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# THE STRESS REDUCING EFFECT OF γ -AMINO BUTYRIC ACID AND APOCYNUM VENETUM LEAF EXTRACT ON CHANGES IN CONSENTRATION OF SALIVARY CHRAMOGRANIN A

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**Abstract**

In this study, we conducted a double-blind placebo-controlled cross-over study in order to examine the stress reducing effect of ingestion of γ -amino butyric acid (GABA), Apocynum Venetum Leaf Extract (AVLE), and concurrent ingestion of both samples. Changes in the concentration of salivary chromogranin A (CgA) and cortisol, scores of the questionnaire, after a stress inducing mental task following every intake were monitored. We found significant difference on CgA activity among the samples. Subject administered with GABA+AVLE exhibited a significantly less increase in CgA concentration compared with that of placebo condition. This result suggested that concurrent ingestion of GABA and AVLE might be able to reduce mental stress in humans more than single intakes.

## 1. Introduction

Being one part of this society, everybody exposed to the daily stress which is caused by family efforts, working environment or by human relationship. And such diseases like, depression, insomnia, suicides have already become one part of our social problem. Consequently, it is important for us to adjust to such a society and acting toward the solution of this problem in order to spend a healthy life. Studies are required to correspond to the physiological activity of the human autonomic nervous activity with the combination of daily intake of foods or food ingredients.

γ -amino butyric acid (GABA ) is known as a food ingredient which gives shooting effect and there have been many study reports which shows it's effectiveness on anti-stress. Meanwhile, since ancient times, the leaf of *Apocynum Venetum* has been used as a tea in Chinese medicine. Chinese Pharmacopoeia refers it as a drug which stabilizes mental condition and helps to treat such illnesses like heart disease, nephritis, and hypertension.

According to the results of recent animal tests on the efficiency of *Apocynum venetum* leaf extract (AVLE), it has been showed that the AVLE has an effective anti depression and anti anxiety actions. However the study of its anti stress effect on human is not yet done.

Furthermore, It can be expected that relax efficiency can be synergistic increased by simultaneous consumption of GABA and AVLE if the AVLE stimulate the action of GABAA receptor.

For the evaluation of nervous stress, biochemical analysis of human saliva, mainly concentration changes of chromogranin A (CgA) and cortisol, are used as one of the non-invasive biometric indicator of autonomic nervous system.

CgA is the major type of protein which is secreted from sympathetic neurons and adrenal medulla chromaffin cells, and considered a good indicator because of its close relationship to an early response of weak mental stress.
Cortisol is a type of adrenal glucocorticoid hormones; which has been used as a indicator to reflect strong physical or chronic stresses.

Here we will discuss about the stress reduction results of GABA and AVLE by determining the concentration changes of Cortisol and CgA before and after consumption of capsules which contains GABA and AVLE.

## 1. Methodology

### 2-1 Participants

In order to be included as a participant in the trial, several things were required. Totally 12 number of students from Shizuoka Prefecture University were enrolled to participate in clinical trial. Before the enrollment, informed consent was obtained from all participants. It was explained to them in advance, that there is no physical danger by participating in this experiment, and sudden dropout in the middle of experiment will not cause any serious disadvantages.

### 2-2 Experimental procedure

From the day before starting the trial, participants were required to get enough sleep and to abstain from food, smoking and severe driving. GABA, AVLE and placebo samples which are used for the trail purpose, were packed to cellulose capsules and contain 25mg of GABA powder (PHI Co., Ltd), 25mg of AVLE (Tokiwa Phytochemical Co., Ltd.) and 25 mg of dextrin, respectively.

The following 4 type of sample combinations were used for the clinical study:
1. GABA+Placebo

2. AVLE +Placebo
3. GABA+ AVLE
4. Placebo+Placebo

The trial period was divided info four days and each participant ingested the samples with 150ml mineral water (“Morino mizudayori ” made by Coca Cola National Beverage Co.,Ltd) per day. The 12 participants were divided into four groups randomly. The sample ingestion order of each group was as follow.

1) 1→2→3→4
2) 2→4→1→3
3) 3→1→4→2
4) 4→3→2→1

The trial was held at the end of September. Average temperature of the room where it was held was 25.7+0.7℃, and average humidity 64.8+3.7％. The trial has started after the participants entered into the room. All participants were required staying in calm and rest condition for 15 min. And then they were required to gargle their mouth, collect the salvia with Salivette device and then ingest the samples. It has been reported that GABA is rapidly absorbed with reaching peak within 30min or 1hour, and then blood level decrease rapidly; therefore it was decided to collect the salvia at 30min after the ingestion. Uchida - Kraepelin test for 15min was started to load stress to the participants at 15min after the sample ingestion, and then collect the salvia immediately. A questionnaire survey upon the participant’s subjective feeling was taken at last. The main purpose of this survey was to evaluate fatigue, relax, sleepiness, pressure, tension (zero point: not felt at all ~ 100 point: felt very strongly). CgA concentration in the frozen salvia samples was determined by YK070 Human Chromogranin A EIA Kit. Total protein for protein correction was determined according to the Bradford’s standard assay method. And the cortisol was determined by 1-3002 salivary cortisol EIA Kit.

**2-3 Statistical analysis**

The differences on the levels of CgA (protein correction), cortisol concentration and the score of survey questionnaire between the groups were evaluated by Dennett’s multiple comparison method, following repeated measure analysis using decomposition SPSS11.0J. The significance level of test was set to 5%. However, the data of one participant was excluded due to the inadequate amount of salvia.

## 3. Results

### 2-4 Subjective evaluation

Table 1 shows the average subjective scores and decomposition analysis results for each item. Significant difference compared with placebo was not found in any items.

Table 1. Average subjective scores and decomposition analysis results

| Evaluation items | average scores for each intake sample (±Standard deviation) | | | | Results of repeated measures ANOVA | | | | Dennett's test result | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | DOF | Error | F value | P value | Sample compare | P value |
| fatigue | 58.945(16.697) | 62.045(17.861) | 57.209(20.331) | 53.873(19.212) | 3 | 30 | 0.507 | 0.68 | | |
| Relax | 50.318(16.564) | 44.191(17.138) | 52.718(15.692) | 55.318(10.927) | 3 | 30 | 1.064 | 0.379 | | |
| | | | | | | | | | Sample 1 | 0.346 |
| Sleepiness | 30.718(18.164) | 63.255(27.549) | 41.700(23.696) | 46.627(30.172) | 3 | 30 | 3.064 | 0.043 | Sample 2 | 0.312 |
| | | | | | | | | | Sample 3 | 0.943 |
| Pressure | 33.455(22.131) | 43.609(21.551) | 37.445(18.411) | 34.091(14.105) | 3 | 30 | 0.692 | 0.564 | | |
| Tension | 29.973(20.286) | 45.345(23.197) | 39.936(22.485) | 31.718(14.219) | 3 | 30 | 1.858 | 0.158 | | |

Sample 1. GABA+Placebo
Sample 2. AVLE +Placebo
Sample 3. GABA+ AVLE
Sample 4. Placebo+Placebo

### 2-5 CgA (Protein correction)

Repeated-measures ANOVA of CgA level change indicated a trend of difference between the samples. (Fig.1 F (3.30) =2.651.p=0.067). According to the result of Dennett's multiple comparison, ingestion of GABA+AVLE lead to a significant decline in CgA concentration change compared with placebo (p=0.028). However, no significant difference was obtained on each single intake of GABA and AVLE compare with placebo (p=0.155, 0.190).

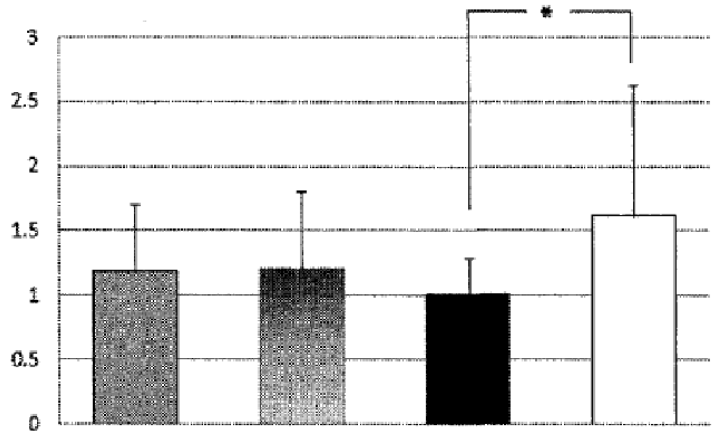


**Figure.1**

**The average of salivary CgA concentration changes before and after intake of the samples (protein correction)(±S.D.)**

The values in the figure were the average of salivary CgA concentration changes of 11 participants, which was determined with YK070 Human Chromogranin A EIA Kit and then corrected for total protein.

＊indicates that the rate of change was significantly lower from placebo due to ingestion of GABA + AVLE (P=0.028, Dennett's multiple comparison).

### 3-3 Cortisol

No differences were observed in cortisol concentration changes between samples by repeated measures ANOVA.

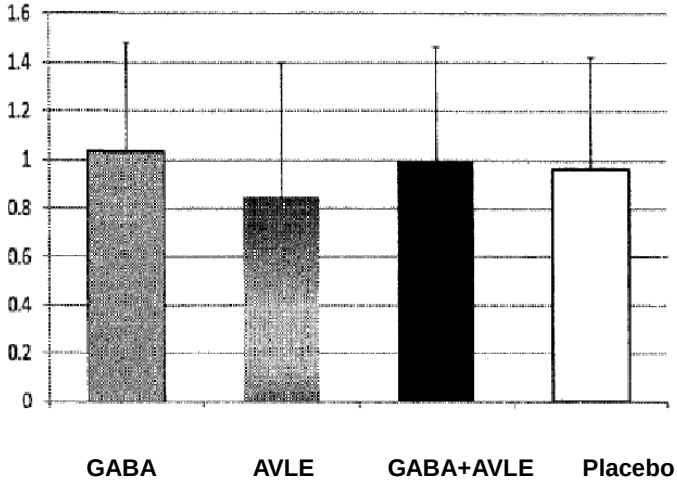


**Figure.2**

**The average of salivary cortisol concentration changes before and after intake of the samples**

The values in the figure 2 were the average of salivary cortisol concentration changes of 11 participants, which is measured by 1-3002 salivary cortisol EIA Kit.

## 4. Discussion

### 4-1 Subjective evaluation

Sympathetic nerve gets excited by physiological stress when the feeling of tension, pressure or fatigue grows up. However, compare with placebo, no significant difference was observed in all samples. As the result, the expected stress reduction effects of GABA and AVLE at 30min after ingestion were not obtained by this study.

And also the variation of scores was large due to the individual difference between the participants. Therefore, we considered the importance of new method of survey, in order to obtain objective information, like to avoid subjective evaluation and increasing the number of participants.

### 4-2 CgA (Protein corrector)

As it mentioned before, ingestion of GABA+AVLE lead to a significant decline in CgA

concentration change compared with placebo (p=0.028) according to the results of Dennett's multiple comparison. In other words, the average level of CgA concentration increased 1.609 times after Uchida - Kraepelin stress load in the placebo group, whereas the CgA concentration almost kept the same level with the pre-test in the group of GABA +AVLE.It has been reported that CgA in saliva is released from salivary gland by the activation of autonomic nervous system, especially sympathetic nervous system by stress load. It was suggested that concurrent ingestion of GABA and AVLE had the stress reduction effect (Figure 3).

Meanwhile, significant difference was not observed in single ingestion of GABA and AVLE, compared to placebo. The commercial available GABA supplement is usually 45mg/tablet or 750mg/tablet, but 25mg was used in this study in order to coincide with the ingestion amount of AVLE. It was reported that the effective dose of GABA was 30~100mg, therefore, the stress reduction effect of GABA was not obtained because the amount of GABA used in this study was not enough.

Although no significant difference was observed in single ingestion of AVLE, it can be expected to obtain the significant effect if we increase the ingestion amount of AVLE in the future research.

### 4-3 Cortisol

No difference was observed in cortisol concentration changes between the samples by the repeated measures analysis. As shown in the figure 1, 15-min stress load test increased the concentration of salivary CgA. However, salivary cortisol level, compare with pre-Uchida−Kraepelin test, didn't show any growing attitude, rather tended to decrease. The reason may be that CgA level shows a decrease tendency in the afternoon, and CgA usually reaches the peak usually at 20~30min after stress load.

In other words, salivary CgA was shown to be a more appropriate index than salivary cortisol in this study design.

## 5. Conclusion

From the above results, the subjective mood changes were not observed by subjective evaluation questionnaire. However, the simultaneous ingestion of AVLE and GABA led to a more effective action against artificially loaded psychological stress at 30min after ingestion.

**Acknowledgment**

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