The Effect of Hypothalamus-Pituitary-Adrenal Axis Treatment by 10.6 *μm* Laser Moxibustion in Kidney Yang Deficiency Syndrome Model

I Dewa Ayu Agra Darmawati[1][a], Pan Wen[2][b], Zhao Ling[2][c], Ida Ayu Putri Wulandari[3][d] and I Ni Kadek Neza Dwiyanti[4][e]

[1]*Applied Bachelor of Acupuncture and Herbal Medicine, Faculty of Health, Institute of Technology and Health Bali, Indonesia*

[2]*School of Acupuncture-Moxibustion and Tuina, Shanghai University of Traditional Chinese Medicine, China*

[3]*Bachelor of Nursing Program, Faculty of Health, Institute of Technology and Health Bali, Indonesia*

[4]*Bachelor of Midwifery Program, Faculty of Health, Institute of Technology and Health Bali, Indonesia*

Keywords: Kidney Yang Deficiency Syndrome (KYDS), Hydrocortisone, HPA Axis, Laser Moxibustion.

Abstract: The damage and functional disorders of different degrees of the hypothalamic-pituitary-target gland axes (adrenal, thyroid, and gonad) are the mechanisms of kidney yang deficiency syndrome. This study aims to assess the effects of 10.6 μm laser moxibustion on rats with kidney-yang deficiency syndrome. The 40 SD rats were randomly divided into a normal group, model group, Sham laser moxibustion (SLM) group, and Laser moxibustion (LM) group. All rats in model KYDS were induced with a high dose of hydrocortisone (25mg/kg.BW) for 15 days to mimic the model. The LM group obtained infrared laser moxibustion irradiation on points GV 4 (Mingmen) and CV4 (Guanyuan) for 10 minutes at each point. The rats in the model showed a reduction of 17-OHCS, CRH, ACTH, and CORT concentration compared with the normal group ($P< 0.01$). These Indicated models have been successfully developed. After the intervention, the biochemistry of the HPA axis indicators in the LM group had shown to enhance ACTH (22.59 vs 18.69), CRH (21.83 vs 18.01), CORT (5.04 vs 4.23), and 17-OHCS (6.81 vs 4.14) all with $P<0.01$ compared with the model group. The 10.6 μm infrared laser moxibustion is effective for the treatment of KYDS by increasing the HPA Axis.

1 INTRODUCTION

According to Traditional Chinese Medicine, kidney yang deficiency is a type of deficient cold pattern which causes kidney yang deficiency to lead to an abnormality in warming and transformation qi Jia-Xu (2011). Studies have shown the damages and functional disorders of different degrees of the hypothalamic-pituitary-target gland axes (adrenal, thyroid, and gonad) are the mechanism for forming "kidney yang deficiency syndrome" (Chen, Wang, Zhan, Zhang, & Wang, 2019; Nan et al., 2016; Reheman et al., 2019; Tan et al., 2014; C. M. Wang et al., 2012; L. Zhao et al., 2013; Zhou et al., 2016).

Based on a certain previous study, the classic method to mimic the syndrome of kidney yang deficiency by injecting a high dose of exogenous glucocorticoid (e.g. Hydrocortisone)(C. M. Wang et al., 2012). Hydrocortisone is a naturally occurring corticosteroid that is produced by the adrenal cortex

The mechanism of moxibustion on the stimulating point is to provide thermal stimulation by burning the herb on the point itself (Jinfeng, Xinjun, Xiaojing, & Zhi, 2016).The research on the effects of moxibustion in KYDS has been limited to English-language articles. Because of the high risk of bias and low reporting quality of these studies, it has been difficult

[a] https://orcid.org/0000-0002-1940-270X
[b] https://orcid.org/0000-0002-2411-0314
[c] https://orcid.org/0000-0002-4017-8167
[d] https://orcid.org/0000-0002-1599-8884
[e] https://orcid.org/0000-0002-0715-2811

Darmawati, I., Wen, P., Ling, Z., Wulandari, I. and Dwiyanti, I.
The Effect of Hypothalamus-Pituitary-Adrenal Axis Treatment by 10.6 μM Laser Moxibustion in Kidney Yang Deficiency Syndrome Model.
DOI: 10.5220/0012016700003576
In *Proceedings of the 2nd Bali Biennial International Conference on Health Sciences (Bali BICHS 2022)*, pages 101-108
ISBN: 978-989-758-625-5

to determine whether moxibustion is an effective and safe treatment for patients with KYDS.

The human body is a biological heater that delivers infrared rays. Infrared rays were transformed into internal by absorbed the objects and penetrate deep into the tissues (Shen et al., 2006). The mechanism of moxibustion on stimulating point is provided thermal stimulation by burning the herb on the points itself. Moxibustion is a therapy in which thermal stimulation is applied to the surface of human skin to activate the thermosensitive TRPV channels. That plays a crucial role in the initial part of the primary afferent nerves, and may also be the effectors of moxibustion (Jinfeng et al., 2016).

Infrared laser moxibustion represents a novel non-invasive and painless technology. Recent research has indicated that moxibustion's specific wavelengths of infrared radiation are as potent as generated thermal radiation. The 10.6 μm infrared laser applied in our research mimics the effects and avoids the shortcomings of traditional moxibustion treatment, such as smoke, unpleasant smell, and difficulty in controlling the dosage (Mao et al., 2016). The study aims to assess the effects of 10.6 μm laser moxibustion on rats with kidney-yang deficiency syndrome.

2 MATERIAL AND METHOD

2.1 Animals Preparation and Groups

A total of 40 males Sprague-Dawley rats (220 ± 20g) were supplied by the Laboratory Animal Center of Shanghai University of Traditional Chinese Medicine. The rats used in this research were housed in a cage and provided with certified standard rat chow and tap water freely available. The room temperature and humidity were regulated at 20 ± 2º C and 60% ± 10% respectively. A 12/12 hour light-dark cycle was set, with the lights on at 8 a.m. After one week of acclimatization in the new environment to prior the experimentation.

Rats were randomly divided into five groups: Normal, model, SLM, and LM. 10 rats in each group by random sampling method. The research protocol was approved by the Laboratory Animal Welfare and Ethics Committee of Shanghai University of Traditional Chinese Medicine with the ethical number is PZSHUTCM190322001.

2.2 Reagents and Instruments

Hydrocortisone injection was purchased from Tianjin Jinyao Pharmaceutical Co., Ltd with National Medicine Standard H12020886, batch number: 1811101 (China). GFDH pill was purchased from Zhongjing Henan Wanxi Pharmaceutical Co., Ltd, approval number: Z41021898. The Enzyme-Linked Immunosorbent Assay kits for ACTH (ZC-36479), CORT (ZC-37082), CRH (ZC-36478), and 17-OHCS (ZC-35937) were provided by Shanghai ZCIBIO Technology Co., Ltd (Shanghai, China).

The treatment was conducted using SX10-C1 laser moxibustion devices (Shanghai Wonderful Opto-Electrics Tech Co Ltd, Shanghai, China) with the following parameters: wavelength 10.6 μm; power output 85 mV; 10 min/ each point; the diameter of spot size is 2 mm. The irradiation was conducted at 2 cm away from the skin surface.

2.3 Modeling Procedure and Treatment

The normal group has been obtained the administration of NaCl (25 mg/kg of BW) by intramuscular injection. However, the rats of model, SLM, LM, and GFDH group induced by injecting intramuscular hydrocortisone at a dose of 25 mg/kg of body weight into a hind limb once daily (08.00-10.00 a.m.) in turn another side respectively for 15 days.

The normal and model group raised as normal without any treatment. The SLM and LM group obtained treatment with 10.6 μm laser moxibustion. The procedures were identical in the SLM, except that the laser output was switched off. The laser probes aligned on GV 4 and CV 4 acupoints and irradiated each acupoint for 10 minutes. Each rat received the treatment every other day, with a total of 10 sessions of treatments.

Moreover, food and water intakes still reported every day. In the last treatment session, all rats have to collect 24 h urine 17-OCHS and ANY-Maze open field. The procedure carried out the same as after mimicked the model. All rats were sacrificed to take blood and organs.

Samples of 24 h period urine were collected on day 15 of hydrocortisone injection and day 20 after treatment. The urine was centrifuged at 4º, 2500rpm for 10 min to remove residues. Afterward, the supernatants were collected. The blood was collected from abdominal aorta into heparinized tubes and immediately centrifuged at 3000rpm for 15 min. The plasma was transferred into a clean tube. All samples were stored at −80ºC until biochemical analysis. The specimen should be slowly equilibrated to room temperature before use, and should not be heated to dissolve. The hemolysis of the specimen will affect the final test results, thus this test should not be

performed on hemolyzed specimens. The level of ACTH, CRH, and CORT was measured using ELISA kits.

2.4 Behavioral Observation

The ANY-Maze open field was used to observation spontaneous rat locomotor activity by using protocols that have been published. The open field is a simple analysis of the general locomotor's ability and anxiety-related emotional behaviours of the rats (Seibenhener & Wooten, 2015). It can plot the animal's track with a simple line or as a heat map. Heat maps would indicate how much time the animal spent in different parts of the apparatus (ANY-maze, 2020). The rats were placed on the box within 100cm x 100cm x 40cm open field apparatus and allowed to move freely. Adjust the indoor light to minimize the shadow in the apparatus. The test duration of rats is 5 min each rat. The infrared camera directly above the open field apparatus to recorded the movement of the rat using the ANY-MAZE software. The apparatus was cleaned by 70% alcohol in between tests

3 STATISTICS

Statistical analyses were performed using SPSS Statistics for Windows (Version 23, IBM corp.). Normally distributed data were expressed as mean value ± standard deviation (SD) and the significant changes of variation between groups after model determined using the independent sample *t-test*. Meanwhile, the evaluation of treatment was analysed by one-way analysis of variance (ANOVA), the least significant difference (LSD) tests were performed for post hoc multiple group comparisons. The one-way non-parametric ANOVA Kruskal-Wallis test was used to analyze the nonnormally distribution of data with the Mann-Whitney test to assess the comparisons between multiple groups. A *p*-value of less than 0.05 was considered significant and the zero *P* value was used in from less than 0.01.

4 RESULTS

4.1 Any-Maze Behavioral Observation

The general behavior or activity changes of rats during the model and treatment have been recorded. The ANY-Maze open field was used to observation spontaneous rat locomotor activity by using protocols that have been published. The open field is a simple analysis of the general locomotor's ability and anxiety-related emotional behaviors of the rats.

In Any-Maze open field, rats in the model presented less movement thus preferred to stay in one zone moreover showed frightened, exhaustion, and increased defecation. It might a presentation of stress because the hydrocortisone induced the adrenal to produce the steroid hormone. Inability to adapt to the new environment when they are separated from the others compared with the normal. While the rats in LM were presented with a desire to identify the new environment with surrounds the area of the Any-Maze field and can recognize the tracks. However, the SLM group's inability to recognize the track plots and less movement (Figure 1).

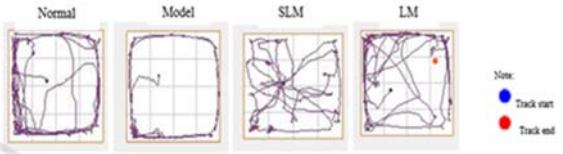

Figure 1: The general locomotor ability of rats by Any-Maze open field analysis.

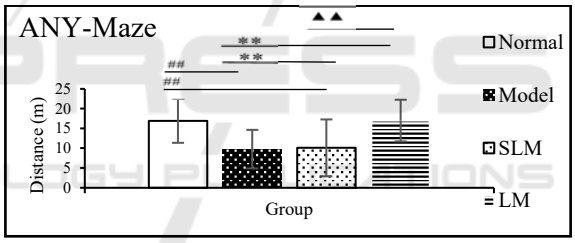

Figure 2: The distance of ANY-Maze open field apparatus in each group. Bars represent means ± SD of 10 rats per group, ## P<0.01, # P<0.05 versus the normal group; ** P<0.01,* P<0.05 versus the model group;▲▲ P<0.01, ▲P<0.05 versus the SLM group.

4.2 The Comparison of the Biochemical Indicator of HPA Axis in Each Group of Rats

The 17-OHCS of the rats in the model group were decreased on day 15 (4.87 vs 7.72 μmol/L; $P < 0.01$) (Table 1). Moreover, the model group has been established successfully from the decreased blood plasma concentration of CRH, ACTH, and CORT with $P < 0.01$ (Table 2). All these results indicated that the KYDS model was successfully established.

Table 1: The 24 h urine 17-OHCS on the 15 days after hydrocortisone withdrawal ($\bar{x} \pm s$).

Group	*n*	17-OHCS (μmol/L)
Normal	10	7.72 ± 1.39
Model	30	4.87 ± 1.81$^{\#\#}$

Note: Compared with the Normal group $^{\#\#}$ *P*<0.01.

The CRH, ACTH, CORT, and 17-OHCS of rats in LM were showed an increase when compared with the model group (P <0.01) (Table 2). On the contrary, in the SLM group was showed a decrease of the ACTH concentration compared with the normal group (20.28 vs 23.40 pg/mL; P<0.01), the 17-OHCS (5.06 vs 7.02) µmol/L, CRH (18.52 vs 21.95) pg/mL, and CORT (4.72 vs 5.50) ng/mL (P< 0.05) when compared with the normal group but had not a significant difference compared with the model group (Table 2).

Table 2: Hormones variation investigation result after the treatment in each group ($\bar{x} \pm s$).

Groups	n	17-OHCS (μmol/L)	CRH (pg/mL)	ACTH pg/mL)	CORT (ng/mL)
N	10	7.02 ± 0.96	21.95 ± 4.44	23.40 ± 2.26	5.50 ± 0.46
M	10	4.14 ± 1.1$^{\#\#}$	18.01 ± 1.91$^{\#\#}$	18.69 ± 1.89$^{\#\#}$	4.23 ± 0.58$^{\#\#}$
SLM	10	5.06 ± 2.45$^{\#}$	18.52 ± 2.51$^{\#}$	20.28 ± 1.74$^{\#\#}$	4.72 ± 0.56$^{\#}$
LM	10	6.81 ± 1.82**	21.83 ± 3.04**	22.59 ± 2.56*	5.04 ± 0.25**$^{\#}$

Note: Normal (N); Model (M); Sham Laser Acupuncture (SLM); Laser Moxibustion (LM). Compared with the normal group (## *P*<0.01, #*P*<0.05), compared with model group (**P*<0.01, * *P*<0.05).

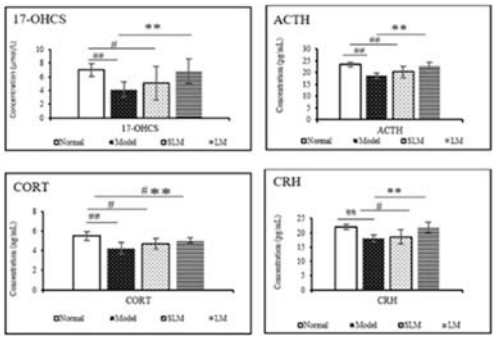

Figure 3: The changes in biochemical parameters related to the HPA axis of kidney-yang deficiency syndrome (KYDS) rats. Bars represent means ± SD of 10 rats per group, ##P<0.01, # P<0.05 versus the normal group, **P<0.01, *P<0.05 versus the model group.

5 DISCUSSION

A large number of exogenous hormones and repeated injections could disturb the stability of energy metabolism and the self-adjusting ability in vivo (Lu, Wang, Zhu, Lin, & Wo, 2012). Subsequently, causing the damage of cellular or function degeneration in the HPA axis. It was found and showed in clinical manifestations of KYDS in humans (Liu et al., 2016). The HPA axis is the main neuroendocrine system that regulates responses to stress. It is well known that the production of high levels of ROS into the glands that comprise the HPA axis is associated with the activation of a stress response system. The hyperactivity of the HPA axis induced by redox imbalance occurs by a reduction in negative feedback through a decrease in GR translocation to the cellular nucleus in corticotroph cells of the pituitary. The hyperactivity of the HPA axis induced by redox imbalance may occur by a reduction in negative feedback through a decrease in GR translocation to the cellular nucleus in corticotroph cells of the pituitary. The reactive oxygen species are ions or small molecules containing oxygen and an unpaired electron, and this free electron confers high reactivity to oxygen. The HPA axis is the main neuroendocrine system that regulates responses to stress. The production of high levels of ROS into the glands that comprise the HPA axis. It was known that associated with the activation of a stress response system (Prevatto et al., 2017)

The deficiency of kidney yang is the result of the hypofunction or dysfunction of many hormones control by the hypothalamus-pituitary-target gland axes. The hypothalamic–pituitary–adrenal axis is a neuroendocrine axis that utilizes three primary structures, allowing it to respond appropriately to stressful life-events. Thus involved in the paraventricular nucleus of the hypothalamus (PVN), the anterior pituitary gland, and the adrenal gland. The PVN is the houses of the key neurons controlling the level of activation of the HPA axis, regulation of metabolism, growth, and immune functions, as well as pre-autonomic control of gastrointestinal, cardiovascular, and renal functions. The rat neuroendocrine neurons of the PVN can be divided into Magno- and parvocellular divisions based on each neuron's size and projections. When once the releasing factor to reach the anterior pituitary, the PVN would trigger the release of hormones into the general circulation. The CRH, thyrotropin-releasing hormone (TRH), oxytocin, dopamine, somatostatin, and vasopressin (AVP) expressing neurons are among those that project to the median eminence.

Additionally, OT and AVP magnocellular neurons project to the posterior pituitary and secrete directly into the general circulation (Burford, Webster, & Cruz-Topete, 2017).

The pituitary gland is the organ that has a function to the secretion of protein hormones, plays a critical role in the maintenance of homeostasis during and after stress, as well as during other physiological processes (growth and metabolism). The pituitary is divided into the anterior lobe (or adenohypophysis) and the posterior lobe (or neurohypophysis). The posterior pituitary receives axonal inputs from magnocellular OT and AVP neurons residing in the supraoptic nucleus and PVN, where they release their secretory product into the general circulation. The corticotrophs are the key anterior pituitary cell type involved in HPA axis regulation and the production of ACTH. These cells contain receptors that bind CRH to activate the synthesis of ACTH in response to humoral signals from the hypothalamus. The adrenal cortex is composed of three distinct concentric zones: the zona glomerulosa, zona fasciculata, and zona reticularis from outside to inside. In addition to the cortical zones, the adrenal medulla is also involved in the regulation of homeostasis. Following the autonomic nervous system activation which secretes epinephrine and norepinephrine. However, the main adrenal region responsible for glucocorticoid secretion is the zona fasciculata. Cells in the zona fasciculata express the melanocortin receptor-2 (MC2R). The regulation basal and reactive hormone is from the allowed of access to the vascular system of the adrenal glands (Oyola & Handa, 2017) .

The CRH is a 41-amino acid peptide and arginine vasopressin (AVP) released from the hypophysiotrophic neurons in the paraventricular nucleus (PVN) of the hypothalamus. Thus stimulate the pituitary to the secretion of ACTH into the bloodstream through the activation of the CRH receptor type 1 (CRH-R1). The binds ACTH and the melanocortin type 2 receptors (MC2-R) in the zona fasciculate of the adrenal cortex, made a trigger the adrenal cortex to synthesize and release of the primary stress hormones. By the exogenous glucocorticoids (Hydrocortisone) directly suppressed ACTH production, the cortex of adrenal glands produces glucocorticoids (cortisol in humans and corticosterone in rodents) (Kokras, Hodes, Bangasser, & Dalla, 2019). The ACTH stimulated CORT production. In the end, it led to a decrease in the content of CORT(Coursin & Wood, 2002).

Moxibustion is one of complementary alternative medicine in China with a technique that applies heat to the acupoints utilizing by burning a compressed, powdered, combustible mass obtained from the young leaves of moxa (Artemisia vulgaris) on the certain locations of the body surfaces to treats and prevents the diseases. The moxibustion is warm in property and can smooth the circulation in the twelve meridians with regulates the qi and blood, as well as expelling the coldness and dampness, warming the meridians, strengthen yang from collapsing, reduce abscesses, dissipate nodules, stopping bleeding maintain health and well-being. Moxibustion is very effective in treating yin syndrome, cold syndrome, and deficiency syndrome(Iravani et al., 2020; Kim, Chae, Lee, Lee, & Park, 2011). It has significant advantages to the treatment of chronic diseases and the prevention of diseases. In modern research, moxibustion therapy can promote metabolism, improve immune system functions, and regulate the physiological function of internal organs. The thermal effect has produced by the moxibustion is beneficial to play a role in the therapeutic. The selection of an appropriate moxibustion material might important for optimal therapeutic effect. The understanding of the mechanism of the moxibustion effect from the following has three aspects was the pharmacological action, thermal effect, and infrared physical properties (Ling Zhao & Shen, 2022).

It presented the thermal stimulation produced by moxibustion significantly increased local blood flux and affected heart rate variability. That found a two-phase response in blood flow, a transient decrease followed by an increase without blood pressure change when applying moxibustion the thermal stimulation to the gastrocnemius muscle. The analysis demonstrated that the increase in blood flow occurred due to an axon reflex that had a reflex arc below the spinal cord, and the transient decrease in blood flow was induced by the excitation of postganglionic muscle sympathetic fibers. Moreover, several studies have been shown the effect of stimulation influenced the modulate autonomic nervous activity, neurotransmitter levels, endogenous substance levels, levels of inflammatory factors, and cardiovascular and renal function, especially the sympathetic nervous system. The sympathetic nervous system is a branch of the autonomic nervous system and plays a crucial role in the control of the cardiovascular system in humans through the regulation of both cardiac function and peripheral blood flow. Based on the fingertip temperature was found the sympathetic nervous induced changes in microcirculation. It used various stimuli of noise, deep breath, and hand cooling to triggering the sympathetic nervous system to induce the decreases in cutaneous microcirculation with the lag phase of approximately 21 and 26

seconds, were record the transient decrease of fingertip temperature. Moreover, found of the first minute of manual acupuncture stimulation, the burst rate of skin sympathetic nerve activity increased accompanied by the reduction of skin blood flow. It was suggested the thermal stimulation probably could trigger the sympathetic nervous system and induce the reduction of microcirculation included by the decrease of fingertip temperature. Furthermore, moxibustion could increase heart rate and mean femoral arterial pressure. It has a regulatory effect on cardiac function in brachycranial rats with the promotion of the degranulation of mast cells. Because of the degranulation of mast cells that play an important role in signal transmission between mast cells and peripheral sensory nerves (Matsumoto-Miyazaki et al., 2016).

The mechanism of action of infrared radiating material is transforming heat energy from the body through (convection and conduction) into radiation within the infrared wavelength range between 3~20 μm to induce homeostasis and photobiomodulation via deeper penetration of IR radiation and water molecule absorption in the skin. It uses to enhance blood circulation and metabolism of the human body(Tsai & Hamblin, 2017).

It was developed the peak of infrared radiation produced by moxibustion which is vigorous as those generated by thermal radiation. The peak wavelength of the infrared radiation spectrum of indirect moxibustion from conventional partitioned moxibustion and acupuncture points are approximately 10 μm. It played an important role when combined with purely the thermal effect from infrared radiation of indirect moxibustion. The wavelength of 10.6 μm laser is similar to the 10 μm from conventional indirect moxibustion and acupuncture points to generate a potent thermal effect (Deng & Shen, 2013; Mao et al., 2016; L. Wang et al., 2013; L. Zhao et al., 2013). The irradiation on GV4 and CV 4 points provides the therapeutic effect by generates a protective response against viral and bacterial infection thus promotes autophagy and the bactericidal function of macrophage that probably contributed to the inhibition of Akt phosphorylation and the activation of eIF2α phosphorylation, these the key signal pathways of variety of stresses. Moreover, by adjusting the 5-HT to release the MC degranulation would trigger the reaction of the body to active the nervous-endocrine-immune system, and regulate the immunity to achieving the target of disease prevention and treatment (Li et al., 2014; Qiong et al., 2018; 沈雪勇, 1996)

Infrared laser moxibustion was represented as a novel noninvasive and painless technology that provides self-regulation within treatment parameters. Recent research has indicated that the specific wavelengths of infrared radiation produced by moxibustion are as potent as generated thermal radiation (Mao et al., 2016). In traditional Chinese medicine, kidney yang deficiency is the condition of yang abnormality in warming and transformation qi functions. This also the main type of deficient of the cold pattern then can be alleviated by warm therapy (沈雪勇, 1996). The laser moxibustion was irradiated on GV 4 and CV 4 to stimulate a warm sensation. The combination of these two points is provided the therapeutic effect of seeking yang from yin, vice versa. Reinforcing yang without damaging yin, may regulate and strengthen both yin and yang also balancing. Moreover, tonifying kidney essence and strengthen primordial qi (Yuan, Yang, Han, & Ni, 2018).

The sham laser treatment, not able to change the condition of the restraining state of the releasing hormones in the hypothalamus, pituitary, and adrenal cortex. Because of the absence of the laser irradiation on the points. The placebo or sham group was used to avoid bias in the treatment.

6 CONCLUSION

The 10.6 μm infrared laser moxibustion is effective for treatment KYDS. It might relieve through increasing the content of ACTH, CRH, CORT concentration of HPA axis. Further study would focus on the histopathology examination of the hypothalamus, pituitary, and adrenal gland organ.

CONFLICTS OF INTEREST

“The authors declare that there is no conflict of interest regarding the publication of this review.”

STATEMENT OF HUMAN AND ANIMAL RIGHTS

“The treatment of animals conformed to the ethical criteria in this experiment.”

ABBREVIATIONS

TCM:	Traditional Chinese Medicine
KYDS:	Kidney Yang Deficiency syndrome
HYD:	Hydrocortisone
SLM:	Sham Laser Moxibustion
LM:	Laser Moxibustion
HPA:	Hypothalamic-Pituitary-Adrenal
ELISA:	Enzyme-Linked Immunosorbent Assay
ACTH:	Adrenocorticotropic Hormone
CRH:	Corticotropin-Releasing Hormone
CORT:	Cortisol (Human) / corticosterone (Rodents)
17-OHCS:	17-Hydroxicorticosteroid
AVP:	Arginine Vasopressin
ROS:	Reactive Oxygen Species
GR:	Glucocorticoid Receptor
MR:	Mineralocorticoid Receptor
FFAs:	Free Fatty Acids
PVN:	Paraventricular Nucleus
MC2-R:	Melanocortin Type 2 Receptor

REFERENCES

ANY-maze. 2020. A detailed description of the ANY-maze measures 2019. from http://www.anymaze.co.uk/index.htm. http://www.anymaze.co.uk/index.htm.

Burford, N. G., Webster, N. A., & Cruz-Topete, D. 2017. Hypothalamic-pituitary-adrenal axis modulation of glucocorticoids in the cardiovascular system. *International journal of molecular sciences, 18*(10), 2150.

Chen, R., Wang, J., Zhan, R., Zhang, L., & Wang, X. 2019. Fecal metabonomics combined with 16S rRNA gene sequencing to analyze the changes of gut microbiota in rats with kidney-yang deficiency syndrome and the intervention effect of You-gui pill. *J Ethnopharmacol, 244*, 112139. doi:10.1016/j.jep.2019.112139

Coursin, D. B., & Wood, K. E. 2002. Corticosteroid supplementation for adrenal insufficiency. *Jama, 287*(2), 236-240. doi:10.1001/jama.287.2.236

Deng, H., & Shen, X. 2013. The mechanism of moxibustion: ancient theory and modern research. *Evidence-Based Complementary Alternative Medicine, 2013*. doi:https://doi.org/10.1155/2013/379291

Iravani, S., Cai, L., Ha, L., Zhou, S., Shi, C., Ma, Y., . . . Zhao, B. 2020. Moxibustion at 'Danzhong'(RN17) and 'Guanyuan'(RN4) for fatigue symptom in patients with depression: Study protocol clinical trial (SPIRIT Compliant). *Medicine, 99*(7).

Jia-Xu, C. F. W., J. 2011. *Diagnostics in Chinese Medicine (International Standard Library of Chinese Medicine)* (C. J.-x. D. J. G. X.-l. J. Y.-m. L. J.-j. L. Y.-y. Y. Li-feng, Trans. J. F. W. L. Deasy Ed. 1 ed.). People's Republic of China: People's Medical Publishing House.

Jinfeng, J., Xinjun, W., Xiaojing, W., & Zhi, Y. 2016. Analysis of factors influencing moxibustion efficacy by affecting heat-activated transient receptor potential vanilloid channels. *Journal of Traditional Chinese Medicine, 36*(2), 255-260. doi:https://doi.org/10.1016/S0254-6272(16)30036-X

Kim, S.-Y., Chae, Y., Lee, S. M., Lee, H., & Park, H.-J. 2011. The effectiveness of moxibustion: an overview during 10 years. *Evidence-Based Complementary Alternative Medicine, 2011*.

Kokras, N., Hodes, G. E., Bangasser, D. A., & Dalla, C. 2019. Sex differences in the hypothalamic-pituitary-adrenal axis: An obstacle to antidepressant drug development? *Br J Pharmacol, 176*(21), 4090-4106. doi:10.1111/bph.14710

Li, X., Guo, G., Shen, F., Kong, L., Liang, F., & Sun, G. 2014. Moxibustion activates macrophage autophagy and protects experimental mice against bacterial infection. *Evidence-Based Complementary Alternative Medicine, 2014*. doi:https://doi.org/10.1155/2014/450623

Liu, Q., Zhang, A., Wang, L., Yan, G., Zhao, H., Sun, H., . . . Wang, X. 2016. High-throughput chinmedomics-based prediction of effective components and targets from herbal medicine AS1350. *Sci Rep, 6*, 38437. doi:10.1038/srep38437

Lu, D.-Z., Wang, P.-E., Zhu, J., Lin, T.-Q., & Wo, X.-D. 2012. *Effect of Hydrocortisone on Liver Mitochondria Proteome of Rats.* Paper presented at the 2012 International Conference on Biomedical Engineering and Biotechnology.

Mao, H., Mao, J. J., Guo, M., Cheng, K., Wei, J., Shen, X., & Shen, X. 2016. Effects of infrared laser moxibustion on cancer-related fatigue: A randomized, double-blind, placebo-controlled trial. *Cancer, 122*(23), 3667-3672. doi:10.1002/cncr.30189

Matsumoto-Miyazaki, J., Miyazaki, N., Murata, I., Yoshida, G., Ushikoshi, H., Ogura, S., & Minatoguchi, S. 2016. Traditional thermal therapy with indirect moxibustion decreases renal arterial resistive index in patients with chronic kidney disease. *The Journal of Alternative Complementary Medicine, 22*(4), 306-314. doi:https://doi.org/10.1089/acm.2015.0276

Nan, Y., Zhou, X., Liu, Q., Zhang, A., Guan, Y., Lin, S., . . . Wang, X. 2016. Serum metabolomics strategy for understanding pharmacological effects of ShenQi pill acting on kidney yang deficiency syndrome. *J Chromatogr B Analyt Technol Biomed Life Sci, 1026*, 217-226. doi:10.1016/j.jchromb.2015.12.004

Oyola, M. G., & Handa, R. 2017. Hypothalamic–pituitary–adrenal and hypothalamic–pituitary–gonadal axes: sex differences in regulation of stress responsivity. *Stress, 20*(5), 476-494. doi:https://doi.org/10.1080/10253890.2017.1369523

Prevatto, J. P., Torres, R. C., Diaz, B. L., Silva, P. M., Martins, M. A., & Carvalho, V. F. 2017. Antioxidant treatment induces hyperactivation of the HPA axis by

upregulating ACTH receptor in the adrenal and downregulating glucocorticoid receptors in the pituitary. *Oxidative medicine cellular longevity, 2017*. doi:https://doi.org/10.1155/2017/4156361

Qiong, L., Wei, Z., Mailan, L., Xia, L., Jianan, C., Shuning, H., . . . Xianghong, J. 2018. A study on the differences of effect on mast cells and serum 5-HT in the acupoint area of "CV 4" of rats by acupuncture stimulus and thermal stimulus. *World journal of acupuncture-moxibustion, 28*(4), 257-262. doi:https://doi.org/10.1016/j.wjam.2018.12.008

Reheman, A., Gao, Z. Y., Tursun, X., Pu, X. P., Wu, T., He, F., . . . Aisa, H. A. 2019. Optimization of Extraction Technology of Majun Mupakhi Ela and its Effect on Hydrocortisone-induced Kidney Yang Deficiency in Mice. *Sci Rep, 9*(1), 4628. doi:10.1038/s41598-019-41006-6

Seibenhener, M. L., & Wooten, M. C. 2015. Use of the open field maze to measure locomotor and anxiety-like behavior in mice. *JoVE*(96), e52434. doi:10.3791/52434

Shen, X., Ding, G., Wei, J., Zhao, L., Zhou, Y., Deng, H., & Lao, L. 2006. An infrared radiation study of the biophysical characteristics of traditional moxibustion. *Complement Ther Med, 14*(3), 213-219. doi:10.1016/j.ctim.2005.09.003

Tan, Y., Liu, X., Lu, C., He, X., Li, J., Xiao, C., . . . Lu, A. 2014. Metabolic profiling reveals therapeutic biomarkers of processed Aconitum carmichaeli Debx in treating hydrocortisone induced kidney-yang deficiency syndrome rats. *J Ethnopharmacol, 152*(3), 585-593. doi:10.1016/j.jep.2014.02.011

Tsai, S.-R., & Hamblin, M. R. 2017. Biological effects and medical applications of infrared radiation. *Journal of Photochemistry Photobiology B: Biology, 170*, 197-207. doi:https://doi.org/10.1016/j.jphotobiol.2017.04.014

Wang, C. M., Xu, S. Y., Lai, S., Geng, D., Huang, J. M., & Huo, X. Y. 2012. Curculigo orchioides (Xian Mao) modifies the activity and protein expression of CYP3A in normal and Kidney-Yang Deficiency model rats. *J Ethnopharmacol, 144*(1), 33-38. doi:10.1016/j.jep.2012.08.020

Wang, L., Wu, F., Zhao, L., Zhang, H., Shen, X., Huang, Y., & Lao, L. 2013. Patterns of traditional chinese medicine diagnosis in thermal laser acupuncture treatment of knee osteoarthritis. *Evidence-Based Complementary Alternative Medicine, 2013*. doi:https://doi.org/10.1155/2013/870305

Yuan, H., Yang, M., Han, X., & Ni, X. 2018. The therapeutic effect of the chinese herbal medicine, rehmanniae radix preparata, in attention deficit hyperactivity disorder via reversal of structural abnormalities in the cortex. *Evidence-Based Complementary Alternative Medicine, 2018*. doi: https://doi.org/10.1155/2018/3052058

Zhao, L., & Shen, X.-Y. 2022. Chemical and physical characteristics of moxibustion. In *Advanced Acupuncture Research: From Bench to Bedside* (pp. 249-272): Springer.

Zhao, L., Wu, H., Qiu, M., Sun, W., Wei, R., Zheng, X., . . . Jia, W. 2013. Metabolic Signatures of Kidney Yang Deficiency Syndrome and Protective Effects of Two Herbal Extracts in Rats Using GC/TOF MS. *Evid Based Complement Alternat Med, 2013*, 540957. doi:10.1155/2013/540957

Zhou, X.-H., Zhang, A.-H., Wang, L., Tan, Y.-L., Guan, Y., Han, Y., . . . Wang, X.-J. 2016. Novel chinmedomics strategy for discovering effective constituents from ShenQiWan acting on ShenYangXu syndrome. *Chinese Journal of Natural Medicines, 14*(8), 561-581. doi:10.1016/s1875-5364(16)30067-x

沈雪勇. 1996. Acupuncture treatment for kidney deficiency with combined application of points mingmen and guanyuan. *中医杂志: 英文版*(4), 275-277.