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Effects of L-theanine on attention and reaction time response

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ABSTRACT

Previous human studies revealed that L-theanine influences brain function. The current study was designed to evaluate the affect of L-theanine (Suntheanine®) on attention and reaction time response in 18 normal healthy University student volunteers. In accordance with preliminary analysis of the manifest anxiety scale (MAS), the subjects were divided into two groups referred to as high anxiety propensity group and the minimal anxiety propensity group. Both groups received L-theanine (200 mg/100 ml water) and placebo (100 ml water) in a double blind repeated measurement design protocol. Assessments were performed for 15–60 min after consumption under a relaxed condition upon exerting an experimentally induced visual attentional task as well as audio response tests. Self-reports of anxiety as State-Trait Anxiety Inventory (STAI) was characterized at post experiments. Alpha bands electroencephalographic activity and heart rate were recorded throughout the trial. The results demonstrate the significant enhanced activity of alpha bands, descending heart rate, elevated visual attentional performance, and improved reaction time response among high anxiety propensity subjects compared to a placebo. However, no significant differences were noticed among subjects with a minimal anxiety propensity. Results evidently demonstrated that L-theanine clearly has a pronounced effect on attention performance and reaction time response in normal healthy subjects prone to have high anxiety.

1. Introduction

The relaxing effects of tea consumption have been known for decades, however, the modern neuropharmacological science has demonstrated that an active constituent of tea, called L-theanine (gamma glutamylethylamide) has modulatory effects on the brain state. L-Theanine is a unique non-proteinic amino acid that has been reported with predominant therapeutic values. It has been shown to regulate oscillatory brain activities in humans with an ability to pass through the blood–brain barrier during cognitive task execution and electroencephalographic (EEG) transcriptions and is believed to cause a relaxation effect (Song, Jung, & Oh, 2003; Yokogoshi, Kobayashi, Mochizuki, & Terashima, 1998). Kobayashi et al. (1998) reported that activity within the brain's neurotransmission rhythm of alpha frequency band (8–14 Hz) increased in reaction to 200 mg L-theanine ingestion when measured during a state of rest. Since the alpha activity is also known for relaxed, alert and low arousal, Juneja, Chu, Okubo, Nagato, and Yokogoshi (1999) have supported the finding that L-thenaine induces an increase in occipital and parietal alpha activity in human participants. Recent theoretical and experimental research has also revealed a leading role of brain oscillation in the alpha

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frequency bands towards physiological and psychological behaviours, which could be influenced by the ingestion of L-theanine (Bryan 2008; Eschenauer & Sweet, 2006; Haskell, Kennedy, Milne, Wesnes, & Scholey, 2008; Rogers, Smith, Heatherley, & Pleydell-Pearce, 2008).

Nobre et al., have investigated the effect of L-theanine on the resting brain activity in young healthy human volunteers and revealed that the ingestion of L-theanine at realistic dietary levels has a significant effect on the general state of mental alertness or arousal (Nobre, Anling Rao, & Owen, 2008). Gomez-Ramirez, Kelly, Montesi, and Foxe (2009) have studied the effect of L-theanine on alpha band oscillatory brain activity during a visuo-spatial attention task, wherein they investigated whether the effects of L-theanine occur also on tonic and phasic alpha activity during visuospatial task, which were found previously during intersensory selective attentional deployment, through the high-density electrical mapping study of the effects of L-theanine (Gomez-Ramirez et al., 2007). Such oscillatory brain activities were expressed not only under relaxed conditions, but also in attention processes, wherein the differential effect of cue information on anticipatory alpha amplitude was found to be larger on ingestion of 250 mg of L-theanine relative to placebo. In addition, Terashima, Takido, and Yokogoshi (1999) revealed that L-theanine was capable of rapidly influence the secretion and function of neurotransmitters in the central nervous system and also the endocrine systems (Isowa, Ohira, & Murashima, 2004). Related reports also support the neuroprotective effect of L-theanine in cortical neurons through an antagonistic role and thus, suggest the functional features of L-theanine in brain dynamics (De Mejia, Ramirez-Mares, & Puangpraphant, 2009; Kakuda, 2002; Kakuda, Nozawa, Unno, Okamura, & Okai, 2000; Kimura & Murata, 1986; Nagasawa et al., 2004).

Additionally, the combined effect of L-theanine and caffeine on cognitive performances clearly evidenced the beneficial impression on recognition, visual information processing, attention and moods (Owen, Parnell, De Bruin, & Rycroft, 2008; Rogers et al., 2008). Haskell et al. (2008) performed a randomized, placebo controlled, double blind, balanced cross over study to investigate the acute cognitive and mood effects of L-theanine (250 mg) with or without caffeine (150 mg) through selective attentional tasks as well as specific semantic memory and semantic reasoning. Kelly, Gomez-Ramirez, Montesi, and Foxe (2008) also investigated the combined effect of L-theanine (100 mg) with or without caffeine (50 mg) on human cognition evidenced by oscillatory alpha band activity and attention task performance studied between 30 and 120 min. Recently, Einother, Martens, Rycroft, and De Bruin (2010) have reported that L-theanine (97 mg) and caffeine (40 mg) improve task switching but not intersensory attention or subjective alertness. Another study examined the acute effects of L-theanine in comparison with a standard benzodiazepine anxiolytic, alprazolam and placebo on behavioural measures of anxiety in healthy human subjects using the model of anticipatory anxiety. This revealed that neither L-theanine nor alprazolam demonstrate any acute anxiolytic effect under the conditions of increased anxiety in the aforementioned model (Lu et al., 2004).

In a competitive world, people are often stressed, and that greatly influences their mental concentration while performing behavioural tasks that demand attention and rapid response. It has been demonstrated that relaxing time coursed features of L-theanine are concerned with psychological and physiological parameters such as stress (Breznitz et al., 1998), anxiety (Shinba et al., 2008; Bishop & Jenkins, 2008), heart rate (Townsend, Baier, Becker, & Ritchie, 2007), and the raising of skin temperature to a higher level (Quinlan, Lane, & Aspinall, 1997). However, the anxiolytic effects of L-theanine alone with suitable dose have not been fully established, particularly in human studies.

In the present study we have, therefore, focused on the relaxing effect of L-theanine using visual attentional task and rapid audio response tasks with L-theanine (200 mg) in two groups of normal healthy subjects stratified into high and minimal anxiety propensity groups according to the prior examination using manifest anxiety scale (MAS) of the anxiety inventory (Taylor, 1953). Both groups received L-theanine (200 mg/100 ml water) and placebo (100 ml water) in double blind repeated measurement design protocol. Assessments were performed under a relaxed condition upon exerting an experimentally induced visual attention as well as audio response quiz.

2. Materials and methods

2.1. *Participants*

Eighteen normal healthy subjects who participated in this study were male University students, aged between 18 and 20 years (average ± 1) with body weight between 57 and 78 kg (average 64.5 ± 6.3 kg). All participants gave written consent to take part in the study protocol approved by the Ethics Committee of University of Shiga Prefecture in Japan. All subjects were considered for selection if they were healthy free from social drugs and non-medicated at least for one month prior to the experiments and had no known personal history of physical or psychiatric disorders. In general, the volunteer subject participation was performed according to the guidelines of the Declaration of Helsinki 2000.

2.2. *Procedural details*

Pure L-theanine (Suntheanine®, Taiyo Kagaku, Yokkaichi, Mie, Japan, 100% purity) was used as active ingredient in this study. All subjects were advised to abstain from all kinds of tea beverages for at least 24 h prior to the start of the experiment. Manifest Anxiety Scale (MAS; Taylor, 1953) enquiry was performed prior the experiments and subjects were assigned to either a high anxiety propensity or minimal anxiety propensity group on the basis of scores obtained on the MAS inventory, wherein the individual's degree of anxiety state was directly proportional to their overall score. Actually, all five levels of anxiety grades of the MAS inventory were covered in the two aforementioned groups as high anxiety propensity group comprised levels 1–3 (8 subjects) and minimal anxiety propensity group consisted of levels 4 and 5 (10 subjects). Both groups received L-theanine (200 mg in 100 ml water) and placebo (100 ml water) in double blind repeated

measurement design protocol. Since L-theanine is a colourless, tasteless, and water soluble powder, the placebo- and L-theanine containing drinks were not perceptibly different in appearance or taste. Subjects were allowed to pre-practice attentional task or response test three times. After appropriate rest for 10 min, baseline conditions of brain rhythms (BR) using electro-encelephalographic (EEG) and heart rate (HR) were recorded. Subjects of both groups were served either the L-theanine solution (200 mg in 100 ml water) or placebo only (100 ml of water) and subjected to attentional tasks session or reaction time response (RTR) performance at baseline and 15, 30, 45 and 60 min after ingestion of treatment solution. Each testing task session lasted approximately 3 min and rest time of 10 min was allowed between the tasks. Brain rhythms (BR) and heart rate (HR) were recorded throughout the experimental tests. Self-reports of anxiety statement using the Japanese version (Nakazato & Mizoguchi, 1982) of State-Trait Anxiety Inventory (STAI) (Speilberger, Gorushi, & Lushene, 1970) was completed after every single task (Fig. 1) to measure state and trait anxiety.

2.3. *Brain rhythms (BR) and heart rate (HR) measurements*

Brain rhythms were measured using electro-encelephalographic (EEG), Biofeedback Training (BFT) system, at alpha (8.2–12 Hz) waves according to the system manual during the relaxing stage (quiet and stillness). Alpha band frequency determined by the ratio of dominant brain wave within 1 s. The speed of A/D conversion was 20 ms. Heart rate (HR) was measured using heart rate monitor accurate plus (POLAR ELECTRO "FINFARAID") on the right position of the chest as per manual's instructions.

2.4. *Attentional tasks studies*

Attention tester (AF type 1997, Inaba Human Engineering Laboratory, Narashino City, Japan) was used for attentional tasks. The attention tester was plugged with display monitor, on which the numbers 1–9 randomly appeared by 2.2 Hz frequency within 20 s. Subjects were asked to press the button

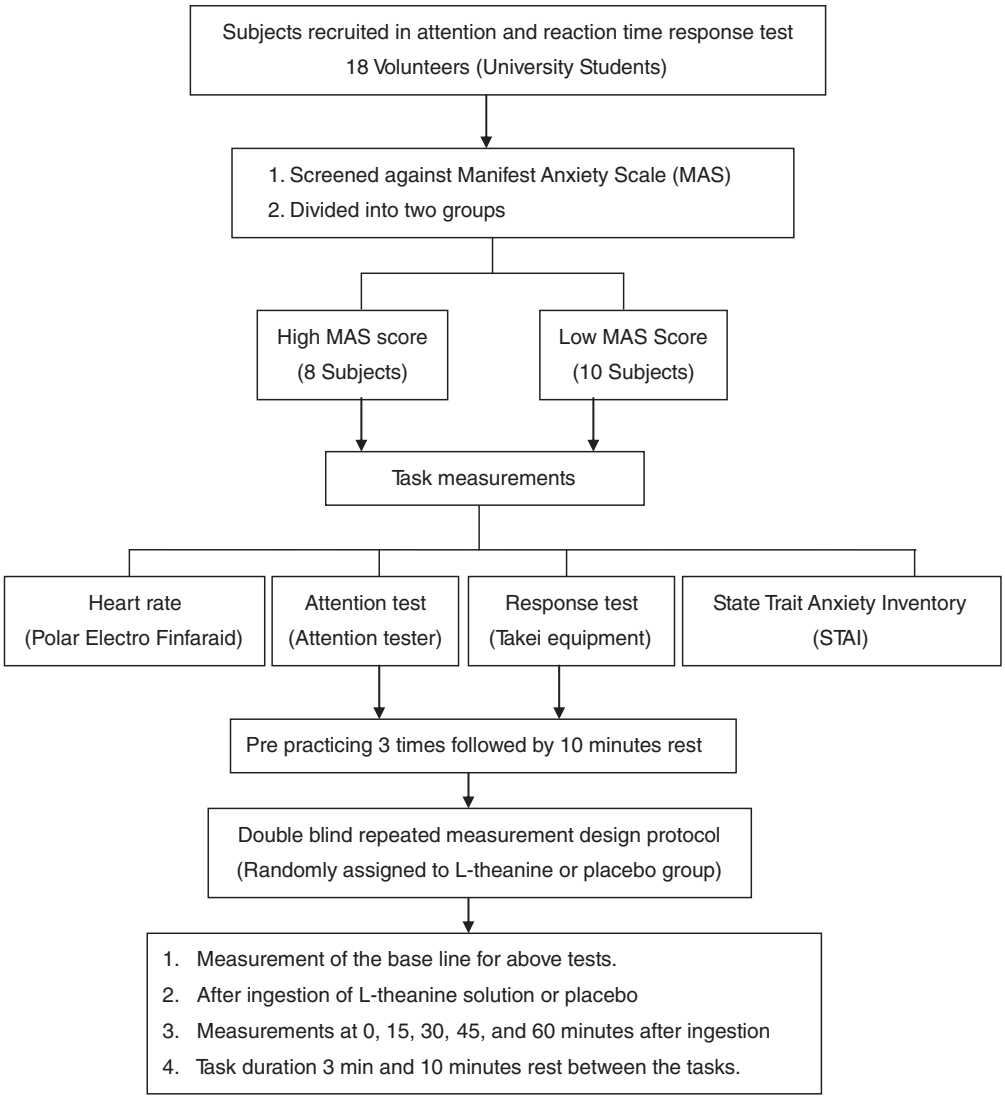

Fig. 1 – Enrollment, randomization, and tests flow diagram.

when the number 2, 5 or 8 appeared on the monitor. Pre-practice was done three times before the experiment began. When the experiment started the numbers 2 or 5 or 8 prompted on the monitor a bit faster than the pre-practice time, through 2.6 Hz frequencies within 20 s. Each experiment lasted 20 s and repeated five times to complete one set. The percentage of concentration was determined as follows:

- S = number of times the signal appeared on the monitor (Signal)
- P = number of times the subject correctly press switch (Pass)
- M = number of times the subject made a mistake (Mistake)
- Percentage of concentration (%) = $\{2S - (P + M)\}/2S \times 100$

2.5. Reaction time response (RTR) studies

Reaction time equipment (Takei equipment company, Niigata City, Japan) was used for sound response test. The equipment was set to two-sound systems at 1000 and 500 Hz frequencies. The sound signals emerged randomly from the speaker five times within 1–5 s. Subjects were asked to carefully listen to the sound signal cued from the speaker and press the button as quickly as possible when they heard the sound of 500 Hz frequency. Pre-practicing was allowed three times, prior to experiment.

2.6. Statistical analyses

Since the study was based on L-theanine intake under relaxed (still) condition, one way repeated measures ANOVA (Stat view for window version 5.0, Hulinks Co., Ltd.) mean values of pre-dose baseline data as well as those after 15, 30, 45 and 60 min upon ingestion of treatment solution. These were conducted to ascertain relevant differences in the performance ability of active L-theanine treatment solution compared to the placebo.

3. Results

3.1. Psychological analyses

The psychological analyses were derived from the anxiety statement (STAI) data collected after each and every attentional task as well as reaction time response test (Table 1). Both placebo- and L-theanine groups showed the time dependent decrement pattern of anxiety, as mean values were found downcast to baseline. However, no substantial difference was observed between the groups because the F values for the hypothesis were quite low and thus expressed the higher p values for both high ($F = 0.012$; $p = 0.913$) and minimal ($F = 2.706$; $p = 0.1035$) anxiety propensity groups. This clearly indicates that subjects were accustomed to the testing environment.

3.2. Physiological measures

Heart rate recorded during the attentional tasks was found to decrease in both of the theanine and placebo treatment groups, which evidently confirms the favourable physiological response during the attentional task. A substantial difference was recorded for subjects of high anxiety propensity group ingested with theanine and placebo treatments. One-way ANOVA revealed appreciable interaction under the condition of attentional behaviour at p value of =0.0016 ($F = 10.82$). Since the F ratio for the said hypothesis is very large with small p value, it is possible to confidently reject the null hypothesis and conclude that the heart rate alters statistically in the subjects of the high anxiety group. However, critically no sizeable variation occurred in minimal anxiety propensity group as of the large p value ($p = 0.6736$; $F = 0.175$).

3.3. Attentional response

Fig. 2 displays the alteration in correct answer ratio against time upon ingestion of L-theanine and placebo treatments in both high and minimal anxiety propensity groups. The re-

Table 1 – STAI, heart rate and alpha2 brain rhythms measured during the attentional or response trials in high and minimal anxiety propensity subjects digesting L-theanine or placebo treatments.

	Treatment	Baseline	15 min	30 min	45 min	60 min	Statistical analysis (ANOVA)
		High anxiety propensity group					
STAI	L-Theanine	1	0.936 ± 0.164	0.895 ± 0.118	0.846 ± 0.176	0.854 ± 0.182	N.S*
	Placebo	1	0.930 ± 0.042	0.886 ± 0.042	0.852 ± 0.045	0.849 ± 0.074	
Heart rate	L-Theanine	1	0.956 ± 0.052	0.921 ± 0.061	0.914 ± 0.065	0.932 ± 0.102	$F = 10.820$; $p = 0.0016$
	Placebo	1	1.011 ± 0.040	0.989 ± 0.049	0.958 ± 0050	0.991 ± 0.055	
Alpha 2 brain wave	L-Theanine	1	1.090 ± 0.143	1.082 ± 0.130	1.029 ± 0.240	1.056 ± 0.117	$F = 3.704$; $p = 0.0548$
	Placebo	1	0.981 ± 0.137	0.936 ± 0.142	1.036 ± 0.173	0.996 ± 0.144	
	Minimal anxiety propensity group						
STAI	L-Theanine	1	0.960 ± 0.092	0.977 ± 0.130	0.925 ± 0.119	0.950 ± 0.179	N.S
	Placebo	1	0.920 ± 0.089	0.918 ± 0.087	0.895 ± 0.100	0.882 ± 0.111	
Heart rate	L-Theanine	1	0.969 ± 0.057	0.975 ± 0.063	0.949 ± 0.076	0.953 ± 0.088	N.S
	Placebo	1	0.956 ± 0.038	0.969 ± 0.040	0.944 ± 0.039	0.952 ± 0.057	
Alpha 2 brain wave	L-Theanine	1	0.986 ± 0.223	0.994 ± 0.176	1.020 ± 0.243	0.992 ± 0.274	N.S
	Placebo	1	1.001 ± 0.253	1.003 ± 0.187	0.969 ± 0.352	0.977 ± 0.231	

* Not significant.

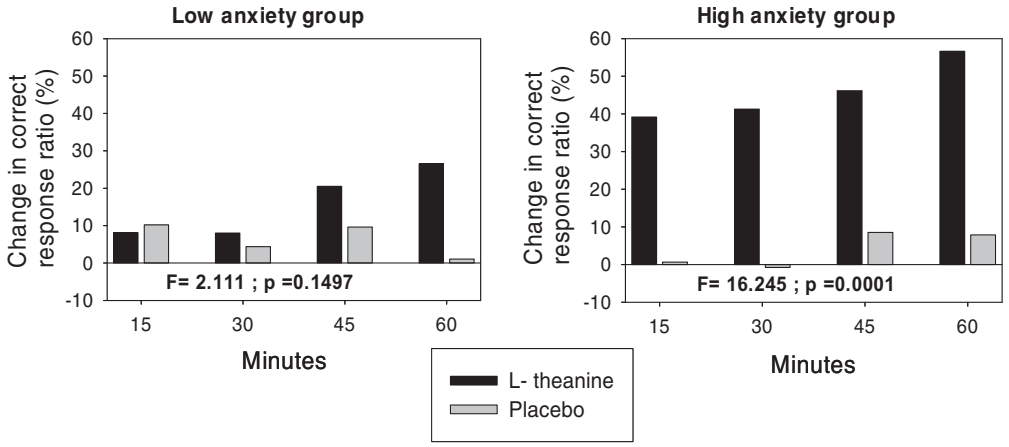

Fig. 2 – Variation in the ratio of attentional task scores in high and minimal anxiety propensity groups upon ingestion of L-theanine or placebo treatments.

sults clearly demonstrate that significant variation could be observed among high anxiety propensity subjects compared to subjects with minimal anxiety propensity in accordance to their MAS score. Fixing the baseline at change level ratio 1, the high anxiety propensity subjects showed a substantial change (~39%) at initial 15 min upon digestion of L-theanine treatment, however with increasing time the influence of L-theanine treatment remained almost constant with slightly upward trend even until 60 min and reached up to 56.6%. Thus, suggests that L-theanine treatment could maintain a prolonged action on the subjects with high anxiety propensity. On the other hand a negligible variation in the change of correct answer response ratio was noticed among the subjects consuming the placebo treatment. Whereas, the subjects with minimal anxiety propensity showed no considerable variation when ingesting L-theanine or placebo treatments at the initial stage except a little change (~26%) could be noticed after 60 min of L-theanine ingestion. Significantly increased attentional task scores were found in the subjects of high anxiety propensity, between L-theanine and placebo treatments by one-way ANOVA. The ANOVA analysis showed a higher F value ($F = 16.245$) and significant p value ($p = 0.0001$) indicating a reasonably high proportion of correct responses after the L-theanine ingestion as compared to placebo. Similarly, ANOVA statistical values did not show any significant effect among minimal anxiety propensity subjects ($F = 2.111$; $p = 0.1497$). Means of the proportion of correct responses did not differ significantly between conditions. Overall results evidently demonstrated a positive effect of L-theanine treatment on normal healthy subjects with high anxiety propensity symptoms.

3.4. *Reaction time response*

The data collected for reaction time response are described in Fig. 3. Change in reaction time response compared to baseline data showed a significant influence of L-theanine treatment compared to placebo among the high anxiety propensity subjects. Unlike the attentional task response, the change in reaction time response was quite fast and significant at initial 15 min after the ingestion of L-theanine treatment and then remained substantially lower than the baseline in a

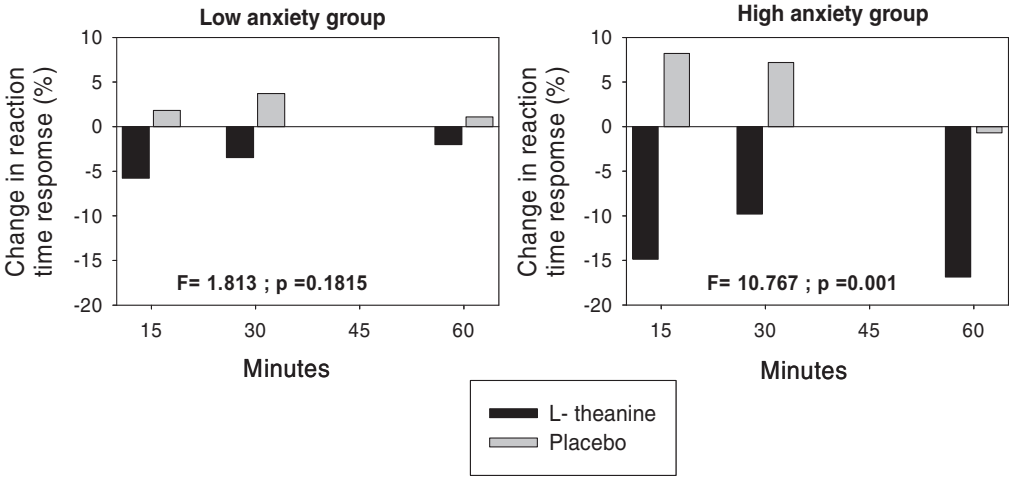

Fig. 3 – Change in reaction time response ratio with time upon ingestion of L-theanine or placebo treatment among high and minimal anxiety propensity subjects.

significant manner throughout the study period. Overall ability to respond quickly was improved nearly 15 min upon L-theanine ingestion. However, the high anxiety propensity group ingested with placebo treatment showed a reverse trend. Statistical values resulted from ANOVA confirmed an attribute significance level of high anxiety propensity subjects at lower p value ($p = 0.001$; $F = 10.767$). While, the minimal anxiety propensity subjects were not significantly different between L-theanine and placebo treatment ($F = 1.813$; $p = 0.1815$), although a noticeable difference was found in 15 and 30 min.

3.5. *Electroencephalographic (EEG) recording*

Table 1 lists the data collected using electroencephalographic recording of alpha band at still conditions during attentional task performance among subjects with high and minimal anxiety propensity symptoms. An increased ratio of alpha bands was recorded in attentional task performance in high anxiety propensity group from baseline at still conditions. The statistical ANOVA analysis results are also included in the Table.

4. Discussion

Green tea components in the recent years have witnessed a tremendous increase in interest among consumers due to their potential health benefits. The important bioactive constituent as a restorative is the amino acid L-theanine, which is also well known as a psycho-physiologically active agent. The relaxation effect of L-theanine is an interesting topic documented worldwide. Also, the anti-anxiety features of L-theanine are achieved through enhanced alpha electric band generation in occipital and parietal region of brain and increased synthesis of γ-aminobutyric acid (GABA). The supportive mechanism behind such activity revealed that enhanced GABA levels helps increase level of brain's dopamine and reduce serotonin levels that ultimately result in general feelings of calm and well being (Juneja et al., 1999; Kakuda, 2002). It is noteworthy to mention that unlike other conventional anti-anxiety treatments, L-theanine did not result in increased drowsiness, slowed reflexes, or impaired concentration. Results of a present study showed that ingestion of 200 mg of L-theanine changes the electrical activity in the brain by increasing alpha electric band measured by EEG. Earlier reports showed the dose dependent features of L-theanine on the brain activity can be prolonged and could typically last 8–10 h. However, the original claims relating to the calming effect of L-theanine show it is usually noted within 40–60 min after L-theanine ingestion at a dose of 50–200 mg. Effective dose of 200 mg of L-theanine in the present study to induce relaxation is well consistent with the work of Kobayashi et al. (1998), and in line with that of Lu et al. (2004). Thus, the data could be recorded during relaxing up to 60 min and time dependently monitored during the visual attentional and audio responds tasks.

The psychological evaluation including mood or behavioural effects covered within STAI state anxiety (STAI) revealed that L-theanine somewhat reduces time dependent attentional anxiety but is not predominately significant to placebo and are also in line with finding of Petrac, Bedwell, Renk, Orem, and Sims (2008). In the first instance, the STAI score signals no proneness to anxiety among the subjects of this study, however it could imply that anxiety level remains almost steady during the task period. Hence, the experimental design of the study allows us to validate each attentional task performed under the relax state and the resulting effect of L-theanine treatment was importantly cleared from placebo on such attention related behavioural tasks, which can be employed to determine the subjective and cognitive descriptive performances.

Usually during the visual behaviour tasks the role of anxiety is detrimental in performance, action or any perception, which tends to be interrupted by an increased anxiety. In a very recent report, Behan and Wilson (2008) tested whether the anxiety had an effect on the gazing behaviour during the execution of a far aiming archery task wherein the accuracy was affected by the duration of quiet eye period, which is an indicator of optimal focus and concentration as well as sensitive to increases in anxiety. It was demonstrated that better performance is associated with prolonged quiet eye time periods. It was very similar to the visual concentration. Visual attentional task results demonstrated in this study prevailed the convincing effect of L-theanine treatment on normal healthy subjects with high anxiety propensity symptoms. However, a less pronounced effect of L-theanine among healthy subjects with minimal anxiety propensity is not very surprising. Such visual attentional behaviour could also be indexed by alpha electric band oscillatory activity measured by EEG, which could be related to deployment of visuospatial attention in a spatial cueing paradigm. Similarly, Worden, Foxe, Wang, and Simpson (2000) studied an anticipatory biasing of visuospatial attention indexed by retinotopically specific alpha band electroencephalography increases over occipital cortex. The statistical values obtained for both visual attentional task and alpha band activity of this study are in line while showing the convincing influence of L-theanine on the subjects with high anxiety propensity symptoms which support the aforementioned relaxation phenomenon.

Further, during the audio reaction time response, the subjects of high anxiety group that ingested L-theanine, demonstrated a faster action and thus, the change in reaction time response ratio remained lower than baseline value throughout the task session. Substantial calming effect could be noticed even after 60 min of L-theanine ingestion, which could be attributed to the unique feature of L-theanine entering the brain through crossing the blood–brain barrier. Wherein, Kakuda (2002) stated that oral administration of L-theanine is first absorbed into the blood from the intestinal tract, and then a little amount of L-theanine in blood flows into the brain through the blood–brain barrier. This increases the level of dopamine in the brain and reduces the serotonin level to generate neuroprotective effect via neurotransmission which resulting in faster responds (Ito, Nagato, & Aoi, 1998; Mason, 2001).

Furthermore, L-theanine modulated heart rate among subjects with higher anxiety propensity symptoms. Heart rate was reasonably lower from baseline during the attentional tasks upon ingestion of L-theanine treatment compared to placebo, which also concluded that the heart rate altered statistically in the subjects of high anxiety propensity group. The

correlation of heart rate and attention task performance could not be explained directly, however it could perhaps be entirely or partly attributed to L-theanine function that lowers systolic and diastolic blood pressure along with reduced serum amyloid-alpha, which is an inflammatory marker associated with cardiovascular disease (Nantz et al., 2007). Since, heart rate is one of the physiological parameters to assure excitation, fear, depression as well as anxiety, the behavioural actions such as watching an interesting program on television, while normal attention, the heart rate is also reported to be descending through physiological response studies (Mujica-Parodi et al., 2009; Petrac et al., 2008; Richards & Casey, 1991; Van der Veen, Lange, van der Molen, Mulder, & Mulder, 2000). In addition, the study indicates that L-theanine positively influences both visual as well as auditory attentional tasks.

5. Conclusion

In conclusion, the tested dose of L-theanine was confirmed as 200 mg, for enhanced performance in visual attention task, and reaction time response, among the subjects with higher anxiety propensity symptoms. Findings of the current study also suggested that L-theanine had no convincing affect in healthy subjects with minimal anxiety propensity levels according to MAS scores. The modulation of alpha band activity in the brain, measured by EEG, also endorses the neuroprotective features of L-theanine, which might be related to both glutamate receptors as well glutamate transporter. Given that L-theanine is a relaxant, it is directly or indirectly involved at the neurochemical level and thus it is impacted by a number of neurotransmitter systems, wherein the major target may include glutamate, gamma-aminobutyric acid (GABA), dopamine, and serotonin. Therefore, 200 mg of L-theanine intake may help normal people with high anxiety propensity to concentrate on their daily activities.

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