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Original Article

Effects of pine nodule extract, Sho-ko-sen, on mental stress-induced changes in the autonomic nervous balance in young students

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ABSTRACT

Extract of pine nodules and its component, SJ-2, have been reported to have an inhibitory effect on catecholamine secretion induced by acetylcholine (a physiological secretagogue) in cultured bovine adrenal medullary cells. The present study aimed to determine the effect of pine nodule extract, Sho-ko-sen, on the autonomic nervous activity induced by mental stress (Uchida-Kraepelin arithmetic test) in healthy young students. Autonomic nervous balance was measured by power spectral analysis of heart rate variability using a standard hexagonal radar chart. Four 15-min repetitions of an arithmetic task served as an acute mental stressor that caused increases in two sympathetic parameters and an increase in one parasympathetic parameter in the placebo group, while no increases in sympathetic parameters but various changes in parasympathetic parameters were observed in Sho-ko-sen group. The present study demonstrated that acute arithmetic stress-induced increases in sympathetic and parasympathetic nervous activity were detected by the autonomic nervous balance assay in the placebo group, but that such increases in sympathetic nervous activity, at least, were cancelled in healthy young students who ingested Sho-ko-sen (pine nodule extract).

KEYWORDS: autonomic nervous balance, heart rate variability, mental stress, extract of pine nodules.

1. INTRODUCTION

Extracts of pine nodules formed by bark proliferation on the surface of *Pinus tabulaeformis* or *Pinus massoniana* trees have been used as an analgesic for joint pain, rheumatism, neuralgia, dysmenorrhea and other complaints in Chinese traditional medicine [1]. Previous studies report that α-pinene, a natural compound isolated from pine needle oil, has anti-liver cancer cell growth activity [2]. Furthermore, pine nodule extract and its component, SJ-2, have been reported to have an inhibitory effect on catecholamine secretion

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induced by acetylcholine (its physiological secretagogue) in cultured bovine adrenal medullary cells [3].

Cardiac activity is usually regulated by the brain via the vagus nerve, which plays an important role in heart rate variability (HRV) [4]. The power spectral analysis of HRV that has been widely performed is usually divided into low-frequency (LF: 0.04-0.15Hz) and high-frequency (HF: 0.15-0.4Hz) components, which are associated with the sympathetic plus parasympathetic and parasympathetic nervous functions, respectively [5, 6].

Goto *et al*. (1999) [7] previously developed a modified power spectral analysis method using a standard hexagonal radar chart composed of six selected parameters (three sympathetic indexes and three parasympathetic indexes). Using this method, we reported an imbalance in the autonomic nervous activity of women with climacteric symptoms [8]. In the present study, we examined the effect of pine nodule extract on mental stress (arithmetic task)-induced changes in the autonomic nervous balance, and found that the pine nodule extract attenuates arithmetic task-induced increases in sympathetic nervous parameter in healthy young students.

2. MATERIALS AND METHODS

2.1. Materials

Sho-ko-sen was provided from Tokujun Co., LTD (Kobe, Japan). Sho-ko-sen (275 mg of powder/ capsule) is composed of extracts of pine nodule (247.5 mg), leaves of willow (16.5 mg), softshell turtle (8.25 mg), and corn (2.75 mg). The lactose was purchased from Maruishi Pharmaceutical Co. Ltd. (Osaka, Japan). Powders of Sho-ko-sen (0.9 g) or lactose (0.2 g for placebo) were dissolved in 150 ml of water.

2.2. Participants

Participants in the present study were twelve healthy undergraduate girl students (aged 20-22 years; 21.2±0.8) who were not suffering from any chronic or acute illnesses and not any taking medication. They were instructed to drink mainly water, and not to take caffeine-rich beverages such as green tea, coffee, and black tea before 4 hours of the experiment. All the participants received verbal and written information about the study and signed an informed consent form before entering the study. This study was approved by the ethics committee of the Kyushu Nutritional and Welfare University.

2.3. Measurement with autonomic nervous activity

The autonomic nervous activity was measured by a power spectral analysis of heart rate variability (HRV), as previously reported [7, 8]. In brief, signals of the R-R interval were recorded in supine position (sup) for 60 seconds and in subsequent standing position (std) for 90 seconds under constant respiratory frequency (15/min) using a heart rate senser measured by Silmee ^{TM} Bar type Lite (TDK, Tokyo, Japan) constantly at 14:30 to 16:30. We used the following statistical non-spectral and spectral analysis values as six parameters of autonomic nervous activity in the HRV analysis: (1) mean R-R intervals in supine position (mRR (sup)) for 60 seconds, which indicates heart rate at supine rest; (2) ratio of the HF components to the total power component (HF/Total ratio) in the supine position as a parasympathetic parameter; (3) SDRR(sup), which represents the vigor of inherent activity or an activity of inherent bio-balance or inherent vegetative power of balance control; (4) difference between the mRR (sup) and the minimal R-R interval in subsequent standing position (RRmin(std)), mRR(sup)- RRmin(std)), which indicates the momentary irritability reaction level by stimulation of standing; (5) difference in the mean R-R interval between supine position and subsequent standing position (mRR(sup-std)), which indicates the mean excitability against stimulation or stress; and (6) ratio of the LF component of the total power component in standing position (LF/Total(std)), shown as a relative sympathetic activity.

2.4. Procedure

This study had a group comparison design and participants were randomly assigned to Sho-ko-sen (n=6, 21.2±0.4 years) or placebo (n=6, 21.5±0.5 years) groups. The participants did not know whether they were consuming pine nodule extract or placebo. All participants were requested

to avoid eating or drinking, except for water, for 2 hr before the start of each trial. The experiment schedule is shown in Figure 1. This experiment performed under the U-K test (an arithmetic task; Nisseiken, Inc., Tokyo, Japan) for 15 min served as an acute mental stressor. After measurements of blood pressure and autonomic nervous balance and sampling of saliva (1-2 ml) for 15 min, participants took a bolus of powder (0.9 g) of Sho-ko-sen or placebo (lactose, 0.2 g) dissolved with 150 ml of water. After 15 min ingestion of Sho-ko-sen or placebo, participants executed fourth-consecutive tasks for 15 min each, which consists of three arithmetic task alternating between one minute of calculation and one minute of break. After U-K test for 1 minute, blood pressure, pulse rate, and autonomic nervous balance of participants were measured and sampled with saliva (1-2 ml).

2.5. Blood pressure

Blood pressure and plus rate were measured by the blood pressure measurement equipment (OMRON HBP-9020, Tokyo, Japan).

2.6. Assay of α-amylase activity in saliva

α-Amylase activity in saliva was measured using Alpha Amylase Test Kits (SOMA Bioscience, Wallingford, Oxon, UK).

2.7. Statistical analysis

Data are expressed as mean ± SEM. Statistical evaluation of the data was performed with the paired *t*-test. Values were considered significant when *P* was less than 0.05. The statistical analyses were performed using SPSS Statistics, Ver.27 (IBM) for Windows.

3. RESULTS

3.1. Effects of the U-K test on the autonomic nervous balance

We examined the effect of the U-K test as a stressor on the autonomic nervous balance (Figure 1). In placebo group, the U-K test with four arithmetic tasks caused significant increases in two sympathetic parameters namely mRR(sup)-RRmin(std) and mRR(sup-std) and one parasympathetic parameter namely mRR(sup) (Figure 2).

3.2. Effects of Sho-ko-sen on the autonomic nervous balance under the U-K test

The ingestion of Sho-ko-sen, however, cancelled the arithmetic task-induced increases in these two sympathetic indexes. On the other hand, Sho-ko-sen caused various changes in parasympathetic parameters; that is an increase in mRR(sup) and a decrease in HF/total (Figure 3).

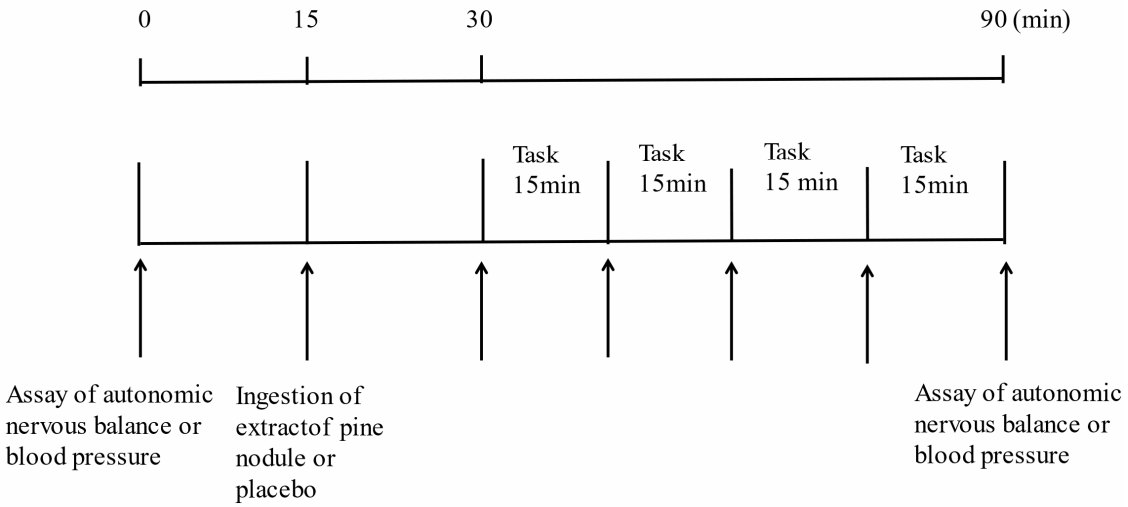
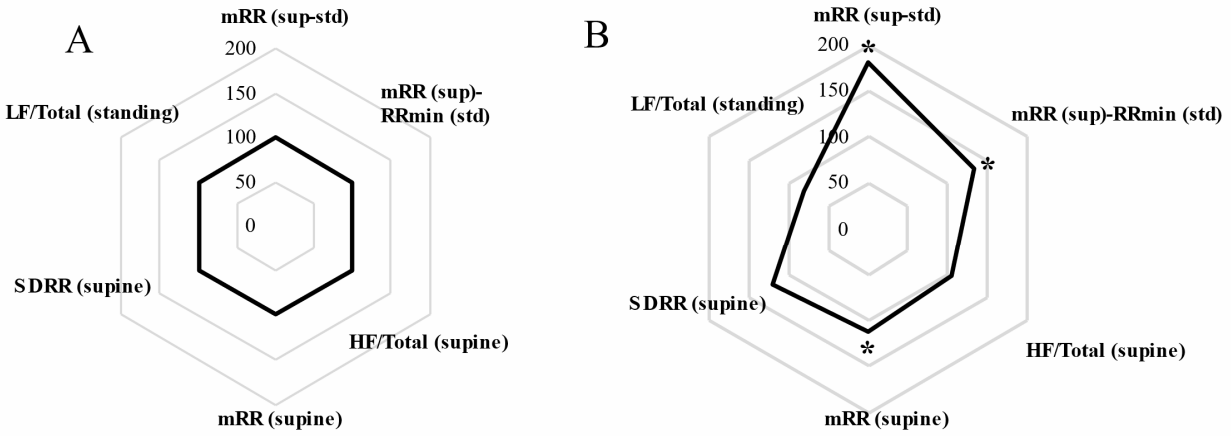


Figure 1. Experimental protocol. The arrowed line indicates the time point at which participants took the water dissolved with Sho-ko-sen (0.9 g) or lactose (0.2 g), autonomic nervous balance and blood pressure were measured, and U-K test was performed.



	Autonomic nervous balance					
	Sympathetic nervous activity			Parasympathetic nervous activity		
	LF/Total	mRR (sup-std)	mRR - RRmin	HF/Total	mRR(sup)	SDRR(sup)
Before (n=6)	0.585 ±0.066	92.2±8.2	213±15	0.733 ±0.041	850±54	58.5±9.3
After (n=6)	0.474 ±0.068	167±21*	282±19*	0.762 ±0.052	952±48*	70.8±10.1

Figure 2. Effects of mental stress (U-K test) on the autonomic nervous balance in placebo group. Upper figure: The autonomic nervous activity before (A) and after (B) performing the U-K test was measured as described in "Methods". Data are expressed as mean ± SEM of Sho-ko-sen group (n=6) and placebo group (n=6). The hexagon radar chart of three sympathetic nervous parameters and three parasympathetic nervous parameters. Lower table: The autonomic nervous activity of control (placebo) subjects was measured as described in 'Methods'. Data are expressed as means ± SEM of control (placebo) subjects (n=6). *P<0.05, compared with each parameter before exposure to the U-K test.

3.3. Effects of the U-K test on blood pressure fluctuation and α-amylase activity in saliva

We also examined the effect of the U-K test on blood pressure fluctuation and α-amylase activity in saliva. After the U-K test, there was little change in systolic or diastolic pressure of blood pressure (Table 1) and α-amylase activity in saliva (Figure 4) in placebo or Sho-ko-sen group.

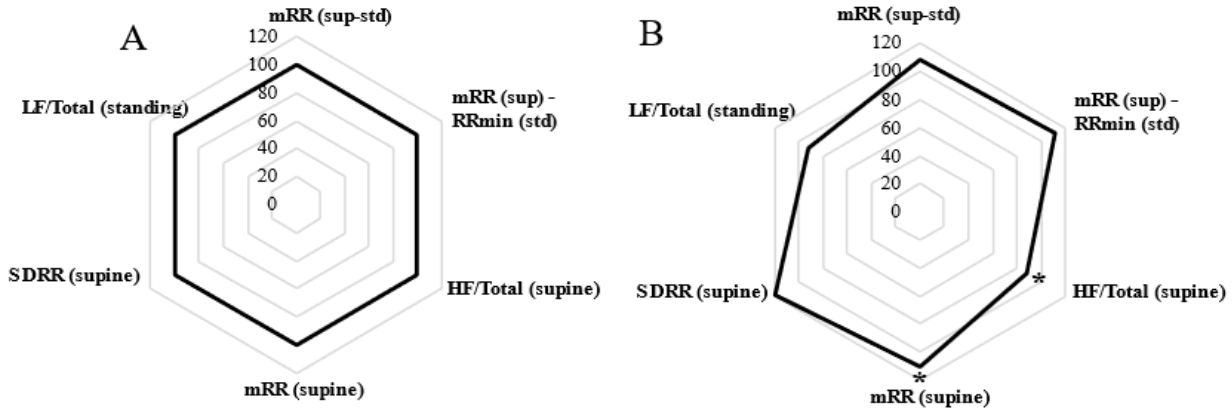
4. DISCUSSION

4.1. Effect of arithmetic tasks on the autonomic nervous balance

In the present study, we demonstrated the effects of Sho-ko-sen on changes in the autonomic nervous balance induced by mental stress (arithmetic tasks) in healthy young students by using power spectral analysis of HRV, as previously reported [7, 8]. The main finding of the present study is that acute mental stress elicited by arithmetic tasks increased the mRR(sup)- RRmin(std) and mRR (sup-std) indexes in the sympathetic nervous activity each of which shows the momentary irritability reaction and the mean excitability induced by standing. These results suggest that arithmetic-induced stress enhances the stimulation of sympathetic nervous activity induced by standing. This is consistent with a previous report that arithmetic tasks adequately functions as an acute stressor [9].

4.2. Effect of Sho-ko-se on arithmetic task-induced changes in the autonomic nervous balance

Sho-ko-sen cancelled these responses of sympathetic nervous activity induced by acute mental stress. To the best of our knowledge, this is the first direct evidence to show that Sho-ko-sen causes



	Autonomic nervous balance					
	Sympathetic nervous activity			Parasympathetic nervous activity		
	LF/Total	mRR (sup-std)	mRR - RRmin	HF/Total	mRR(sup)	SDRR(sup)
Before (n=6)	0.737 ± 0.053	202 ± 82	286 ± 22	0.799 ± 0.043	851 ± 36	55.8 ± 4.2
After (n=6)	0.678 ± 0.056	219 ± 36	320 ± 37	0.701 ± 0.047*	942 ± 49*	66.3 ± 5.4

Figure 3. Effects of mental stress (U-K test) on the autonomic nervous balance in Sho-ko-sen group. Upper figure: The autonomic nervous activity before (A) and after (B) performing the U-K test was measured as described in 'Methods'. Data are expressed as mean ± SEM of Sho-ko-sen group (n=6). Lower table: The autonomic nervous activity of Sho-ko-sen group was measured as described in 'Methods'. Data are expressed as means ± SEM of Sho-ko-sen group (n=6).

Table 1. Effects of mental stress on systolic and diastolic blood pressure in placebo and Sho-ko-sen groups. The systolic and diastolic of blood pressures of healthy young students were measured as described in 'Methods'. Data are expressed as mean ± SEM of Sho-ko-sen (n=6) and placebo group (n=6).

	Systolic blood pressure (mmHg/dl)		Diastolic blood pressure (mmHg/dl)	
	Before	After	Before	After
Placebo	104 ± 2	106 ± 4	63 ± 3	63 ± 2
Pine nodule	101 ± 5	103 ± 3	63 ± 4	64 ± 2

a reduction of the stress responses-induced increases in sympathetic nervous activity by acute arithmetic tasks.

The inhibitory mechanism of Sho-ko-sen on the stimulation of sympathetic nervous activity induced by arithmetic stress, however, remains to be determined. In our previous study, pine nodule extract and its component, SJ-2, which were main components in Sho-ko-sen, were found to inhibit acetylcholine-induced catecholamine synthesis and secretion through suppression of nicotinic acetylcholine receptor-ion channels in cultured bovine adrenal medullary cells [3]. Since catecholamines play a very important role in modulating heart rate, blood pressure, and blood glucose levels, as well as the general reactions of

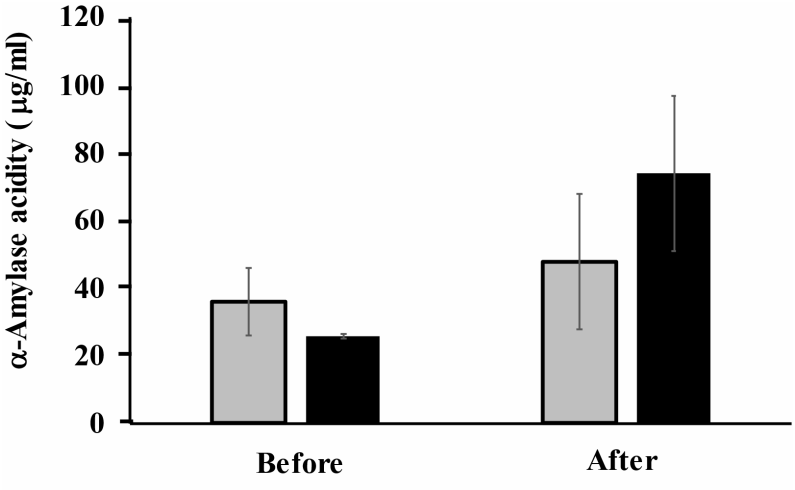


Figure 4. Effects of mental stress on α-amylase activity in saliva in placebo and Sho-ko-sen groups. α-Amylase activity in saliva before (A) and after (B) performing the U-K test was measured as described in 'Methods'. Data are expressed as mean ± SEM of Sho-ko-sen (n=6) and placebo group (n=6).

the sympathetic nervous system [10], we speculate that pine nodule extract attenuates arithmetic stress-induced activation in the excitability index of sympathetic nervous activity, in part, *via* the suppression of nicotinic acetylcholine receptors in the peripheral and/or central sympathetic nervous system. Further *in vivo* and *in vitro* studies are required to confirm this possibility.

In placebo group, arithmetic tasks also increased mRR(sup) which shows a decrease in heart rate, suggesting, in part, an increase in parasympathetic nervous activity. These results give rise to the possibility that arithmetic tasks influence both sympathetic and parasympathetic nervous activities. On the other hand, Sho-ko-sen caused various changes in parasympathetic parameters, that is an increase in mRR(sup) and a decrease in HF/total. The former change in mRR(sup) was also observed in placebo group, suggesting a common phenomenon induced by arithmetic stress. The mechanism of latter change in HF/total induced by Sho-ko-sen, however, is not explainable at present. Further experiments would be required to resolve this complex phenomenon.

4.3. Effect of arithmetic tasks on blood pressure and α-amylase activity in saliva

Regarding fluctuations in blood pressure and α-amylase activity in saliva, the arithmetic tasks had little effect on systolic or diastolic blood pressure and α-amylase activity in saliva. Based on these results, it is possible to infer that the assay system used to test the autonomic nervous balance in the present study has more sensitivity than that of assays of the blood pressure and α-amylase activity in saliva to detect small changes in the autonomic nervous balance induced by mental stresses.

5. CONCLUSION

In conclusion, we detected increases in two parameters of the sympathetic nervous system induced by the mental stress in placebo group, which were cancelled by ingesting Sho-ko-sen.

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AUTHOR CONTRIBUTIONS

N. Y. designed this research and wrote the paper; M. T., H. A., T. K., and T. H. helped to perform research; H. S., N. S., M. Tsutsui and K. A. analyzed data and discussed research. M. T. and H. A. equally contributed to the work. All authors revised the paper and approved the final version.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest regarding the publication of this paper.

ABBREVIATIONS

HR, heart rate; HRV, heart rate variability; s-IgA, salivary immunoglobulin A; sup, supine position; std, standing position; mRR(sup-std), difference in the mean R-R interval between supine position and subsequent standing position; SDRR (sup), the standard deviation of mean R-R intervals in spine position; mRR (sup), mean R-R interval in supine position; HF/Total ratio, ratio of the high frequency components to the total power component in the supine position; mRR(sup)-RRmin (std), difference between the mRR (sup) and the minimal R-R interval in subsequent standing position; LF/Total (std), ratio of the low frequency component of the total power component in standing position; S/P ratio, the ratio of sympathetic/parasympathetic nervous activity. U-K test, Uchida-Kraepelin arithmetic task test.

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