EFFECTS OF KIDNEY TONIC AND LIVER DISPERISING FORMULA ON HPA AXIS FUNCTION OF SENILE DEPRESSION RATS

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ABSTRACT

Objective*: To explore the effects of Kidney Tonic and Liver Dispersing formula on Hypothalamus-pituitary-adrenal cortical (HPA) axis function of senile depression rats.*

Methods: *Fifty-two male Sprague-Dawley (SD) rats were randomly divided into four groups. Normal, Senile depression. Kidney Tonifying and Liver Dispersing (KTLD) and Fluoxertine. There were ten rats in every group. All the rats in the 4 groups were fed naturally until 16 months old. From the age of 16 months. Depression model was induced by chronic unpredictable mild stress (CUMS) in the other three groups of rats except the control group. At the same time, KTLD group and FH group rats were accordingly administered with KTLD soup (14.62g/kg/d) and FH (1.8 mg/kg/d) for a total of 21 days. Rats in blank control group and senile depression group were given the same amount of normal saline respectively. Open field experiment was used to observe the behavior of rats. Reverse transcription quantitative polymerase chain reaction (RT-qPCR) were used to detect the level of corticotropin releasing hormone (CRH) mRNA in hypothalamus and adrenocorticotropic hormone (ACTH) mRNA in pituitary. Quantity of CRH, ACTH and corticosterone (CORT) in serum were detected by enzyme-linked immunosorbent assay (ELISA).*

Results: *Compared with Control group , the level of CRH mRNA in hypothalamus, ACTH mRNA in pituitary and the quantity of CRH , ACTH and CORT in serum were all increased in Senile depression group (P<0.05). Compared with Senile depression group , the level of CRH mRNA in hypothalamus , ACTH mRNA in pituitary and the quantity of CRH, ACTH and CORT in serum were all decreased in KTLD group and FH group rats (P<0.05). Compared between KTLD group and FH group, there were no evident difference (P>0.05).*

Conclusion: *KTLD formula can significantly downregulate the hyperfunction of HPA axis in senile depression rats.*

Keywords: *Senile Depression, kidney tonic and liver dispersing, HPA axis.*

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Introduction

Senile depression refers to the onset of depression in the age of 60 years of age or older. Due to the physical weakness of the elderly, it is easy to induce stroke, heart disease and Alzheimer's disease in addition to the symptoms of general depression.

In the elderly, the prevalence of depression is about 9%~18%[(1)]. Seriously affect the physical and mental health of the elderly. Hypothalamic pituitary-adrenal (HPA)-axis is implicated in the pathway to depression[(2)].

Overactivity of hypothalamic- pituitary- adrenal (HPA) axis function has been implicated in depression and suicidal behavior. The study has indicated that the method of kidney tonifying and soothing liver and relieving depression can improve the function of HPA axis in aging and depression[(3-5)]. According to the theories of traditional Chinese medicine, "Depression may be induced by stagnation of liver qi" and "kidney deficiency can lead to aging". We combined the aging, depression and HPA axis dysfunction and observed the effect of KTLD recipe on HPA axis in senile depression rats, and fur-

ther explored the pathogenesis of senile depression and the mechanism of KTLD recipe, so as to provide experimental basis and effective method for the treatment of senile depression.

Materials and methods

Animals

Fifty-two male 16-month old SD rats were housed in 5/cage and acclimated to the temperature-controlled (18~25°C) and humidity-controlled (60%-70% relative humidity) for 1 week under natural light and dark cycle. The rats had free access to water and food throughout the study.

All animal experiments were performed in accordance with the guidelines for the Care and Use of Laboratory Animals, and experimental protocols were approved by the committee of Ethics of Animal Experimentation of Henan University of Chinese Medicine. All efforts were made to minimize animal suffering and to reduce the number of mice used. Rats were provided by Shandong Experimental Animal Center. licence number: SCXK 2014-0007. Certificate number: 37009200004477.

Drug and reagent

Raw materials of KTLD were purchased and concentrated to oral liquid in Dongsheng Pharmaceutical Co, Ltd. The consists of KTLD formula are shown in Table 1. Fluoxetine tablet(10 mg) were purchased from Changzhou Fourth Pharmaceutical Co, Ltd. Lot number H1998013.

Chinese name	Botanical name	Amount (g)
Shu Di	*Rehmannia glutinosa*	24
Shan Yao	*Dioscorea oppositifolia L Lycium*	12
Gou Qi Zi	*Cornus officinalis Sieb. et Zucc*	12
San Zhu Yu	*Cyathula officinalis Kuan*	12
Chuan Niu Xi	*Cuscuta chinensis Lam*	9
Tu Si Zi	*CollaCornusCervi*	12
Lu Jiao Jiao	*Chinemys reevesii*	12
Gui Ban Jiao	*Bupleurum chinense DC*	12
Chai Hu	*Citrus reticulata Blanco*	6
Chen Pi	*Ligusticum chuanxiong hort*	6
Chuan Xiong	*Cyperus rotundus L*	4.5
Xiang Fu	*Citrus aurantium L*	4.5
Zhi Que	*Paeonia lactiflora Pall*	4.5
Bai Shao	*Glycyrrhiza uralensis Fisch*	4.5
Zhi Gan Cao		1.5

Table 1: Components of KTLD formula.

Animal experiment

Animal group

The rats were fed adaptively for a week. Then all rats were estimated by Open Field Test. The rats with a score of 30 to 120 were randomly divided into 4 groups. That was Normal, Senile depression, Kidney Tonic and Liver Dispersing (KTLD) and Fluoxertine. There were thirteen rats in every group.

Model replication

From the age of 16 months, the classical method of chronic unpredictable mild stress(CUMS) was used in rats[6].There were seven stressors in the course of CUMS. including food and water deprivation, nip tail for one minute, pairing with another stressed animal for forty eight hours, tilted cage (45°), wet cage, circadian disturbance. One of the above stressors were randomly selected to stimulate the rats. During the whole experiment, each stressor was given three times for twenty-one days. Senile depression was confirmed with Open Field Test.

Drug administration and intervention

The rats of all groups were given intragastric administration from the first day of the stress stimulation for twenty-one days. KTLD group rats were given a dose of KTLD with 14.62 g/kg/d.

Fluoxertine group rats were given a dose of Fluoxertine suspension with 1.8 mg/kg/d. The rats of Normal and Senile depression groups were both given equivalent saline. The intragastric administrations were all after stress intervention in every day.

Open field test (OPT)

The open-box device was made of wood opaque material, with a square of 100cm × 100cm on the bottom. The bottom surface was divided into 5 × 5 squares of equal area with a non-decolorizing pen. The height of the device was 50 cm. The test is carried out in a relatively quiet laboratory. The Test time was fixed from 8:00 to 12:00 am each day.

During the experiment, the rats in each group were placed in the center of the open-box device in turn. The activity of the rats was observed and the number of traversing grids (all four claws entered into the square could be recorded as 1 point) was recorded as the horizontal movement score in five minutes. The number of times to stand upright (marked by two claws leaving the bottom, no matter how long an animal stood until the two claws were lowered as 1 point) was a vertical motion score in five minutes.

Behavioral scores were measured once before stress intervention, and then measured once a week for a total of four times during the experiment.

Tissue collection

After the last OPT were carried, the rats were anesthetized by intraperitoneal injection of 10% chloral hydrate, and the blood was collected from the abdominal aorta to separate the serum. After the blood was taken, the rats were quickly decapitated, and the hypothalamus, pituitary and adrenal glands were separated by disinfecting apparatus on ice. All the tissues were stored at -80 °C for later use.

Histology observation of hypothalamus, pituitary and adrenal cortex

The isolated fresh tissue was fixed in 4% paraformaldehyde at room temperature for three days and then dehydrated gradiently by acetone and ethanol and embedded in paraffin.

Slice thickness was 6μm. The slice was stained with hematoxylin and eosin(HE). The slides were sealed with neutral gum. Histological morphology of hypothalamus, pituitary and adrenal cortex was observed under microscope.

Concentration detection of serum CRH, ACTH and CORT

Concentration of serum CRH, ACTH and CORT was deteced with enzyme-linked immunosorbent assay (ELISA). The measurement was according to the manufacturer's instructions of ELISA (Suzhou Calvin Biotechnology Co, Ltd. China).

Statistical analysis

The data was analyzed by SPSS17.0 statistical software. Data of groups are presented as means ± SEM. One-way analysis of variance (ANOVA) and t-test were used for comparison among and beteen groups respectively. a = 0.05 was selected as level of inspection. P<0.05 was considered statistically significant.

Results

KTLD formulation improved body weight

Table 2 shows the change of body weight. There was no significant difference in body weight of each group before the experiment. The weight of the rats in Senile Depression, KTLD, and Fluoxertine was lower than that of Normal (P<0.05). The weight of the rats in KTLD and Fluoxertine was higher than that of Senile Depression (P<0.05). These datas showed KTLD Formulation could improve body weight of senile depression rats.

Groups	Before Experiment	One week	Two weeks	Three weeks
Normal	563.31±11.093	594.76±10.001	628.92±10.980	667.84±13.409
Senile Depression	563.92±12.579	575.84±10.245▲	587.07±11.353▲	597.38±11.786▲
KTLD	565.00±12.675	584.92±12.906	607.38±14.239▲*	632.38±15.272▲*
Fluoxertine	562.30±10.680	583.02±9.275▲	603.53±10.112▲*	629.92±11.842▲*

Table 2: Change of body weight (n=13, $\bar{x}\pm s$, Unit: g). *F=0.116, P=0.927, P>0.05. Compared with normal, ▲P<0.05; Compared with senile depression, *P<0.05.*

Open field test

Table 3 and Table 4 show the level and vertical score of open field test. There was no significant difference in OFT Behavioral score (P>0.05) among all group rats before the experiment. Compared with the Normal, the behavioral level and vertical score of OFT in the Senile Depression rats decreased significantly (P<0.05). Compared with the Senile Depression, the behavioral level and vertical score of OFT in the KTLD and Fluoxertine rats were higher (P<0.05). There was significant difference between the Fluoxertine and the KTLD rats (P<0.05).

Groups	Before Experiment	One week	Two weeks	Three weeks
Normal	33.23±3.059	32.46±3.332	32.15±3.508	34.84±1.951
Senile Depression	32.15±2.303	19.53±2.066▲	15.15±2.230▲	12.85±1.993▲
KTLD	32.61±3.428	24.84±1.573▲*	24.31±2.097▲*	25.07±2.564▲*
Fluoxertine	33.92±2.596	25.23±1.877▲*	24.07±2.396▲*	26.07±2.660▲*

Table 3: Level score of open field test (n=13, $\bar{x}\pm s$, Unit: point). *F=0.541, P=0.818, P>0.05. Compared with Normal, ▲P<0.05; Compared with Senile Depression, *P<0.05.*

Groups	Before Experiment	One week	Two weeks	Three weeks
Normal	12.15±2.034	13.07±1.037	14.15±1.143	12.23±2.35
Senile Depression	12.07±1.497	6.92±0.954▲	4.92±0.759▲+	3.23±0.926▲+
KTLD	11.61±2.022	8.76±1.300▲*	8.38±0.767▲*	7.69±1.031▲*
Fluoxertine	11.92±1.705	9.07±1.320▲*	9.23±0.926▲*+	7.53±1.126▲*

Table 4: Vertical score of open field test (n=13, $\bar{x}\pm s$, Unit: point). *F=0.377, P=0.863, P>0.05. Compared with Normal, ▲P<0.05; Compared with Senile Depression, *P<0.05; Compared with KTLD, +P<0.05.*

Histomorphology

KTLD protects pathological structure of hippothalamus

Figure 1 shows histomorphology of hypothalamus. In Normal group, the neuroendocrine cells in

paraventricular nucleus of hypothalamus were regular in shape, moderate in size, compact in level, intact in cell structure, clear in nucleolus and nucleolus, large in nucleus, relatively small in cytoplasm and oval in nucleus.

In Senile Depression group, most of the hypothalamic cells were irregular in shape, disorder in arrangement, different in size, light-stained in some cells, pyknosis and deep staining in some cells, and vacuole-like changes were observed in some of them. In Fluoxertine group, the hypothalamus cells were more regular, a few nuclei were stained deeply, and the cytoplasm was increased. In KTLD group, The morphology of hypothalamic cells in KTLD group rats was regular, uniform in size, with a small amount of cytoplasm and clear nucleolus.

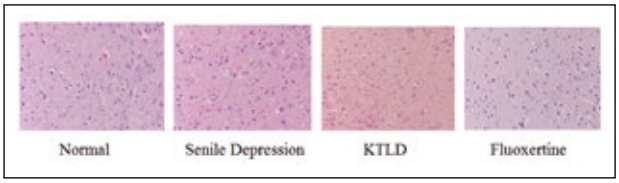

Figure 1: Histomorphology of Hypothalamus (HE×400).

KTLD protects pathological structure of pituitary

Figure 2 shows histomorphology of pituitary. Basophilic cells and eosinophil cells were observed in the pituitary of the Normal group rats. The cells were arranged closely, the nucleolus was clearly seen, the outline of the cells was clear, and the cells were round. In the Senile Depression group, pituitary cells were closely arranged, irregular in shape and different in size. A few blood sinuses were found among the cells, some of the cells were deformed or even necrotic, and the nuclei were stained deeply and condensed. In the Fluoxertine group, the pituitary cells were more regular and arranged closely, the outline of the cells was clear, and a few blood sinuses appeared between the cells. The pituitary cells in KTLD group rats were relatively regular and arranged closely, with a small amount of blood sinus.

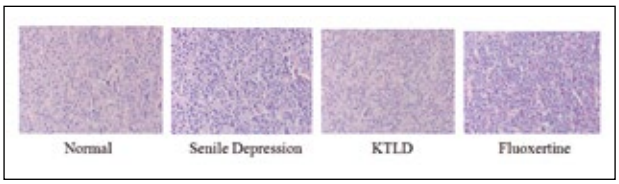

Figure 2: Histomorphology of Pituitary.

KTLD protects pathological structure of adrenal cortex

Figure 3 shows histomorphology of adrenal cortex. The adrenal gland cells in the Normal group rats were relatively intact and arranged in bundles, showing foam shape and light staining of nucleus. In the Senile Depression group rats, most adrenal gland cells were destroyed, a large number of blood sinuses and flake blood samples were observed. In the Fluoxertine group rats, the adrenal gland cells were basically intact, and a few blood sinuses were found in the adrenal gland cells of the rats.

The glandular cells of adrenal gland of rats in the KTLD group rats were relatively intact, with a small amount of blood sinus, cortical edema and no obvious infiltration of inflammatory cells.

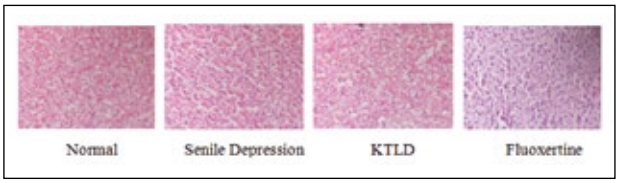

Figure 3: Histomorphology of Adrenal Cortex (HE×400).

KTLD regulates function of HPA Axis

Table 5 shows Serum Content of CRH, ACTH and CORT. Compared with Normal, the levels of serum CRH, ACTH and CORT in the Senile Depression group rats were significantly higher ($P<0.05$). Compared with Senile Depression, the levels of serum CRH, ACTH and CORT in KTLD group and Fluoxertine group rats were significantly lower ($P<0.05$).

Groups	CRH	ACTH	CORT
Normal	61.46±7.385	43.02±6.135	30.58±3.277
Senile Depression	108.37±15.918▲	80.647±12.644▲	57.46±8.458▲
KTLD	70.66±8.585*	50.50±5.509▲*	31.46±4.440*
Fluoxertine	69.55±10.524*	47.57±5.343*	33.34±4.102*

Table 5: Serum content of CRH; ACTH and CORT (n=10, $\bar{x}\pm s$, Unit: pg/ml). *Compared with Normal, ▲P<0.05; Compared with Senile Depression, *P<0.05.*

Discussion

The response of the body to stress is a dynamic balance, which is the regulation and adaptation to the stress. If such a balance is broken, that adaptive response will become an adaptive non-benign reaction, causing damage to the body. Hypothalamic-pituitary-adrenal (HPA)axis is the most important axis of stress. Corticotropin-releasing hormone (CRH) plays a central role in the regulation of the HPA-axis. ACTH release results in the release of corticosteroids from the adrenal that, subsequently, through mineralocorticoid and glucocorticoid receptors, exert negative feedback on the pituitary and the

hypothalamus. The most important glucocorticoid in humans is cortisol and in animals is corticosterone. HPA axis function changes during the course of aging in rodent animal and humans.

Aging is a process of degenerative changes in the tissues and organs of the body with the increase of age[7]. According to the neuroendocrine theory of aging, HPA is the main regulator of aging. The disorder of terminal hormone GCs secretion of HPA axis is involved in the occurrence of many elderly diseases, such as depression, cognitive impairment, Alzheimer's disease and so on[8]. The study has found that both in physiological and pathological brain aging usually keep up with the modulation of the HPA axis. Basal cortisol levels increased in the elderly[9]. The axial activity of HPA in aged rats increased with the increase of age, and the level of corticosterone in peripheral blood increased abnormally[10].

Exposure to stress is also thought to play an important role in the etiology of depression. For example, Dysfunction of the HPA axis may contribute to aging-related diseases like depression, cognitive deficits, and Alzheimer's disease in some older individuals[11]. The HPA-axis is hyperactive in depression[12]. It is hypothesized that particularly a subgroup of CRH neurons that projects into the brain is activated in depression and induces the symptoms of this disorder[13]. During aging, the activation of the CRH neurons is modest compared to the extra activation observed in Alzheimer's disease and the even stronger increase in major depression. CRH neurons are strongly activated in depressed patients, and so is their HPA-axis, at all levels, but the individual variability is large. corticosterone is a well-known inducer of depression-related behavior[14]. The occurrence of major depression or even only of depressive symptoms seems to amplify the changes of the adrenal steroidal secretory pattern, already present in physiological aging[15] both in depressed males and females the HPA-axis is increased in activity[16].

Older participants suffering from depression show a high degree of dysregulation of HPA axis activity, with differences compared with younger adults[17]. Individuals diagnosed with major depressive disorder (MDD) are associated with high levels of stress, implicating dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis and anomalous levels of cortisol secretion[18]. But there are also experiments that prove that Late-life depressive symptoms are associated with both hyperactivity and hypoactivity of the HPA axis, which suggests distinct mechanisms for these associations. For example, Older persons in the lowest and highest urinary cortisol deciles were 2.2 and 1.9 times more likely to have significant depressive symptoms than older persons in all other deciles. Depressed persons with low cortisol presented more physical frailty than depressed persons with high cortisol[19]. Beta amyloid can exert an inhibitory effect on HPA axis activation to provoke depressive-like profile in rats[20]. Prolonged HPA axis overactivity occurs in normals and some depressed patients[21]. There was a positive correlation between the cognitive impairments and increased activity of the HPA axis[22]. Based on the correlation between hyperfunction of HPA axis and aging and depression, it has been proved to be effective by a large number of clinical and experimental studies to delay aging and treat depression by regulating the function of HPA axis. In this experiment, the method of kidney tonifying and liver soothing was used to intervene the senile depression model rats, and remarkable results were obtained.

In the course of the onset of senile depression in the elderly, aging is the premise of the onset of senile depression. Aging is an important cause or inducement of the incidence of depression in the elderly. Aging increases the probability of depression or aggravates the condition of depression. The results of our study show that actively improving the aging state is helpful to the treatment of senile depression, which provides a new way of thinking and method for the treatment of senile depression or elderly-related diseases.

Conclusion

The hyperfunction of the HPA axis in the senile depression model rats can be attenuated and the pathological changes of the morphology of the hypothalamus, the pituitary, and the adrenal cortex can be improved by the kidney-tonifying and liver-soothing prescription.

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