ORIGINAL ARTICLE

Effect of Rhodiola Extracts on Learning, Memory Deficits and Micro Structure of Brain Cells Induced by Sleep Deprivation

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ABSTRACT

Objective：To Study the effect of *Acanthopanax senticosus* extract on the brain cell ultrastructure of rats model of sleep deprivation and its learning and memory deficits.

Material and Methods: Brain cells ultrastructure were observed by transmission electron microscopy, rat model of sleep deprivation were made by using a modified multiple platform water environmental law, learning and memory ability were assessed by a maze test in rats.

Results: In rat model of sleep deprivation group (SD group) there was obvious brain cell injury, learning and memory deficits and increased reaction time as compared to cage control group (group CC) and tank control group (group TC) ($p<0.05$, $p<0.01$). In treatment group (ST group) with *Acanthopanax senticosus*, the brain cell injury was lighter, learning and memory deficits were reduced and reaction time was shortened ($p<0.05$) than that in SD group.

Conclusion: The results showed that extract of *Acanthopanax senticosus* can improve brain cells injury induced by sleep deprivation and can improve learning and memory deficits in sleep deprived rats.

Key words: sleep deprivation; Rhodiola; brain cells; micro structure

INTRODUCTION

Acanthopanax senticosus (Rhodiola rosea L.) belongs to Araliaceae perennial xylophyta [1]. Modern pharmacological research shows that its main effective component has the ability to improve work efficiency, delay aging, and it has been already accepted as a health care drug and food resource [2]. Sleep deprivation (SD) is due to various external and internal factors that induce sleep loss and loss of normal sleep routine [3]. At present, the research on sleep deprivation has been paid more and more attention, but the role of *Acanthopanax senticosus* extract on sleep deprivation in rats has not been reported. This study tries to find the effect of *Acanthopanax senticosus* extract on sleep deprivation, its preventive and therapeutic effects for sleep deprivation, and offers a new strategy for providing theoretical support in clinical application.

MATERIALS AND METHODS

The selected experimental animals were adult Wistar rats, 120 in total out of which 80 rats qualified, weight of 190 ~230g (the animal experimental center of Jiamusi University, Certificate No.: SYXK (black) 2006-004).

Sleep deprivation box (170cm× 70cm× 50cm, homemade); adjustable pipette (type SOCOREX); electronic analytical balance (MP120-2); laboratory pH (PHSJ-4A); medical image acquisition and analysis system (BL-2000 type).

Y-Maze Test

Experiment apparatus : Y-maze test consists of Trisection labyrinth box (type MG-2) and control instruments.

Electrical stimulation parameters: voltage:50-70V, extend time:2-5S.

Preliminary Selection: Rats were initially put into the labyrinth box for 3-5min for adaptation, repeated electric stimuli were given to make sure that the rats explore into all three channels. The rats which were more sensitive to shock and quick in response to escape were chosen, while rats less sensitive to stimuli or lagging in response were eliminated.

Selection criteria

Correct response: Rats escape from primary area to safe area directly after shock.

Error response: Rats escape from primary area to other channel than the safe area. If rats escape not only from the primary area to safe area but also from safe area to primary area is also error.

Running to safe area in 10S after electric stimulus is standard which reflects rats reaction time.

The standard of trained rat is to have correct response continuously 18 times or more out of 20 (accuracy of reaction≥90%).

Training tests were performed for 20 times, error number(EN)≤2, total reaction time(TRT) ≤120s as the standard of trained rats. EN reflects accuracy of rats reaction whereas TRT reflects time of rat's reaction; both are used to evaluate the ability of learning and memory synthetically.

Method of Training

After preliminary selection rats were put into the labyrinth box for 3-5 min for adaptation and begin to train by using random testing. Safe areas were changed in random order to train rats ability of distinguishing light stimulation and safe direction. The light was kept for 10-15 seconds till the rats escape to safe area after shock . Once the training was over the area where the rats escaped to, was the starting point of the next training session. The interval between two training sessions was 30S to 1 min. The training continued untill the standard of accuracy (18/20) was achieved.

The Incubation period of Reaction time begins with turnning on the power switch to brighten the light and ends at rats escaping to safe area. Sports stopwatch was used for reaction time readings. This time indicator reflects the length of rats reaction,which can evaluate the ability of learning and memory. The rats underwent " Y " maze training for 5 days, to achieve the standard rat investment experiment. The qualified rats were randomly divided into 4 groups, named as the sleep deprivation group (SD group) and Salidroside treatment group (ST group) having 35 rats in each group. Environmental controls/ tank control (TC group) and control group/ cage control (CC group) having 5 rats in each group.

***Acanthopanax senticosus* Extract**

Acanthopanax senticosus extracted in concentrated alcohol solution and diluted by water (Xi'an Industrial Biotechnology Limited) was derived from the *Acanthopanax senticosus* roots, brown powder, no special smell, its main effective pharmacological composition Eleutheroside (Salidroside) content of 8.8% (*Acanthopanax senticosus* extract quality standards: GYSW-04028, specification: HPLC).

Animal Model of sleep deprivation

Improved preparation of animal model of sleep deprivation by using a modified multiple platform water environmental law (Modified multiple platform method, MMPM REM) was used. Mouse box 170cm ×70cm× 50cm, built 18 in number with a diameter of 6.5cm, height 8.0cm platform, 15cm space between each platform, filled with water. The water was 1cm below the platform, water temperature was maintained to 22 ℃. Rats on the platform can be fed freely. When the rat enters REM sleep, due to reduction in systemic muscle tone, its body gets unbalanced and hits the water, to make sure that rats cannot enter REM sleep period. Indoor temperature control: 22.0~24.0℃, 40W light time was 08:30~20:30. Before the experiment, rats were placed in the same cage for 1 week, in this week rats were placed on a platform for 1H every day. TC group was put in the same size box as sleep deprivation rat box, but there was no platform, instead a dense meshwork/net and the rats were placed on the net, while the water was 1cm below the net, rest of the conditions like temperature and light is same with those in SD group. Group CC was kept together but were not placed in the platform or the net for training, feeding conditions were the same as for SD groups and TC groups. In SD group and ST group sleep deprivation was induced for 6h 12h, 1d, 2d, 3d, 5d, 7d, a total of seven times, each time 5 rats and were weighed, while CC group rats were just weighed. Besides the normal feeding, ST group was given *Acanthopanax senticosus* intervention of 180mg/kg/d, 10 days before the start of experiment till the end.

Specimen Preparation

In each group after specified time brain tissue was taken under ether anesthesia by decollation, selecting the target site, $2mm^3$ tissue was taken and placed in 2.5% glutaraldehyde. After processing semithin slices of 0.5μM thickness were stained with blue azure III and observed under light microscope for positioning of hippocampus, basal ganglia and cortex. These ultra thin slices were studied under transmission electron microscopy (JEX-1200).

Statistical processing

All data was expressed as $\bar{x} \pm s$; all groups were compared using a one-way ANOVA, $P<0.05$ for

the difference is of statistical significance, while P<0.01 shows more significant difference; all data was obtained using SPSS13.0 statistical software.

RESULTS

1- Behavior and Weight change

There was no death or escape of the subject animals through the entire experiment. Animals in sleep deprivation in early (6~48h), SD group and CC group compared with TC group, showed some euphoric/exciting behavior such as increased activity, external acoustic and light stimuli sensitive. In the mid of the experimental (3~4d), animal behavior gradually changed from excitement to the state of suppression, fatigue and sleepiness as the degree of sleep deprivation period gradually increased, manifested as recurrent bow, scattered fur, dull hair color, anorexic, reduced consumption, decreased activity to external stimuli, weight loss, and reduced growth. The later period of the experiment (5d~7d) the rats got extremely weak, minimum to no response to external stimuli, but aggressive behavior and reactivity increased, fall of hair in some, injured tail and claws, diet was increased but weight loss and severe fatigue state.

As observed in the experiment, in SD and ST group animals; as sleep deprivation varied with time manifested a gradual progressive fatigue and drowsy/sleepiness state, the most obvious manifestation of mental state was low and reduced activities of experimental period, body weight was significantly lower than that of CC and TC group (P<0.01), ST group was also induced REM sleep deprivation, and showed deterioration in normal functioning. The degree of fatigue assessed by rats eating routines and reduced to no activity to external stimuli, was more in SD group as compared to ST group and it appeared late in ST group, in addition weight loss was more than that in CC group and TC group (P<0.05), but higher than those in the SD group, had significant difference (P<0.01), the results of the experiment are shown in table 1.

2- Changes of learning and memory ability

Learning and memory ability in comparison to each other before the start of experiment were the same (P > 0.05). There was no impairment of learning and memory ability in CC group and TC group from the beginning to the end of experiment. In SD group with deprivation time increased, after the electric shock as an external stimuli, reaction time was significantly prolonged as compared with CC group and TC group (P<0.05, P<0.01) indicating learning and memory disorder. ST group as compared to SD group had short reaction time (P<0.05, P<0.01) better learning and memory ability, but as deprivation increased with time, prolonging the reaction time and reducing learning ability. The experimental results are shown in table 2.

Table 1: Body mass changes of rats after SD in different period（m/*g*, $\bar{x}$ ±*s*, *n*=5）

groups	1d	2d	3d	5d	7d
SD	2.00±0.21	3.2±0.37*	4.62±0.49*	2.61±0.21**	1.13±0.11**
ST	2.01±0.24	3.77±0.32	5.47±0.52△	5.14±0.57**△△	4.87±0.42**△△
TC	2.01±0.19	4.21±0.40	5.89±0.57	8.97±0.92	12.07±1.71
CC	2.03±0.22	4.01±0.35	5.90±0.58	9.11±1.01	12.87±1.83

** p<0.05，** p<0.01，vs CC group and TC group；△p<0.05，△△p<0.01，vs SD group*

3- Brain tissue ultrastructure changes

In normal rat brain neurons are regularly arranged with complete clear edged large and round nucleus, uniform chromatin nucleolus, cells with abundant endoplasmic reticulum, high numbers of nuclear mitochondria, clear structure axon. Morphologically neurofilament along the axonal shaft are arranged in parallel, the axoplasm organelles, myelin uniforms myelin membrane, and shaft sealed together. SD group at 5D after sleep deprivation showed occasional increased vascular swelling around the gap, nucleolus appeared notch shaped, dark cells with twisted wire arrangement more than the normal no. of axons. There is nerve edema with appearance of dense and the blank areas, but still along the axonal shaft are still arranged in parallel. Granular disintegration of neurofilaments causing tear in the part of the shaft

membrane disolving nuclear myelin invagination. A significantly reduced number of mitochondria and mitochondrial swelling, fuzzy crest structure irregular shape, un even nucleoplasm, some showed vacuolar degeneration, dilation of endoplasmic reticular lacunes though endoplasmic reticulum is an intensive and complex structure. Sleep deprivation after 7d showed obvious above mentioned changes; ST group also showed the same above mentioned changes but to a lesser degree, CC group and TC group had no obvious change, the experimental results are shown in figure 3-A~F.

Table 2: Reaction time after SD in different period（*t/s,* $\bar{x}$ *±s, n=5*）

Groups	1d	2d	3d	5d	7d
SD	0.79±0.11*	1.98±0.27**	2.83±0.33**	4.89±0.53**	6.07±0.63**
ST	1.01±0.24	1.69±0.30	2.36±0.36**△	3.49±0.34**△△	4.47±0.46**△△
TC	1.17±0.15	1.37±0.10	1.12±0.13	1.13±0.14	1.18±0.17
CC	1.18±0.15	1.30±0.15	1.14±0.17	1.19±0.18	1.16±0.20

* *p<0.05*，** *p<0.01*， *vs* CC group and TC group；△*p<0.05*，△△*p<0.01*，*vs* SD group

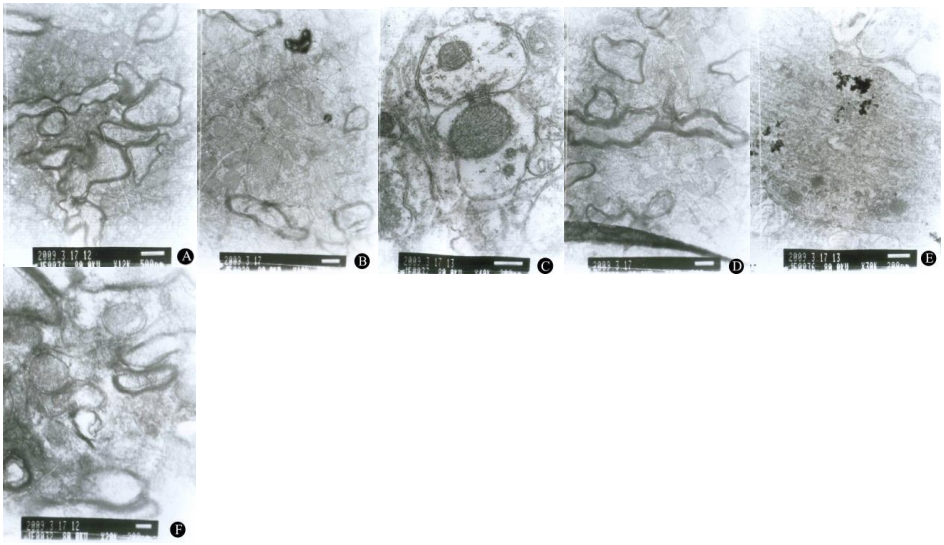

Fig3-A~F micro structure changes of brain cells after SD
Fig 3-A.CC group Myelin and Mitochondria are normal ×12K
Fig 3-B.ST 7d group Myelin disintegrate slightly , Mitochondria are normal ×10K
Fig 3-C.ST 7d group Mitochondrial structure is not clear ×40K
Fig 3-D.SD 7d group Myelin disintegrate ×10K
Fig 3-E.SD 7d group Neurofilament disorder ×30K
Fig 3-F.SD 7d group Mitochondria edema×20K

DISCUSSION

Sleep deprivation is a compound stress, including autophobia, drowsiness, fatigue and increased metabolism. SD can interfere with neurotransmitters in the brain of [4], neuropeptide substance [5], and a variety of cytokines levels [6]. Another report indicated that short-term SD can result in aberrant gene expression [7], and long SD can induce gene change [8], caused by reduced alertness and judgment, memory and decreased immunity changes [9,10].

The experimental results show that after SD in rats body weight and growth rate is apparently slow, each subject rat showed weight loss.

Probably due to sleep deprivation, energy consumption increased, but the synthetic metabolism was decreased. Brain on the body is very sensitive to tissue energy metabolism, oxygen consumption and high rates of metabolism. It weighs less than 3% of body weight, whereas oxygen consumption is 20% of the total oxygen consumption. Brain of SD rats with increased time maintained wakefulness, consumption of material was increased, increased skeletal muscle consumption to maintain posture to prevent the fall into water and long time to maintain the tension state inducing mental anxiety. Energy consumption continued to increase in great quantities, resulting in a catabolic over synthetic metabolism, leading to increased food intake and weight loss. *Acanthopanax senticosus* obviously improves the non-specific resistance, regulating organ physiological function, so that varies the morbidity index to the normal state change enhancing the body's physical and mental activity efficiency adapted to the physiological function to effectively improve the subject rats physical exhaustion state.

In ST group rats learning and memory ability prolonged, the maze reaction time was shortened and brain tissue ultrastructure changes showed that the symptoms improved with *Acanthopanax senticosus* treatment.

Learning and memory is one of advanced brain function, the process to synaptic plasticity based on learning and memory is the essence of signal transmission and processing process, [11,12]. Studies suggest that changes in synaptic plasticity of rats hipocampus acquires the spatial learning and memory function. Increase in the number of synapses in brain increasing presynaptic active zone membrane area, synaptic vesicles number and volume increase with a series of morphological changes, these changes can be considered as spatial learning and memory [13]. Synaptic long-term potentiation (LTP) is considered to be the molecular mechanism of learning and memory function [14]. LTP process needs new RNA and protein synthesis, a variety of substances are required to establish new synaptic contacts, inhibition of protein or mRNA synthesis can weaken or block long memory.

Acanthopanax senticosus can enhance memory and concentration, reducing mistake especially in the fatigue condition. *Acanthopanax senticosus* can effectively inhibit NOS, thereby inhibiting the excessive NO production ensuring cerebral protection. To protect the brain tissue the role of *Acanthopanax senticosus* active components is to act on the phenolic hydroxyl group. A large number of studies show that phenolic hydroxyl free radical scavenging by binding EAA as main functional group to eliminate free radicals, can significantly reduce in the body lipid peroxidation reducing membrane peroxidation degree and the body metabolism during redox reactions so as to protect the brain tissue [15].

CONCLUSION

Acanthopanax senticosus can significantly improve the symptoms of the cognitive impairment, weight loss and brain tissue ultrastructure changes of rats in REM sleep deprivation with increased time.

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