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Effects of Nodus Nelumbinis Rhizomatis Extracts on Sociopsychological Stress in Mice

Ho-Young Won · Su-Yeon Kim · Eun-Jeong Kim · Dong-Won Lee
Dept. of Neuropsychiatry, College of Oriental Medicine, Dongshin University

蓮根 추출물이 생쥐의 사회·심리적 스트레스에 미치는 영향

원호영 · 김수연 · 김은정 · 이동원
동신대학교 한의과대학 신경정신과학교실

만성적이거나 반복적인 스트레스는 우울증이나 심신병 등의 발병 기전과 밀접한 관련이 있다. 전기적 자극(electric foot shock), 한랭 자극, 속박(immobilization), 투쟁(fighting), 에테르 노출 등과 같은 심한 스트레스 자극이 뇌 조직의 시상 및 시상하부, 편도체 등에서의 노르아드레날린(noradrenalin) 방출을 유의하게 감소시키는 동시에 혈청 corticosterone을 증가시키는 것으로 알려져 있다. 한의학에서는 스트레스 인자에 대한 신체반응을 氣의 변화로 인식하고 있고, 이러한 자극요인들은 신체에 대하여 병적 요인을 제공하여 제반 질환을 야기한다. 또한 질병 발생에 대한 근거로 正氣와 邪氣의 상호 관계 위주로 인식하는데, 내부의 氣의 變調는 질병의 발생을 야기하는 기초가 된다.

실험에 사용된 蓮根은 연꽃과에 속한 수생초본인 연꽃의 뿌리부분으로 藕 또는 藕節이라는 명칭으로 불리기도 한다. 性味는 甘·澁·平·無毒하고 肺·胃·肝經으로 들어가며 收斂止血·化瘀의 효능이 있어 吐血·咯血·尿血·便血 등의 각종 출혈 증상에 사용되어져 왔다. 최근 연꽃의 부위별 추출물이 흰쥐의 지질 과산화 생성을 효과적으로 억제하는 것으로 보고되고 있어 蓮根에 대한 다양한 약리 효능의 검색이 필요할 것으로 생각되며 연꽃의 씨앗인 蓮子肉이 養心安神의 효능이 있어 스트레스성 질환에 많이 응용되고 있는 것에 착안하여 본 연구를 착수하였다. 실험 동물은 ICR계 마우스를 이용하였으며, 사회·심리적 스트레스는 옆쪽 cage에서 다른 마우스의 신체에 가해지는 전기 충격을 하루 1시간 동안 지켜보게 하는 것으로 유발하였으며 이 상태에서 약물을 투여한 그룹을 실험군, 그렇지 않은 그룹을 대조군으로 하였다. 정상군은 아무런 자극 없이 하루 1시간 동안 일정 공간에 가두어 두는 것으로 하였다. 실험 결과 사회·심리적 스트레스를 받은 경우에 아무런 처치를 하지 않은 대조군에 비해 연근 추출물을 100㎎/㎏/day 용량으로 5일간 투여한 실험군에서 혈청 중 corticosterone 함량이 유의하게 감소하였으므로 매우 효과적으로 스트레스를 해소하였음을 알 수 있다. 뇌세포 중에서 신경전달물질로 분비되는 노르아드레날린의 분비량은 전반적으로 증가되는 경향을 나타내었으나 유의성은 없었다. 신체적 또는 사회·심리적 스트레스가 간 조직 내 지질의 과산화를 유발하는 것으로 나타났으며 연근 추출물이 이 결과에는 영향을 주지 못하였다. 사회·심리적 스트레스로 인하여 간 조직 내 지질과산화 정도가 증가하였으므로 혈청 내 ALT 함량도 따라 증가할 것으로 추정되었으며 이에 대한 연근 추출물 경구 투여가 간 조직을 보호할 수 있는지를 확인하기 위해 분리한 혈청으로부터 ALT 함량을 측정한 결과 대조군에 비하여 유의한 감소를 나타내었다. 또한 연근 추출물이 혈청 내 지질 과산화물의 생성을 억제할 수 있다면 질병의 예방과 치료에 효과적일 것으로 추정할 수 있으므로 그 생성량을 측정하여 보았으나 대조군과의 차이가 나타나지 않았다. 이상의 결과들을 종합하여 보면 스트레스가 부하된 5일 동안 蓮根 추출물을 함께 투여한 결과 혈청 corticosterone 함량을 유의하게 감소키켰고 뇌 조직내 noradrenaline 함량을 증가시키는 경향을 나타내어 스트레스 해소에 도움이 될 수 있음을 시사하였다. 또한 혈청 내 ALT 함량을 유의하게 감소시켜 스트레스로 인해 발생하는 간 기능의 손상도 어느 정도 억제시키는 것을 확인할 수 있었는데 앞으로 蓮根의 이러한 작용에 대한 보다 자세한 연구들이 필요한 것으로 생각된다.

Key words : 연근, 사회심리적 스트레스, 코티코스테론, 노르아드레날린, 지질과산화, 알라닌 아미노트랜스퍼라제

교신저자 : 원호영, 전남 순천시 조례동 1722-9 동신대학교 순천한방병원 신경정신과학교실
Tel. 061)729-7166, E-mail : leedww1@kornet.net
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Ⅰ. Summary

The effects of Nodus Nelumbinis Rhizomatis extract(NNR) were tested for the anti-sociopsychological stress action. 250g of crude drug of NNR was extracted with 4300㎖ of pure water and the total crude extractive powder 41.9g. ICR male mice(20±2g) were fed orally with the dose of 100㎎/㎏/day for five days. Mice were exposed to sociopsychological stress by restraining and seeing foot shock stressed mice for one hour for five days. NNR administered group showed a significant decrease of serum corticosterone secretion compared with control group. Noradrenaline secretions in the dorsal cortex of brain showed no change. Lipid peroxidation and cytotoxicity of the liver of mice were tested by measuring malondialdehyde and serum level of alanine aminotransferase(ALT), and NNR admistration decreased serum level of ALT. Serum level of malondialdehyde did not changed either. Above results suggest that Lotus Root extract can effectively rid the sociopsychological stress.

Ⅱ. Introduction

It is well recognized that stress is one of the deleterious factors for health. Actually, many diseases or disorders such as hypertension, gastric ulcer, affective disorders, and hyperthermia are likely to be related to stress. It is also believed that chronic stress is more frequent and deleterious to human health than acute stress.

In animal studies, it has been proposed that repeated stress exposure produces a useful model of depression in animals[1]. This model has been used to mimic human abnormality of the hypothalamic-pituitary-adrenocortical system, i.e., elevated levels of glucocorticoids (GCs)[2], and impairment of the sleep-wake cycle[3], which is a typical circadian rhythm parameter. As to the influence on the rhythmic activity of the brain, it has been suggested that chronic stress itself causes disturbances of the circadian rhythm in physiology, endocrinology, and behavior[4-9]. Accordingly, an intimate relationship between chronic stress and circadian rhythm disturbance has been suggested. Furthermore, chronic stress exposures have also been reported to cause behavioral and physiological abnormalities, such as decreased food intake, decrease in some types of behavior, hyperthermia, and an elevation of plasma GCs[10, 11]. However, little information is available on the physiological and behavioral consequences following a long-term period after chronic stress exposure.

Recently, the communication box, developed by Ogawa and Kuwabara[12], has been introduced to study behavioral and physiological changes in rats or mice under physical or psychological stress. The psychological stress is generated by an exposure to emotional responses arising from physically stressed animals. In any case, these stress exposures cause behavioral and physiological changes in animals[13-17].

In the present study, the effects of lotus root extract were tested for the anti-sociopsychological stress action. Lotus

roots are tuberous roots of the lotus water lily, *Nelumbo nucifera* GAERTN., family Nymphaeaceae. In oriental medicine, lotus root has the effect of hemostasis with astringents and promoting blood circulation by removing blood stasis[18].

we examined whether lotus root have anti-sociopsychological effects in mice following exposure to sociopsychological stress induced by the communication box.

Ⅲ. Materials and Methods

Animals

Adult male ICR mice at the body weight of 20±2g were obtained commercially(Daehan experimental animal, Korea) and used. All animals were housed under standard conditions of lights and controlled room temperature, and received food and water ad libitum. The stress exposures were carried out in a separate room.

Nodus Nelumbinis Rhizomatis extract preparation

Nodus Nelumbinis Rhizomatis were purchased in the special herb market(Songsan Herb, Gwang-Ju, Korea) and good samples carefully selected. To fractionate the aqueous extract, 250g of the dried herb of Nodus Nelumbinis Rhizomatis was boiled with 4300㎖ of pure water at 100℃ for 2 hours. After filteration, the filtrate was evaporated under reduced pressure and then freeze-dried to yield the aqueous extract. And the total crude extractive powder was 41.9g. The extract stored in deep freezer when unused, and freshly diluted for experiment. Mice were fed orally with the dose of 100㎎/kg/day for five days.

Chronic stress exposures

Stress was applied by the methods which Ogawa[12] developed. The communication box was equipped with a grid floor composed of 0.5㎝ diameter stainless rods placed 1.3㎝ apart. The box was divided into sixteen smaller compartments(16×16㎝) by transparent plastic sheets(Fig. 1). An electric shock generator, with a timer produced electric foot shocks(2 mA) for 10 sec at intervals of 120 sec and lasted for 1 hour. A plastic plate was placed on each of the grid floors of four or five compartments to avoid the electric shock.

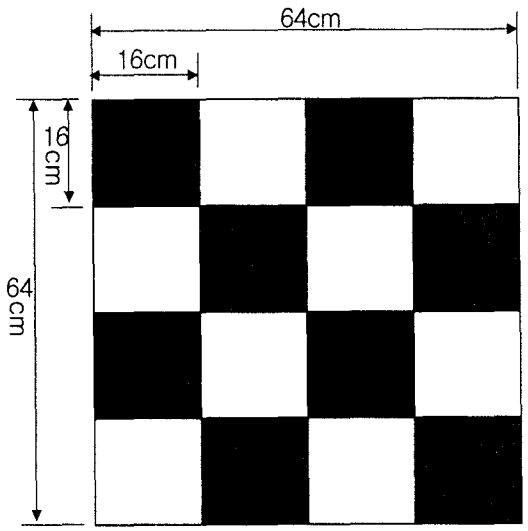

Fig. 1. Scheme of the communication box. Foot shock mice were placed individually in the eight shaded areas(foot shock compartments). Sociopsychological mice were placed in the eight solid areas(nonfoot shock compartment). Foot shocks were delivered in shaded areas.

Mice were divided into three groups, i.e., a control group and two chronically stressed groups. The first group was physically

stressed mice that were given foot shock(FS) stress, the second and the third group was for psychologically stressed rats that stayed in the same communication box as the FS group, but did not receive foot shock but received emotional stimuli from the rats in the FS group, that is, non-foot shock stress(NFS). The second group(control) had no herbal remedy, but the third group(sample) had remedy of NNR for 5 days. Mice in the FS group could sometimes avoid receiving the electric shock for a moment by jumping up. These stress exposures were performed for 1 h/day and lasted for 5 days. Immediately after the stress exposure, all mice were returned to their home cages in their room.

Measurements

After the termination of the stress sessions, we measured serum level of corticosterone, alanine aminotransferase(ALT) and lipid peroxidation and noradrenaline secretions in the dorsal cortex of brain, lipid peroxidation of liver tissues.

Serum level of corticosterone were determined by indirect radioimmunoassay. Circulating corticosterone were quantified by radioimmunoassay using the direct reading assay kits from ICN Biomedicals, Inc. (Costa Mesa, CA, USA). This assay kits have been previously validated for the mouse[19-21].

Serum level of ALT were determined spectrophotometric method using the direct reading assay kits from Asan Pharmaceutical(Asan Pharmaceutical Co. Korea).

Noradrenaline level of brain tissues were measured by ion pairing reverse phase high pressure liquid chromatography(HPLC) with electrochemical detection[22-24].

Lipid peroxidation in hepatic tissue and serum was estimated by measuring the content of malondialdehyde(MDA) according to the method of Uchiyama and Mihara[25]. Samples were homogenized in ice-cold 1.15% KCl(5% wt/vol). A 0.5 mℓ of homogenate was added to 3mℓ of 1% phosphoric acid and 1mℓ of 0.6% thiobarbituric acid. The mixture was heated for 45 min on a boiling water bath. After addition of 4mℓ of n-butanol, the contents were vigorously vortexed and centrifuged at 200×g for 20 min. The absorbance of the upper, organic layer was measured at 535 and 520 nm with diode array spectrophotometer, and was compared to results obtained using freshly prepared malondialdehyde tetraethylacetal standards. MDA values were expressed pmoles per mg protein. Protein was measured by the method of Bradford[26].

Statistical analysis

The data are expressed as the mean±SE. The differences between groups were analyzed by Student's t-test. The significance level was set at $p<0.05$.

Ⅳ. Results

Effects on serum corticosterone level

NNR reduced serum level of corticosterone. As appearing in Fig. 2, physically FS stressed mice showed high level of corticosterone. This result indicates physical stress shows higher level of serum corticosterone than sociopsychological stress. The numerical values of FS, control

and sample were 298.1±6 ng/㎖, 228.4±7 ng/㎖ and 172.2±4 ng/㎖.

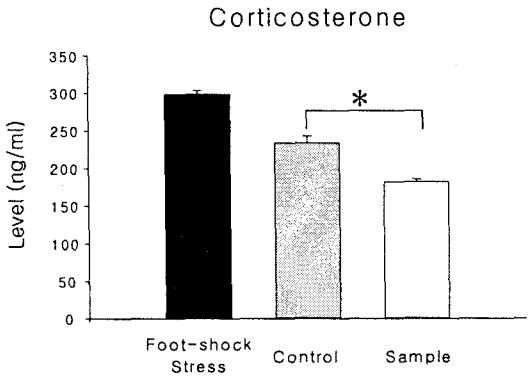

Fig. 2. Effect of Nodus Nelumbinis Rhizomatis extract(NNR) on corticosterone level of ICR-mice for 5 days. Control, psychological stress group. Sample, sociopsychological stress group, and were administered NNR containing 100㎎/㎏/day. *, significantly different when compared with control($p<0.05$).

Effects on noradrenaline level in brain

NNR showed no significant changes in noradrenaline level in brain. As appearing in Fig. 3, physically FS stressed mice showed low level of noradrenaline. The numerical values of FS, control and sample were 60.1±7.8 ㎍/g brain, 133.1±10.1 ㎍/g brain and 129.4±9.8 ㎍/g brain.

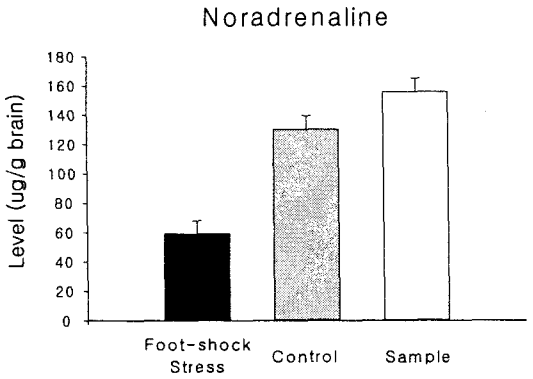

Fig. 3. Effect of NNR on noradrenaline level in brain dorsal cortex area of ICR-mice for 5 days. Control, psychological stress group. Sample, sociopsychological stress group, and were administered NNR containing 100㎎/㎏/day

Effects on lipid peroxidation in liver

NNR showed no significant changes on lipid peroxidation in the liver of mice. As appearing in Fig. 4, physically FS stressed had potential influence of elevating lipid peroxidation in the liver. The numerical values of FS, control and sample were 175.2±7.8, 138.2±16.2 and 143.3±14.1 pmole/㎎ protein.

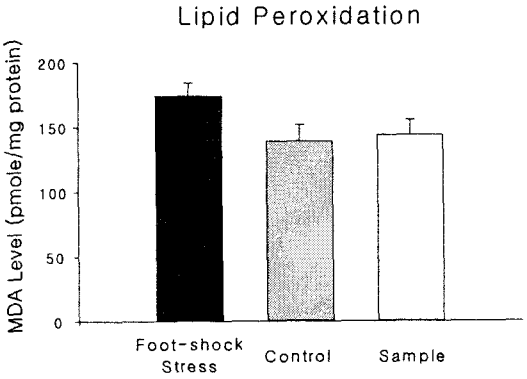

Fig. 4. Effect of NNR on lipid peroxidation in the liver of ICR-mice for 5 days. Control, psychological stress group. Sample, sociopsychological stress group, and were administered NNR containing 100㎎/㎏/day.

Effects on serum alanine aminotransferase

NNR showed significant decrease on serum level of ALT. As appearing in Fig. 5, physically FS stressed had potential influence of elevating ALT level. The numerical values of FS, control and sample were 32.1±1.2 Karmen/㎖, 27.5±2.1 Karmen/㎖ and 22.1±1.2 Karmen/㎖.

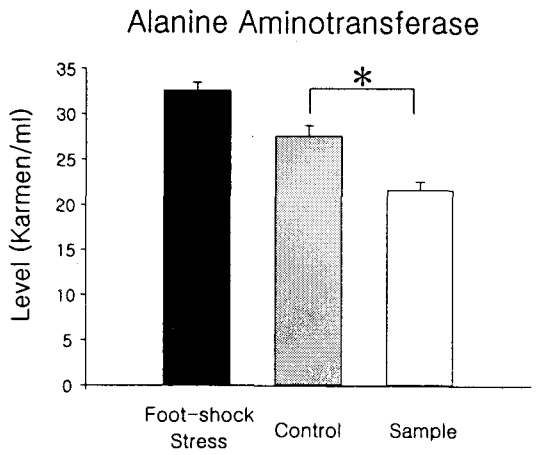

Fig. 5. Effect of NNR on serum alanine aminotransferase(ALT) level of ICR-mice for 5 days. Control, psychological stress group. Sample, sociopsychological stress group, and were administered NNR containing 100㎎/㎏/day. *, significantly different when compared with control($p<0.05$).

Effect on serum lipid peroxidation

NNR showed no significant changes on serum lipid peroxidation as appearing in Fig. 5. The numerical values of FS, control and sample were 3.3±0.3, 2.8±0.3 and 2.7±0.3 pmole/㎎ protein.

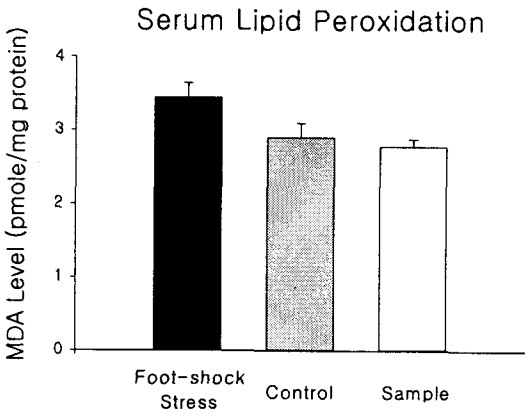

Fig. 6. Effect of NNR on lipid peroxidation in the serum of ICR-mice for 5 days. Control, psychological stress group. Sample, sociopsychological stress group, and were administered NNR containing 100㎎/㎏/day.

V. Discussion

Nodus Nelumbinis Rhizomatis(NNR) are tuberous roots of the lotus water lily, *Nelumbo nucifera* Gaertn., family Nymphaeaceae. In oriental medicine, NNR was considered having sweet taste, acts on lung, stomach and liver, and has the effect of hemostasis with astringents and promoting blood circulation by removing blood stasis. Uncooked lotus root juice can clear heat and stop all, internal bleeding; cooked lotus root can promote blood, treat women of anemia due to heavy menstruation, clear and improve energy[18].

The lotus root is about 8 inches long, and about two inches in diameter. On the outside, the skin of the lotus root is smooth and green in color; on the inside, several large air pockets run the length of the tuber for buoyancy in the water. When a root is sliced in half it resembles a wagon wheel because of these large air pockets. Essentially all parts of the water lily plant are edible - roots, stems, leaves, and seeds[27].

Numerous observations exist to indicate alterations in behavioral and biochemical characteristics in depressed patients. The present study shows that NNR resulted changes in an animal model of sociopsychological stress. Various kinds of methods, such as immobilization, cold water immersion and predictable or unpredictable electric foot shock have been used as those for loading psychological stress inducing anxiety to animals. The influence of non-physical factors of stress including anxiety and fear on animal behaviors is, however, difficult to demonstrate objectively by utilization of these methods. Therefore,

the communication box method was applied to load only psychological stress to animals(Fig. 1). The application of foot shock to the sender mice induced remarkable conditioned emotional stimuli to the responder mice, such as visual, auditory and olfactory sensations from the sender mice.

Plasmatic levels of corticosterone display a circadian rhythm, with the higher values occurring during the dark phase in nocturnally feeding animals. Stressful situations induce a rise of corticosterone levels and this endocrine response to stress also presents circadian variations[28]. NNR reduced serum level of corticosterone, and physical stress may induce higher level of serum corticosterone than sociopsychological stress(Fig. 2).

The effect of sociopsychological stress on central neurotransmission was evaluated in mice. Noradrenaline levels were measured in discrete brain regions following exposure to stress. Noradrenaline levels decrease by stress in the dorsal cortex of mice[29]. NNR showed no significant changes in noradrenaline level in brain. And physically FS stressed mice showed low level of noradrenaline(Fig. 3).

Lipid peroxidation is a free radical-related process that may occur in biologic systems. Free radicals circulate through the body and attack macromolecules like DNA, lipids in membranes, and cellular proteins of the body. The antioxidant system present in the body controls the damage caused by free radicals[30]. Free radical activity is involved in the pathogenesis of many diseases including heart and cardiovascular system[31]. NNR showed no significant changes on lipid peroxidation in the liver and serum. And FS stress have potential influence of elevating lipid peroxidation in the liver(Fig. 4, 5).

ALT is found primarily in the liver and kidneys, with lesser amounts in the heart and in skeletal muscles[32-34], and has been regarded as one of the most sensitive indicators of hepatocellular damage. NNR showed significant decrease on serum level of ALT(Fig. 6).

In general, chronic stress exposure has an influence on the circadian rhythms on various conditions including body temperature, sleep/wake cycle, and food intake, which should be normally observed in human and animals.

The present study suggests that an exposure to sociopsychological stress, but not physical stress, causes a significant changes in pathological animal model. And NNR administration played a positive role in above stress condition.

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