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Research Article

Aqueous Extract of Asparagus (Instant Asparagus Powder®) Reduces Psychological and Physiological Stress Responses

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Abstract

The present study was designed to investigate the anti-stress activity of Aqueous Extract of Asparagus (AEA) in a laboratory setting by the Trier Social Stress Test. Twenty four subjects were randomly divided into three groups: aqueous extract of asparagus, placebo and control treatment. The experimental sessions were performed by single-blinded, and the order of them was counterbalanced. The experiment protocol began with a 30 min rest period (baseline), followed by the Trier Social Stress Test task for 20 min and two rest periods. The saliva samples and psychological measure (state anxiety scores) were obtained at the end of each period. Heart Rates (HR), Systolic and Diastolic Blood Pressure (SBP, DBP) were measured using an Omron electronic blood pressure monitor. The results showed that aqueous extract of asparagus intake resulted in a significant reduction of state anxiety score, HR and SBP responses to an acute stress task relative to the placebo control condition. Moreover, analyses of saliva cortisol level indicated that the reductions in saliva cortisol level was likely attributable to an attenuation of sympathetic nervous activation. Thus, it was suggested that the oral intake of aqueous extract of asparagus could cause anti-stress effects via the inhibition of cortical neuron excitation. These results might suggest AEA consumption as an innovative and effective approach to reduce psychological and physiological stress responses.

Introduction

According to the "Anxiety and Depression Association of America (ADAA)" anxiety disorders are the most common mental illness in the U.S.A. with 18% (40 million) of the adult population being affected [1,2]. The total annual cost of anxiety disorders has been estimated to be between $42.3 billion and $46.6 billion, of which more than 75% can be attributed to morbidity, mortality, lost productivity, and other indirect costs [3-5]. Anxiety disorders are treatable, and the vast majority of people with an anxiety disorder can be helped with professional care. There is an ever-growing body of scientific evidence about Complementary and Alternative Medicine (CAM), which is an approach to health care that exists outside conventional medicine practiced in the United States [6]. CAM is increasing in interest as consumers and health care professionals search for additional ways to treat health disorders. Interest in alternative medicine and plant-derived functional food to conquer stress and promote relaxation has increased recently. For example, kava, a plant found in the South Pacific, has been shown to be safe and effective in treating anxiety and improving mood [7]. Therefore, the search for new therapeutic agents from traditional Chinese medicine is a good strategy.

Asparagus *officinalis* L., a well-known nutritious and healthy vegetable, was applied widely in the traditional Chinese medicine clinic practice for thousands years. In recent years, various healthful effects of asparagus have been scientifically verified by the research reports on neural and mental [8-11]. The woody part (inedible bottom part) is always discarded. However, this woody stem of asparagus still contains many bioactive substances which suggest its potential application in functional food for its therapeutic effects. Asparagus industrial product development is in the ascendant, in China. In early 2013, the Chinese mainland authorized the aqueous extract of asparagus woody stem (AEA, Instant asparagus powder®) as a natural functional food and beverage ingredient. The present study was designed to investigate the activity on reducing the psychological and physiological stress of AEA in healthy volunteers.

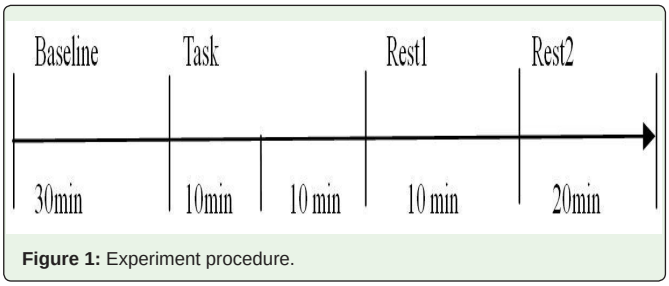

Figure 1: Experiment procedure.

Materials and Methods

Plant material

Asparagus *officinalis* L. (Green Asparagus, cv. Grande F1) was collected from cultivation farm in Hangu administration zone of Tangshan, a vegetation zone that belongs to Qinhuangdao Changsheng Agricultural Technology Development Co. Ltd. Botanical samples were identified by Professor Peng of Hebei Normal University of Science & Technology, Qinhuangdao, China.

Extract preparation and character

Aqueous extract from the woody stem of Asparagus *officinalis* L. (Green Asparagus, cv. Grande F1) was provided by Qinhuangdao Changsheng Agricultural Technology Development Co. Ltd, Hebei Province, China. To obtain the aqueous extract from the woody stem of asparagus, fresh plant material (60.0 kg) was cleaned and crushed, then extracted with drinking water (plant/water ratio 1:8 w/v; water temperature: 85±5 °C) by dynamic maceration for 1.5 h.

Table 1: Nutrient and chemical compositions of the AEA.

No	Compositions	Content	Unit
1	Carbohydrates	44.4	g/100g
2	Proteins	24.5	g/100g
3	Lipids	3.0	g/100g
4	Calcium	3.84	g/100g
5	Potassium	1.12	g/100g
6	Magnesium	0.33	g/100g
7	Sodium	0.28	g/100g
8	Ferrum	0.06	g/100g
9	Copper	0.19	g/100g
10	Zinc	0.09	g/100g
11	Vitamin E	0.17	mg/100g
12	Vitamin B1	0.39	mg/100g
13	Vitamin B2	0.89	mg/100g
14	Selenium	0.14	mg/100g
15	Saponins	17.80	g/100g
16	Flavone	2.91	g/100g
17	Polyphenol	5.93	g/100g
18	Polysaccharide	11.21	g/100g
19	GABA	0.82	g/100g

AEA: Aqueous Extract of Asparagus; GABA: Gamma-Aminobutyric Acid.

Table 2: Amino acid analyses of the AEA.

No	Amino acid	Content (g/100g)
1	ASP	5.95
2	THR	0.29
3	SER	0.51
4	GLU	4.77
5	GLY	0.51
6	ALA	0.48
7	VAL	0.53
8	MET	0.07
9	ILE	0.16
10	LEU	0.41
11	TYR	0.23
12	PHE	0.15
13	LYS	0.29
14	HIS	0.11
15	ARG	0. 41
16	PRO	2.60
17	TRP	0.11
18	CYS	0.28
19	Total	17.86

AEA: Aqueous Extract of Asparagus.

After filtering, the solution of asparagus stem was sprayed drying to dryness [12], yielding extract powder (1.0 kg AEA).

The nutrient and chemical components of this studied extract were identified and quantified (shown in Table 1). Amino acid composition analysis was performed by a fully automatic amino acid analyzer (Hitachi L-8900, Japan). The data were shown in Table 2. The main biologically active constituents of asparagus are the steroidal saponins and flavonoids. For quantitative determination of the steroidal saponins, the AEAS were refluxed with hydrochloric acid solution. The sarsasapogenin from asparagus was determined by reversed phase high performance liquid chromatographic method with evaporative light scattering detection (RP-HPLC-ELSD). The content of sarsasapogenin was 17.8%. The fingerprint of the flavonoids from AEAS was established by HPLC-UV (shown in Table 3). Asparagus extract solution were mixed with diethyl ether then stirred. The diethyl ether layer was separated and evaporated to dryness. The resulting residue was dissolved in methanol as sample solution for fingerprint analyses.

The analyses were performed using a Zorbax C18 column and a diode array detector in an Agilent1100 HPLC system. The mobile phase was 0.5% formic acid solution (solvent A) and methanol (solvent B), at a flow rate of 0.7 mL/min. The gradient elution was 0–20 min, 80%–70% A, 20%–30% B, 20–45 min, 70%–60% A, 30%–40% B, 45–60 min, 60%–50% A, 40%–50% B, 60–70 min, 50%–30% A, 50%–70% B, 70–75 min, 30%–0% A, 70%–100% B. The UV detector wavelength set at 320 nm. Five compounds were identified by the spectral feature of UV, MS and MS/MS (Table 3). The main 8 peaks were quantified by comparing their peak areas with the refer substance (Peak 1).

Table 3: The spectrum data of the AEA.

No	UV (nm)	MS	MS/MS	Chemical Name	Relative peak area
1	270nm	595	[595]:331(100),535(19.65),287(8.19)	Kaempferol-3-O-β-rutinoside	1.00
2	270nm	268	[268]:136(100)	7-hydroxy-4'-methoxyisoflavone	0.08
3	330,260	611	[611]:591(100),467(38.97)	Rutin	0.65
4	340,260	317	[317]:298(84),198(100)	Isorhamnetin	0.72
5	320,280	301	[301]:269(100),241(99)	Quercetin	0.67
6					0.63
7					0.46
8					0.33

Experiment 1

Participants: Twenty-four healthy male undergraduate students (age range, 18-21 years; mean = 19.5, S.D. = 1.8) who were not taking any medication known to influence mental participated in the experiment. The study protocol and material were approved by an Institutional Review Board (Asparagus Engineering Research Centers of Hebei Province) and all subjects provided written informed consent prior to participation. This study was conducted in accordance with Good Clinical Practice Guidelines of China. Healthy volunteers were recruited from Hebei Normal University of Science & Technology. The interventions were conducted at the Asparagus Engineering Research Centers of Hebei Province located in Qinghuangdao, China. They were asked to refrain from consuming alcohol and caffeine, exercising rigorously before and during their participation. All participants received a detailed explanation of the study and gave their informed written consent to participate.

Procedure: All testing was conducted individually during the afternoon hours (between14 and 15:30) to minimize the effects of individual differences in the diurnal rhythm of cortisol. The experimental sessions were performed by single-blinded, and the order of them was counterbalanced. After arrival at the laboratory, the first saliva sample (baseline) was obtained prior to treatment. Then participants were asked to complete the State-Trait Anxiety Inventory (STAI) [13]. Heart rates (HR), systolic and diastolic blood pressure (SBP, DBP) were measured using an Omron electronic blood pressure monitor (HEM-7051). 6g of the aqueous extract of asparagus woody stem (Instant asparagus powder*, Qinhuangdao Changsheng Agricultural Technology Development Co. Ltd) dissolved in 150 ml of water was used as a treatment. The safety of the oral administration of AES was reported in Evidence-Based Complementary and Alternative Medicine.

Twenty-four participants were assigned randomly to the treatment, control or placebo groups: (1) oral administration AEA (6g AEA per subject) immediately after first saliva sample collecting (treatment condition); (2) oral administration a cup of water at the same time (placebo condition); (3) no treatment, and rest in place of the task periods (control condition). After the treatment (AEA, placebo or no treatment), participants rested for 30 min. The acute stress responses were triggered by the Trier social stress test task for 20 min, followed by two rest periods (Shown in Figure 1). The saliva samples and psychological measures were obtained at the end of each period. The cardio-dynamic activity was monitored as well as throughout the experimental session.

Trier Social Stress Test (TSST): The TSST was performed similarly to the description provided by Kirschbaum et al. [14,15]. The participant was told to introduce him to a selection committee. After an initial preparation period of 10 min participants had to give a free speech. During the speech (10 minutes duration) he had to convince the committee that he was the perfect applicant for a vacant position (his 'dream job'). The committee was dressed in white coats and was introduced as consisting of psychologists who are specially trained to monitor and analyze verbal and nonverbal behavior. Furthermore it was announced that the participant's performance was recorded on the video-cassette-recorder to later analyze the interview and the nonverbal behavior. If the participant finished his speech in less than 10 min, standardized questions were used.

In the second part, the participant was asked to serially subtract the number 17 from 2043 as fast and as accurately as possible within 10 min. On every failure the psychologist interfered and the participant has to start again at 2043. Both members of the committee acted in a very cold and reserved manner.

Measures: Saliva samples were collected to detect the cortisol levels [15] as biomarkers of stress. The samples were obtained using Salivette sampling devices (SARSTEDT, Germany) before and after treatment. Cortisol levels were measured using a commercially available highly sensitive chemiluminescent immunoassay kits. Inter and intra-assay coefficients of variation were below 15%.

Participants' state anxiety scores during the experiment were measured using the Chinese version of "State Trait Anxiety Inventory (STAI)" questionnaire. This questionnaire consists of a validated 20 item self report assessment, developed in accordance to the English version.

The cardio-dynamic activity (HR, SBP and DBP) was monitored throughout the experimental session using an Omron electronic blood pressure monitor (HEM-7051).

Table 4: Means scores of state anxiety before, during and after Trier social stress test.

Group	Baseline	Task	Rest1	Rest2
Control	38.5±5.0	37.4±5.1	36.9±4.4	40.9±6.5
Placebo	38.1±4.7	49.1±4.4 †	41.9±3.6 †	38.1±4.7
AEA	39.5±3.1	44.5±4.8 † *	40.8±3.1 †	40.6±6.0

Different from placebo group: * P < 0.05. Different from control group: † P < 0.05. Results are expressed as mean±SD, n=8 /group.

Experiment 2

Sixteen volunteer (age range, 44-65 years; mean = 56.5, S.D. = 9.8) who were not taking any medication known to influence mental participated in the experiment. The study protocol and material were approved by an Institutional Review Board (Asparagus Engineering Research Centers of Hebei Province) and all subjects provided written informed consent prior to participation. This study was conducted in accordance with Good Clinical Practice Guidelines of China. Adult volunteers were recruited from Qinghuangdao Hebei province. The interventions were conducted for 21 days. They were asked to refrain from consuming alcohol and caffeine, keeping the life style during their participation. All participants received a detailed explanation of the study and gave their informed written consent to participate.

All patients received an oral dose of 12.0g AES (2.13 g sarsasapogenin) per day, once a day. They were reassessed 21 days after treatment start. Current treatment was not discontinued. Serum samples were collected to detect the cortisol levels [15] as biomarkers of stress.

Blood samples were collected in the morning (between 0730 h and 0800 h) in a sitting position after resting and relaxing for 30 minutes without heavy exercise and were separated immediately by centrifugation. The obtained serum was stored at –80 until hormonal assaying. The serum concentrations of hypothalamic releasing hormones (CRH), pituitary hormones (ACTH), and target gland hormones (cortisol,) were examined in all subjects using the ELISA method (Microplate reader, Labsystems Multiskan MS 352, USA; Wellwasher, Thermo Labsystems AC8, USA; ELISA reagents,Shanghai Jianglai Co., Ltd., China). All sample measurements were run in duplicate, and the averages were calculated for analysis. Intra and inter-assay coefficients of variation were less than 10% and 15%, respectively.

Statistical analysis

The data of cortisol level, state anxiety scores, HR, SBP and DBP were analyzed using one-way Analysis of Variance (ANOVA) with repeated measurement. Data are presented as mean ± SEM. All analyses were two-sided, with the level of significance set at $p < 0.05$.

Table 5: Means heart rates before, during and after Trier social stress test.

Group	Baseline	Task	Rest1	Rest2
Control	73±13	79±10	79±8	83±12
Placebo	71±11	127±11 †	102±36 †	82±19
AEA	70±10	113±12 † *	103±15 †	88±15

Different from placebo group: * P < 0.05. Different from control group: † P < 0.05. Results are expressed as mean±SD, n=8 /group.

Table 6: Means systolic blood pressure before, during and after Trier social stress test.

Group	Baseline	Task	Rest1	Rest2
Control	102±8	103±9	108±11	105±7
Placebo	106±14	134±6 †	126±9 †	111±12
AEA	107±8	124±7 † *	119±10 †	113±7

Different from placebo group: * P < 0.05. Different from control group: † P < 0.05. Results are expressed as mean±SD, n=8 /group.

Results

Psychological measures

For the baseline values of state anxiety score, one-way ANOVAs revealed no significant difference between groups (showed in Table 4). For the TSST task period, remarkable increase in state anxiety score were noted in the AEA and placebo group, compared with the control group. Moreover, there was significant difference between the AEA and placebo group. Significant decrease in state anxiety score were observed in the AEA group, compared with the placebo group.

In brief, the STAI state anxiety score was remarkably higher during the task period than during the rest periods, and the level of state anxiety was significantly higher under the placebo condition than under the AEA treatment condition during the task period.

Physiological measures

The data on HR and SBP were illustrated in Table 5 and Table 6. For the baseline values, one-way ANOVAs revealed no significant difference between groups. One-way ANOVAs yielded a significant difference between the conditions in TSST task period. For the TSST task period, remarkable increase of HR and SBP were noted in the AEA and placebo group, compared with the control group. The heart rate was remarkably quicker under the placebo condition than under the AEA treatment condition. The systolic blood pressure was significantly higher in the placebo group than the AEA treatment group. During the rest period (Rest 1), there were significant decline of HR and SBP in the AEA and placebo group, compared with the TSST task period. Moreover, analyses revealed that the diastolic blood pressure no significantly increased between the condition and the period in TSST (in Table 7).

As expected, the TSST caused an activation of the HPA system. Salivary cortisol concentrations of participants exposed to the TSST showed an upward tendency during the course of the experiment. The data on saliva cortisol level were illustrated in Table 8and Table 9. While AEA-treated group showed the decreasing of cortisol level compared with the placebo-treated. One-way ANOVA revealed a significant difference between groups in the period of Rest1 after the TSST.

Table 7: Means diastolic blood pressure before, during and after Trier social stress test.

Group	Baseline	Task	Rest1	Rest2
Control	73±13	74±10	75±11	78±8
Placebo	71±10	78±16	79±11	72±9
AEA	70±10	80±13	77±12	72±8

Different from placebo group: * P < 0.05. Different from control group: † P < 0.05. Results are expressed as mean±SD, n=8 /group.

Table 8: Means salivary cortisol levels before, during and after Trier social stress test.

Group	Baseline	Task	Rest1	Rest2
Control	8.01±2.04	8.25±3.08	8.23±3.02	8.03±2.66
Placebo	7.65±2.63	11.99±5.07	14.71±2.73 †	9.01±3.64
AEA	7.89±4.05	8.52±2.73	10.73±2.56 † *	8.87±3.50

Different from placebo group: * P < 0.05. Different from control group: † P < 0.05. Results are expressed as mean±SD, n=8 /group.

Table 9: Means serum hormones levels before and after AEA treatment.

Treatment	CRH (ng/l)	ACTH (ng/l)	Cortisol (μg/l)
Before	118.1±20.4	8.25±3.08	188.2±23.0
After	67.65±26.3 *	11.99±5.07	147.1±27.3 *

Different from before treatment: * P < 0.05.
Results are expressed as mean±SD, n=16.

Discussion

Stress-related research has employed several procedures to activate the human stress system. Two of the most commonly used laboratory paradigms are the Trier Social Stress Test and the Cold Pressor Test [16]. The current studies confirmed the effectiveness of the TSST as a straightforward laboratory stress test capable of eliciting subjective, autonomic, and HPA axis stress responses. The Trier Social Stress Test was applied in this study to investigate the anti-stress effect (reduction of psychological and physiological stress responses) of AEA in a laboratory setting.

Consistent with previous reports [15,16], the TSST, as an effective and economical protocol, induced significant increases in cortisol level, heart rate, blood pressure and the state anxiety score. The result indicated the mean of state anxiety score, systolic blood pressure and the salivary cortisol level in the AEA-treated group showed the significant decreasing, compared with the placebo-treated.

The main findings in this study were that the acute stress responses elicited by the TSST task were reduced by the oral administration of AEA. Moreover, this effect of AEA was consistently observed not only in the psychological stress response but also in physiological stress responses such as HR and salivary cortisol level. To eliminate the placebo effect, placebo group and single-blinded design were set in this investigation. The previous results showed that AEA exhibited anxiolytic-like activity and mediate the secretion of crotisol and 5-HT in tow animal models. This reduction of psychological and physiological stress responses effect of AEA was consistent with the previous results in animal model. The present findings may also provide important scientific evidence for the application and development of anti-stress functional food to conquer the anxiety.

A few limitations of the current studies need to be acknowledged. First, although this study strongly indicated that the aqueous extract of asparagus has anti-stress effects, several limitations must be acknowledged. First, we examined only 24 male participants, whereas the previous study [17] reported sex differences in some responses to acute stressor. Thus, the generalizability of the present findings must be further tested using a larger sample composed of both sexes. Second, relatively few parameters were measured in the present study. Because we did not measure noradrenaline and adrenaline, we can only speculate about activation and reduction of the sympathetic nervous system on the basis of the HR, SBP, DBP and saliva cortisol level data. Further studies investigating such neural mechanisms are needed.

As traditional medicine spreads extensively worldwide, edible herbs have become an increasingly important ingredient in functional foods and therapeutic drugs. Component analysis showed that asparagus are rich in saponins from Asparagus *officinalis* (17.8%), aspartic acid (6.1%, accounting to one third of total amino acid) and gamma-amino butyric acid (1.0%). Reports suggest that sarsasapogenin from Anemarrhena asphodeloides BUNGE (Liliaceae) exile antidepressant activity by mediation the central monoaminergic neurotransmitter systems [18,19]. The content of steroidal saponins was quantified by determination of sarsasapogenin, as an index of active constituents of asparagus.

In conclusion, despite the limitations mentioned, our results suggested that AEA was effective for reducing the stress responses elicited by the TSST task. These results suggest that AEA exerts anti-stress effects during an acute stress challenge and may be of interest to generally healthy adults in stressful situation.

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