the three brain tissues in the expression level. This suggests that up-regulation of the expression levels of 5-HT_{1A} mRNA in the brain, and down-regulation of the expression level of 5-HT_{2A} mRNA in the brain (mainly the hypothalamus and brainstem) is also one of the mechanisms of E.*rutaecarpa* acupoint application in the treatment of insomnia, fully demonstrating the scientific nature of the clinical use of acupoint applications to treat insomnia using Chinese medicine.

The research results also indicate that the anti-insomnia effect of the high-dose Evodia acupoint application group is similar to that of the diazepam group, which verifies the good clinical value and scientific value of the traditional treatment technology of Evodia acupoint application for insomnia from an experimental point of view. This research will open up a new research direction for finding ideal anti-insomnia drugs that are convenient to use, have few adverse reactions, and have better curative effects.

CONCLUSION

E.*rutaecarpa* acupoint application had a good therapeutic effect on insomnia induced by PCPA in rat models. The effect was equivalent to diazepam, and there was no significant difference. According to the results of this study, its mechanism of action may be related to the up-regulation of 5-HT_{1A} mRNA expression levels in the hypothalamus, brainstem, and hippocampus and the down-regulation of 5-HT_{2A} mRNA expression levels in the hypothalamus and brainstem, showing good clinical application value and scientific research potential.

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