Piperis Nigri Fructus Extract Ameliorates Psychological Stress in Mice

Lee So Young[1] · Choi Jae Hong[2] · Jeong Hyang Sook[3] · Kim Young Gyun[4] · Cho Su In[1]*

생쥐의 심리적 스트레스에 대한 胡椒의 효과

이소영[1] · 최재홍[2] · 정향숙[3] · 김영균[4] · 조수인[1]
[1]부산대학교 한의학전문대학원 · [2]최 한의원 · [3]대구한의대학교 일반대학원 ·
[4]동의대학교 한의과대학

스트레스로 인한 인체의 반응은 중추신경계, 시상하부, 변연계 및 기타 표적기관으로부터 시작되는데, 자율신경계 반응, 내분비계 반응, 면역계 반응 등을 통하여 복합적인 신체 증상으로 발현되며, 스트레스 상황에서는 시상하부-뇌하수체-부신 축 (HPA axis)과 교감신경계의 작용으로 여러 신경전달물질 방출에 변화가 생기며 이러한 변화는 면역기전에 중요한 역할을 하고 일부는 면역세포의 활성에 직접적으로 영향을 미쳐 신체 각종 질병의 원인이 될 것으로 추정된다.

한의학에서는 天人相應의 관점에서 六氣를 생체자극의 외적 요인으로 간주하고, 생체내적 현상인 정신이 외적 자극을 통하여 나타나는 생체반응을 七情으로 보았으며, 이러한 관점에서 스트레스는 신체에 五臟의 虛實, 血虛, 精損, 氣逆, 氣의 순환장애, 痰涎, 火 등의 병적인 요인을 만들어 준다

본 연구에서 재료로 사용된 호초 (Piper nigrum Linne)는 후추나무의 과실을 말린 것으로서 세계적으로 널리 사용되는 향신료이며, 한의학에서는 溫中除寒下氣, 快膈消痰, 解毒등의 효능으로 寒痰食積 脘腹冷通 癩亂 吐瀉등의 치료에 활용되어 왔다 특히 快膈消痰하는 작용은 정신적 스트레스에 유효할 것으로 생각되므로 본 연구에 이용하게 되었다. 실험동물은 ICR계 생쥐를 이용하였으며, 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, 胡椒 추출물을 100㎎/㎏/day 용량으로 5일간 투여한 실험군은 아무런 처치를 하지 않은 대조군에 비해 혈장 중 corticosterone 함량이 유의하게 감소되었고, 뇌에서의 noradrenalin 분비량이 유의하게 증가되었으며, plus maze test에서의 머무름 시간이 연장되는 것으로 나타나 胡椒가 심리적 스트레스를 효과적으로 억제하고 진정작용이 있는 것으로 사료되나 구체적인 작용기전 및 인체에서의 효과에 대해서는 향후의 보다 자세한 연구가 필요할 것으로 생각된다.

Ⅰ. Introduction

Psychological stress triggers a number of physiological responses that can be deleterious under some circumstances and stress signals activate the hypothalamus-pituitary-adrenal (HPA) axis and the sympathetic nervous system, and also intensive stress that response results in creation of reactive oxygen species and oxidative stress is generated from the toxic

* 교신저자 : 조수인. 부산대 한의학전문대학원.
E-mail : sicho@pusan.ac.kr
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levels of oxygen-derived reactive oxygen species (ROSs)[2]. These ROSs are the result of normal metabolism, including the energy generation process of aerobic respiration, and the β-oxidation of fatty acids[3], and the produced ROSs can lead to damage of a wide range of cellular biological molecule[4].

The communication box method can produce psychological stress in animals, since they can perceive the responses of other animals exposed to physical stress delivered through an electric foot shock and this kind of intra-species psychological stress was detected in earlier studies and further confirms that animals subjected to experimental anxiety within the communication box have increased stress hormones[5].

Piperis Nigri Fructus has been used spice as well as herbal medicine in worldwide and has function of anti-oxidant, anti-inflammation, anti-cancer, bioavailability of drugs[6,7].

The present study was designed to induce experimental psychological stress, such as anxiety, in mice using an emotional stress paradigm called intra-species emotional communication within a communication box, and we investigated anti-psychological activity of Piperis Nigri Fructus extract (PNFe) in mice following exposure to psychological stress induced by the communicaiton box.

Ⅱ. Materials and methods

1. 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. The animals had free access to standard pellet chow (Daehan experimental animal, Korea) and tap water given through drinking bottles. Experiments were conducted between 9:00 and 14:00 h and the stress exposures were carried out in a separate room.

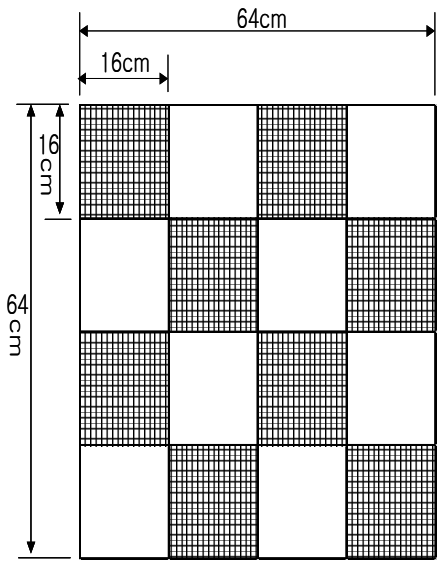
2. PNFe preparation

Piperis Nigri Fructus were purchased in the special herb market (Songsan Herb, Gwang-Ju, Korea) and good samples carefully selected. In order to fractionate the aqueous extract, 300g of the dried herb of Piperis Nigri Fructus was boiled with 5,000 ㎖ 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 46.7g. The extract stored in deep freezer when unused, and freshly diluted for experiment. Mice were fed orally with the dose of 100㎎/㎏ /day for five days.

3. Chronic stress exposures

Stress was applied by the methods which Ogawa[8] developed. Mice were divided into four groups, i.e., a normal group, foot shock (FS) group and two chronically stressed control and sample groups. The first group was non stressed mice, the FS group was physically stressed mice that were given foot shock stress, the third and the fourth group was for psychologically stressed mice 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). Control group had no herbal remedy, but the fourth group (sample) had remedy of PNFe 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 1h/day and lasted for 5 days. Immediately after the stress exposure, all mice were returned to their home cages in their room.



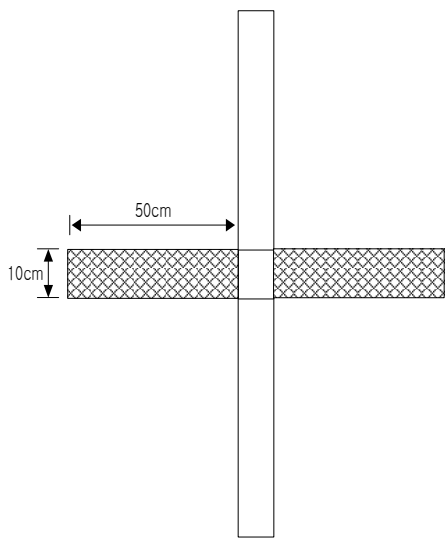
Scheme 1. Scheme of the communication box. Foot shock mice were placed individually in the eight shaded areas (foot shock compartments). Sociopsychological mice were placed in the eight solid areas (non-foot shock compartment). Foot shocks were delivered in shaded areas.

4. Preparations of brain homogenates

At the end of the experimental periods, mice were deprived of food for 24h and then prepared for experimental procedure under ether anesthesia. The whole brain was immediately removed and washed with cold saline. Brain was homogenized with homogenizator in cold potassium phosphate buffer (50mM; pH 7.4). Some of the homogenate was centrifuged at 700×g for 20min in order to determine thiobarbituric acid-reactive substances (TBARS) levels.

5. Transfer latency in elevated plus-maze

The plus-maze (Scheme 2) consisted of two opposite open arms, 50×10㎝, crossed with two enclosed arms, of the same dimensions with 40㎝ high walls. The arms were connected with a central square(10×10 ㎝) to give the apparatus a plus sign appearance. The maze was kept in a dimly lit room elevated 50㎝ above floor level.



Scheme 2. A bird's eye view of plus-maze. Solid area consisted of two opposite open arms, and shaded area crossed with two enclosed arms with 40㎝ high walls. The arms were connected with a central square

(10×10㎝) to give the apparatus a plus sign appearance.

6. Measurement of plasma corticosterone

The whole blood was centrifuged at 1500×g for 10 min at 4℃ to separate plasma from erythrocytes. Plasma was used for the measurement of corticosterone levels. The ImmuChem 125I Corticosterone RIA kit (ICN Biomedicals, Costa Mesa, CA) that is designed for use in laboratory mice and rats were used for measuring plasma corticosterone levels in all experimental groups. The plasma and standard samples that were diluted with steroid diluent incubated with 125I-corticosterone and corticosterone antiserum for 2 h. After the precipitation step, the samples were centrifuged at 1000×g and 4℃ for 15min. The supernatant was removed. The radioactivity of the remaining pellet was counted by using Gamma-counter GC-20. The resulting concentrations of plasma corticosterone were expressed as ng/㎖ using corticosterone standards prepared in different concentrations.

7. Measurement of noradrenalin level of brain tissues

Noradrenalin level of brain tissues were measured by ion pairing reverse phase high pressure liquid chromatography (HPLC) with electrochemical detection[9-11].

8. Thiobarbituric acid-reactive substances (TBARS) assay

TBAR level was measured by a fluorometric method described by Wasowicz et al.[12] and Gumuslu et al.[13] using 1,1,3,3-tetraethoxypropane as standard, and the results were given as nmol MDA/mg protein

9. Transfer latency in elevated plus-maze

A mouse was individually placed on the end of one of the open arms, facing away from the center, and the time taken by the animal to enter one of the closed arms (transfer latency, TL) and frequency of entries during 300sec were recorded with the help of a stop watch.

10. 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 $p<0.05$.

Ⅲ. Results

1. Effect of PNFe treatment in the plasma corticosterone

PNFe reduced plasma level of corticosterone. As appearing in Fig. 1, psychologically stressed mice showed high level of corticosterone. This result indicates physical stress shows higher level of plasma corticosterone than sociopsychological stress. The numerical values of FS, normal, control and sample were 642±17ng/㎖, 162±14ng/㎖, 411±21ng/㎖ and 226±34ng/㎖.

2. Effects on noradrenalin level of brain tissues

PNFe showed significant changes in noradrenalin level in brain. As appearing in Fig. 2, physically FS stressed mice showed low level of noradrenalin. The numerical values of FS, normal, control and sample

were 72.3±8.7㎍/g brain, 182.6±8.4㎍/g brain, 141.2±14.6㎍/g brain and 178.2±10.2 ㎍/g brain.

3. Thiobarbituric acid-reactive substances (TBARS)

TBARS levels were found to be increased in all stress groups. TBARS level in sample group was higher than in control group (Fig. 3). The highest increased TBARS level was observed in FS group. The numerical values of FS, normal, control and sample were 6.2±0.8, 1.9±0.4, 4.6±0.7 and 4.6±0.5nmol MDA/㎎ protein.

4. Effect of a PNFe treatment in the elevated plus-maze

PNFe treatment showed an anxiolytic-like effect at 100㎎/㎏. The vehicle treated control group typically avoided spending time on or entering into open arms (Fig. 4). Vehicle treated mice remained for 70.1±10.6sec in the open arms, whereas PNFe treated mice spent significantly more time in the open arms.

In addition, PNFe treated mice made less entries into the open arms than the vehicle-treated mice (Fig. 5). However, no significant change was observed between groups.

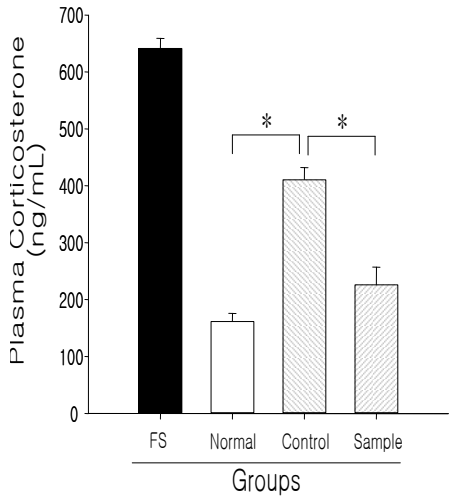


Fig. 1. Effect of Piperis Nigri Fructus extract (PNFe) on corticosterone level of ICR-mice for 5 days. Normal, normal group. FS, foot shock stress group. Normal, normal group. Control, psychological stress group. Sample, psychological stress group, and were administered PNFe containing 100㎎/㎏/day. *, significantly different when compared ($p<0.05$).

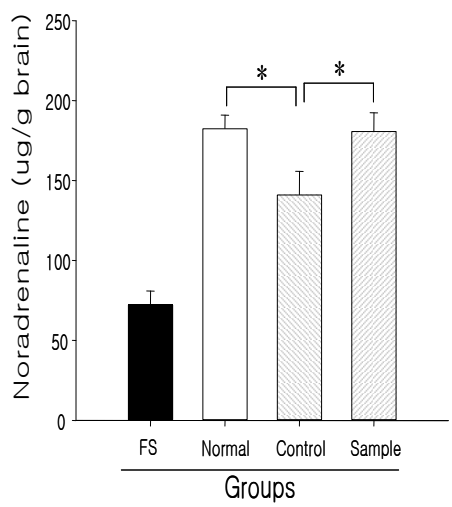


Fig. 2. Effect of PNFe on noradrenalin level in brain dorsal cortex area of ICR-mice for 5 days. FS, foot shock stress group. Normal, normal group. Control, psychological

stress group. Sample, psychological stress group, and were administered PNFe containing 100㎎/㎏/day. *, significantly different when compared ($p<0.05$).

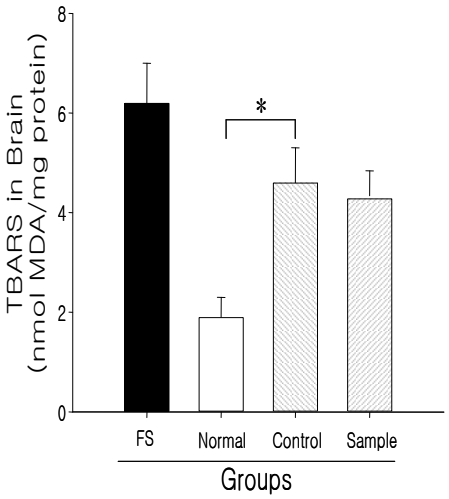


Fig. 3. Effects of PNFe on the psychological stress on the brain thiobarbituric acid-reactive substances (TBARS) levels. Values represent mean±S.E. of eight mice per group. FS, foot shock stress group. Normal, normal group. Control, psychological stress group. Sample, psychological stress group, and were administered PNFe containing 100㎎/㎏/day. *, significantly different when compared ($p<0.05$).

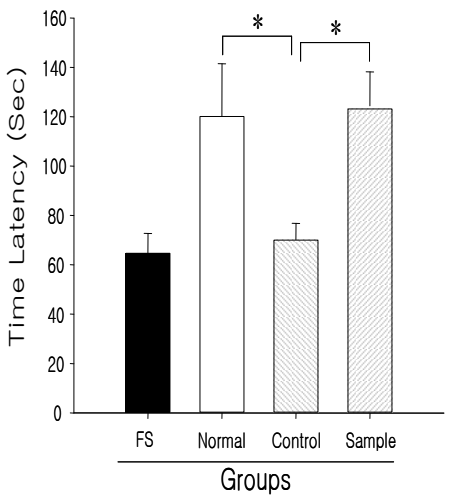


Fig. 4. Effects of PNFe on the psychological stress on the time spent in open arms of the elevated plus-maze test in mouse. Values represent mean±S.E. of eight mice per group. FS, foot shock stress group. Normal, normal group. Control, psychological stress group. Sample, psychological stress group, and were administered PNFe containing 100㎎/㎏/day. *, significantly different when compared ($p<0.05$).

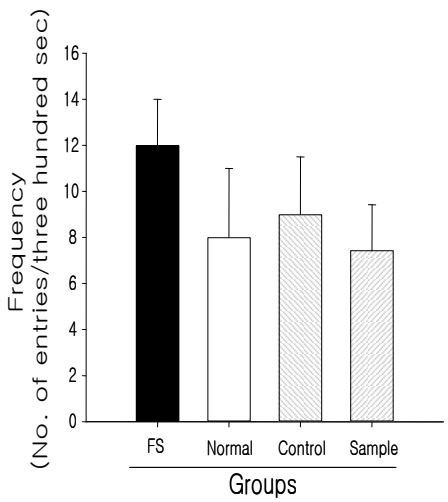


Fig. 5. Effects of PNFe on the psychological stress on the number of arm entries into the closed arms of the elevated plus-maze test in mouse. Values represent mean±S.E. of eight mice per group. FS, foot shock stress group. Normal, normal group. Control, psychological stress group. Sample, psychological stress group, and were administered PNFe containing 100㎎/㎏/day.

Ⅳ. Discussion

The accumulating evidence that stress-related factors contribute to the development of cardiovascular disease[14]. Concerning stress in animal model, The most widely used animal models of anxiety in the last two decades have been the elevated plus mazes[15].

Using elevated plus maze, several measures of anxiety and/or locomotion: percent time spent in the open arms, percent entries into the open arms, number of entries into both the open and closed arms, and number of entries only into the closed arms[15].

Piper nigrum is a perennial vine with a trailing or climbing stem, round, smooth, shrubby, flexuose, dichotomously branched, jointed, swelling at the joints, and often throwing out radicles there which adhere to bodies like the roots of ivy, or become roots striking into the ground, and a native of the East Indian continent, notably the Malabar coast, as well as of many islands in the Indian ocean, where it is extensively cultivated, as well as in the West Indies. Commercial grades are known as Malabar, Singapore, Penang, Sumatra pepper, etc.. Recent studies on Piper nigrum were carried out by Reddy and et al.[16] and other researchers[17-21].

The hypothesis of this study was that psychological stress could induce changes in mice's behavoir and serum level of hormones which are related stress, and an additional goal of this study was to determine whether The mouse on the psychological stress could return to normal after PNFS was treated.

After treated PNFe, PNFe reduced serum level of corticosterone, and physical stress may induce higher level of serum corticosterone than sociopsychological stress (Fig. 1).

The effect of psychological stress on central neuro-transmission was evaluated in mice. Noradrenalin levels were measured in discrete brain regions following exposure to stress. Noradrenalin levels decrease by stress in the dorsal cortex of mice[22]. PNFe showed significant changes in noradrenaline level in brain. And physically FS stressed mice showed low level of noradrenaline (Fig. 2).

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[23]. Free radical activity is involved in the pathogenesis of many diseases including heart and cardiovascular system[24]. PNFe showed no significant changes on lipid peroxidation in the serum and brain (Fig. 3).

The elevated plus-maze test is designed to detect the effect of anxiolytic drugs[25]. Inexperienced mice will normally prefer to spend much of their allotted time in the former. This preference appears to reflect an aversion towards open arms, generated by fear of space. PNFe administration showed significant increase of latency time (Fig. 4). No significant result was shown in arm entry test (Fig. 5) but decreasing tendency of entrance frequency was observed, and this suggest PNFe might have sedative effect.

In general, chronic stress 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. Our study suggests that an exposure to psychological stress, but not physical stress, causes a relevant changes in pathological animal model. Natural anxiolytic agents feature in such research because herbs have been used to treat psychiatric disorders and generally have fewer harmful effects[26], and PNFe administration played a positive role in above stress condition.

Ⅴ. Conclusion

The experiments in this study indicated that psychological stress could induce behavior change through exposure to repeated stress in mouse. We also find out that PNFe could return close to normal level on the psychological stress in the elevated plus-mazed test in mice. Further studies concerning the mechanisms of PNFe in induced psychological stress condition would be revealed.

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