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Article

Versatile Effects of GABA Oolong Tea on the Improvement of Diastolic Blood Pressure, Alpha-Brain Wave and Quality of Life

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Abstract: Emerging evidence demonstrated that using a new manufacturing technology to produce γ -aminobutyric acid (GABA)-fortified oolong (GO) tea could relieve human stress and exert versatile physiological benefits. The purpose of this human research aims to investigate therapeutic effects of daily GO tea consumption on the improvement of blood pressure, relaxation-related brain waves and quality of life (QOL) during a period of 28 consecutive days. Total polyphenols, major catechins and free amino acids were analyzed via HPLC assay. Changes of heart rate, blood pressure, α -brain wave (index of relaxation) and eight-item QOL score were investigated on day 0, 7, 14, 21 and 28, respectively. The chemical analysis results showed that GO tea contained the most abundant amino acids and GABA, contributing to the relaxation activity. Among all study participants, daily drinking GO tea could reduce systolic blood pressure on day 21 and diastolic blood pressure on day 28 ($p < 0.05$, respectively). For participants with pre-hypertension, GO tea could effectively reduce heart rate, systolic and diastolic blood pressure on day 28 ($p < 0.05$). At the end of the study, incremental changes in alpha-brain wave and QOL scores were also demonstrated ($p < 0.05$, respectively). This study recommended that GO tea might potentially serve as a natural source of alternative therapy to benefit blood pressure, stress-relief and QOL.

Keywords: γ -aminobutyric acid; oolong tea; blood pressure; brainwave; quality of life

1. Introduction

The maintenance of autonomic nervous system (ANS) homeostasis was prone to be disturbed due to stress and the intricate comorbidities with generalized anxiety disorder and long-term depression [1]. The mental stress could alter autonomic control of the heart and evoke an increase in heart rate (HR) and blood pressure [2]. In light of this, a pathophysiological link may exist between psychology and hypertension. Indeed, evidence indicates that anxiety is intricately associated with depression, hypertension and ANS imbalance [3]. Moreover, anxiety plays a more pivotal role than depression in the development of hypertension [3].

The ubiquitous non-protein amino acid, γ -Aminobutyric acid (GABA), is an inhibitory neurotransmitter to improve insomnia, depression, anxiety and thereby reduce stress-induced blood pressure elevation [3-5]. The underlying physiological roles of GABA are associated with the modulation of neurohumoral transmission, attenuation of sympathetic activation, incremental changes in alpha waves, and the prevention of neurological disorders [3-5]. Our prior human research proved that abundant contained GABA and oligopeptides derived from the mackerel extract could mitigate fatigue, blood pressure and improve quality of life (QOL) in the study participants [6]. Nonetheless, the information regarding therapeutic effects of GABA on human mental stress remains scarce. Among four-type brain waves (alpha, beta, delta, and theta) for mental stress measured by the electroencephalogram, alpha brain wave activation resulting from a wakeful relaxation serves as an index of anxiety relief [7]. Under stress conditions, GABA administration not only reduces anxiety but also enhances immune modulation [8]. Accordingly, it is of great importance to investigate versatile roles of GABA in foods for mood stabilization, stress reliever, autonomic balance and anti-hypertensive effect to benefit human health and QOL.

Teas containing abundant polyphenols and amino acids are proven to have many health-beneficial bioactivities. The therapeutic applications of teas to promote feelings of calmness, decrease excitation, and relieve stress are considered to correlate with their content of amino acids generated from anaerobic fermentation, especially L-theanine and arginine [9][10]. In addition, semi-fermented Oolong tea is a popular beverage consumed in Taiwan. Compared with the common oolong tea, GABA-fortified oolong (GO) tea is found to exhibit more bio-active effects on relaxation [11]. In people with acute stress, GO tea effectively alleviated autonomic imbalance and improved heart rate variability [11]. Therefore, we developed a new type of GO tea synergistically using anaerobic fermentation to make more GABA content in Oolong tea extract (above 150 mg/100g). Collectively, the aim of this study was to investigate bioactive compositions (polyphenols, catechins and amino acids) and influences on the blood pressure, alpha brain waves and QOL between Taiwan famous oolong teas and GO tea extracts in human research.

2. Materials and Methods

2.1. Teabag preparation and comparison of pH values between various teas

Various tea products were obtained from the local market for teabag preparation, including Wenshen Paochong (WP) tea (New Taipei, Taiwan), High-mountain Oolong (HO) tea (Chiayi, Taiwan), Oriental Beauty (OB) tea (Hsinchu, Taiwan), Tongding Oolong (TO) tea (Nantou, Taiwan). The GO tea derived from Sijichun tea (Nantou, Taiwan) was obtained from EVER HARVEST Biotech. Co. Ltd. (Taipei, Taiwan). The above tea products were used to make tea bags (4 grams in each tea bag). All study participants consume two tea bags per day and brew tea by adding 200 mL of boiling water in each bag. GABA tea was manufactured through the enzymatic decarboxylation from glutamic acid under anaerobic conditions [12]. Given the biosynthesis of GABA is tightly correlated with the pH level in the environment, the pH values between various teas were also compared.

2.2. Measurement of phytochemicals

The amounts of total phenolics and catechins in the various tea extracts were determined by high-performance liquid chromatography (HPLC) and colorimetric assay as reported previously [13]. The total content of phenolic compounds and catechins teas were then determined by a standard curve prepared with gallic acid and expressed in terms of milligrams of gallic acid equivalents (GAE) per gram of extract solid. The free amino acid contents were measured according to the previous research [14] and expressed as milligrams of theanine equivalents (TE) per gram of dry weight.

2.3. Human study

Online public advertisements were posted on the social media to recruit research participants. Adults aged between 40 and 85 years without pregnancy, record of brain surgery or taking any medications that affect to nervous system fulfilled our inclusion criteria. Exclusion criteria included

left handedness; history of medical and neurologic diseases; psychiatric disorders; head trauma; assumption of central nervous system active drugs in the two weeks prior to study entry; and presence of electroencephalographic abnormalities at the baseline electroencephalographic recording. Informed consent was obtained for all participants, and the study was approved by the Institutional Review Boards of Yuanpei University (YPU-IRB-1110426). Personal information of all participants including age, gender, education, and history of illness were recorded before starting the study. In this study, the electroencephalography (EEG) was applied in order to record the brainwaves of all participants. Thirty-eight healthy volunteers (5 males and 33 females) were included, along with their characters described in Table 2. Heart rate, systolic and diastolic blood pressures were measured before and after drinking tea infusion at each fixed time point (0, 7, 14, 21, 28 days).

2.4. Alpha-brain wave measurement

The α -brain wave data were wireless collected from study subjects using the frontal EEG devices in accordance with our previous study [15]. The NeuroSky® MindWave Mobile headset (NeuroSky Inc., San Jose, CA, USA, henceforth MindWave) was used in our research, because it is one of the most popular, convenient and affordable EEG devices with low cost that is accessible for neuroscientists (Supplemental figure 1). The EEG frequency analysis was performed by means of a Fast Fourier Transform (FFT) algorithm, with a 2-second interval on the EEG signals. A single-channel EEG was designed to collect brain activity data from the participants' brain. With reference to ear electrode, the EEG data were wirelessly acquired from FP1 with 128 Hz sampling rate (FP1: Front point 1, 1 cm above the midline of left eyebrow) (Supplemental figure 2). Simplicity and efficiency were the main reasons for designing a single-channel EEG monitoring system. The electroencephalographic data was analyzed offline and the following frequency bands were computed: delta ranging from 0.1 to 3.0 Hz, theta (4.0 to 7.0 Hz), alpha (8.0 to 13.0 Hz), beta (14.0 to 30.0 Hz), and gamma (31.0 to 47.0 Hz), respectively. The electric activities of α -brain wave (μV) during alpha frequency bands were measured at baseline, and after drinking tea infusion at different time points (0, 30 minutes and 28 days). The values of α -brain wave activities (between 0.01 μV and 1.00 μV) were converted to α -brain wave score (1-100).

2.5. QOL questionnaire

The eight-item QOL questionnaires (scores ranged from 1 to 5) were also completed at day 28 after daily drinking tea infusion. The QOL questionnaire was evaluated with five-point Likert scale for eight-dimensional objects: (1) Euphoria. (2) Feeling of relaxation. (3) Better sleep. (4) Less headache. (5) Less muscle tension. (6) Less physically discomforts. (7) Improvement of concentration. (8) Helpful for subjective enjoyment of life.

2.6. Statistics

All data are expressed as mean \pm standard deviation (SD) unless otherwise stated. Differences between the groups were calculated using One-way ANOVA with Dunnett's multiple comparisons test. All statistical analysis was performed using SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. The comparison of pH values between GO tea and various tea products

Various tea products obtained from the local markets were described in Table 1. The pH values in various teas were also analyzed. The pH values in WP, HO, OB, TO and GO teas were 5.84, 5.93, 4.95, 5.89, and 5.07, respectively. Notably, the GO and OB teas exert more acidic pH than other teas (p value < 0.05). According to the prior research, an acidic condition could improve GABA production [12]. Our results were in accordance with the previous study that an acidic condition (pH 4.5–5.5) could improve GABA production [12].

Table 1. Comparison of pH values between various tea products.

Tea	abbreviation	Tea cultivar	Place of origin	pH
Wenshen Paochong tea	WP	Chin-Shin-Oolong	New Taipei	5.84
High-mountain Oolong tea	HO	Chin-Shin-Oolong	Chiayi	5.93
Oriental Beauty tea	OB	Chin-Shin-Dapan	Hsinchu	4.95
Tongding Oolong tea	DO	Sijichun	Nantou	5.89
GABA Oolong tea	GO	Sijichun	Nantou	5.07

Note that the OB and GO teas exert more acidic pH than other teas ($p < 0.05$).

3.2. The comparison of phytochemicals between various tea products

The contents of total phenolic, total catechins and free amino acid in the various Taiwan tea products are shown in Table 2. Most tea extracts contained high concentrations of total phenolic compounds that were rich in total catechins. However, GO tea appeared to contain less polyphenols and catechins than other Taiwan tea products. By the comparison of amino acid content between various teas, we found that there is the most abundant level of free amino acid in the GO tea ($p < 0.05$). HO tea also contains high quantity of amino acid that could demonstrate the difference between the high altitude tea and other ordinary tea products. An increase in cultivation altitude attenuates total polyphenol contents but accentuates free amino acid concentration. This result is in accordance with the previous study about Lushan Mountain tea in China by Han et al. [16]. Collectively, higher amino acid content in GO may arise from cultivation of the tea plant in high altitude using more nitrogen fertilizer.

Table 2. Comparison of phytochemicals between various Taiwan tea extracts.

	<u>Total Phenolic</u> mg GAE/g	<u>Total Catechins</u> mg GAE/g	<u>Free amino acid</u> mg TE/g
WP	404.4 ± 0.8	164.9 ± 4.0	73.4 ± 3.4
HO	326.6 ± 1.8	140.9 ± 1.8	93.7 ± 4.6
OB	328.6 ± 0.6	147.2 ± 5.1	68.4 ± 1.5
DO	353.2 ± 2.7	195.4 ± 1.2	65.6 ± 2.3
GO	263.0 ± 4.4	91.0 ± 3.2	99.0 ± 4.0

^a Abbreviation: GAE, gallic acid equivalents; TE, theanine equivalents; WP, Wenshen Paochong tea; HO, High-mountain Oolong tea; OB, Oriental Beauty tea; TO, Tongding Oolong tea; GO, GABA Oolong tea. Each value is expressed as mean ± standard deviation ($n \geq 3$). Note that GO tea contains the most abundant level of free amino acid.

3.3. The comparison of compositions for eight catechins among various Taiwan tea extracts

As our prior study, the identification of major eight catechins was determined by HPLC according to retention times obtained from authentic standards run under identical conditions [13]. The major catechins in all tea leaves include (+)-catechin (C), gallic catechin (GC), (-)-epigallocatechin (EGC), (-)-epicatechin (EC), (-)-epicatechin gallate (ECG), and (-)-epigallocatechin gallate (EGCG), while EGCG and EGC being the most abundant in the tea products (Table 3). All of the contents of the various catechins in the ordinary oolong teas were higher than the GO. Compared with the results of total polyphenols and total catechins in Table 2, there should be many unidentified polyphenolic compounds or catechins in the GO tea.

Table 3. The comparison of compositions for eight catechins among various Taiwan tea extracts.

	Content (mg/g extract)				
	WP	HO	OB	DO	GO
Catechin	4.4 ± 0.1	4.2 ± 0.1	4.1 ± 0.0	5.2 ± 0.1	3.5 ± 0.1
EC	10.6 ± 0.3	11.2 ± 0.4	9.7 ± 0.1	12.9 ± 0.1	6.2 ± 0.4
ECG	9.5 ± 0.6	8.2 ± 0.4	11.7 ± 0.2	17.2 ± 1.0	5.8 ± 0.4
EGC	66.1 ± 0.8	74.6 ± 0.6	9.0 ± 0.5	52.3 ± 1.3	12.1 ± 0.5
EGCG	64.1 ± 0.6	61.7 ± 1.6	18.7 ± 0.2	87.1 ± 0.9	11.7 ± 0.8
GA	1.3 ± 0.0	0.8 ± 0.0	10.8 ± 0.2	0.9 ± 0.1	7.6 ± 0.2
GC	25.4 ± 0.6	25.1 ± 1.8	7.4 ± 0.7	19.1 ± 0.7	7.3 ± 0.4
GCG	20.8 ± 1.9	16.1 ± 1.45	5.5 ± 0.2	23.4 ± 2.7	4.9 ± 0.1

Tea abbreviation: WO, Wenshen Paochong tea; HO, High-mountain Oolong tea; OB, Oriental Beauty tea; TO, Tongding Oolong tea; GO, GABA Oolong tea. Catechin abbreviation: EC, Epicatechin; ECG, Epicatechin gallate; EGC, Epigallocatechin; EGCG, Epigallocatechin gallate; GA, gallic acid; GC, Gallocatechin; GCG, Gallocatechin gallate.

3.4. The anti-hypertensive effects of GO tea extracts

GABA has been evidenced as a powerful bioactive compound with anti-hypertensive effects [17]. We conducted an interventional prospective cohort study design to investigate anti-hypertensive effects of GO tea in the middle-aged population. Our Table 4 demonstrates the baseline characteristics of the study subjects (n = 38) before consuming the GO infusion. The duration of follow-up was twenty-eight days. The mean age of whole study participants were 55.4 ± 10.4 years. Approximately 51.5 % of the study participants had a history of pre-hypertension. Table 5 indicates that interval changes of the study subjects' heart rate, systolic and diastolic blood pressure exhibited descending trends through daily consumption of the GO tea. Among all study participants, daily drinking GO oolong tea could reduce systolic blood pressure on day 21 and diastolic blood pressure on day 28 (p < 0.05), respectively. To investigate it further, data in subjects with pre-hypertension were extracted. Their baseline mean systolic blood pressure was 137.2 ± 15.6 mmHg. In this subgroup analysis (Figure 1), systolic and diastolic blood pressure were significantly reduced by 11.8 and 8.4 mmHg after 28-day consumption of GO tea, respectively (p < 0.05). Moreover, their heart rates were also significantly reduced by 5.9 bpm after four weeks (p < 0.05). The current results confirm that daily supplementation of GO tea could effectively reduce heart rate and blood pressure in middle-aged adults with pre-hypertension.

Table 4. Baseline characteristics of the study subjects (n = 38).

Variables	Average
Age (years)	55.4 ± 10.4
Gender (female; n (%))	33 (86.8)
History of pre-hypertension (n (%))	17 (51.5)
Daily sleep time (hour)	7.00 ± 1.06
Heart rate (beats per minute)	74.9 ± 9.2
Systolic blood pressure (mmHg)	120.0 ± 20.0
Diastolic blood pressure (mmHg)	77.5 ± 12.4
Alpha brain wave score	35.0 ± 17.3

Education (Bachelor's degree or higher)	21 (63.6)
Types of works (white-collar worker)	25 (75.8)

Note: The α -brain wave score (1-100) were converted from values of α -brain wave activities (between 0.01 μ V and 1.00 μ V).

Table 5. Changes in heart rate and blood pressure of GABA oolong tea consumed during 28 days.

Variables	Before		GO consumption		
	0	7	14	21	28
Heart rate (bpm)	74.87 \pm 9.18	73.00 \pm 9.89	72.30 \pm 8.68	73.52 \pm 9.05	72.76 \pm 8.38
SBP (mmHg)	120.00 \pm 20.04	117.33 \pm 16.65	116.39 \pm 12.69	114.52 \pm 14.53*	115.76 \pm 12.89
DBP (mmHg)	77.5 \pm 12.35	75.06 \pm 9.02	74.55 \pm 7.27	74.12 \pm 8.49	73.52 \pm 7.99*

^aSBP, Systolic blood pressure; DBP, Diastolic blood pressure. ^b Each value is expressed as described in Table 3.

* indicates a significant difference at the level of $p < 0.05$.

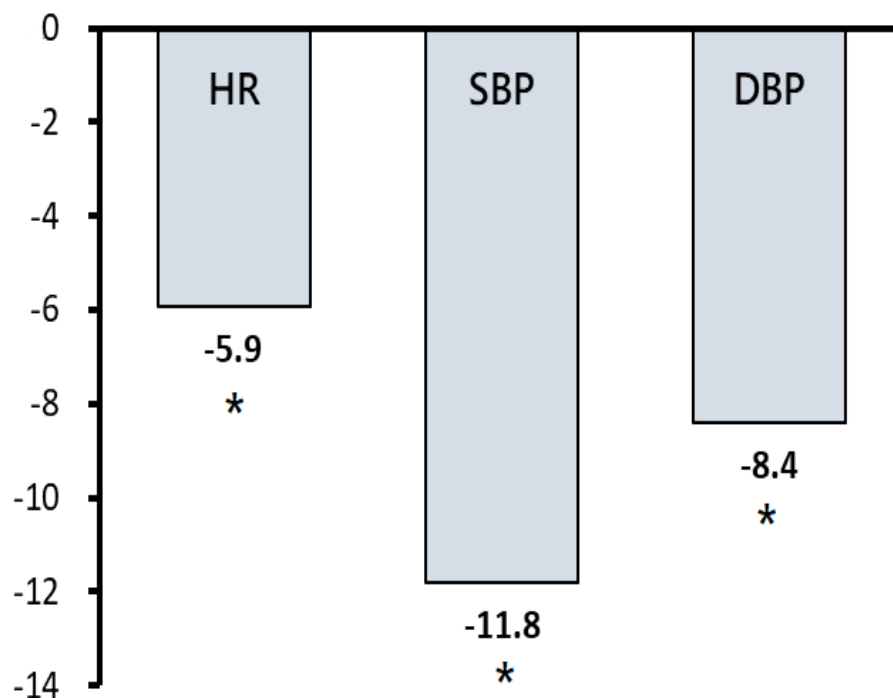


Figure 1. Effect of daily consumption of GABA oolong tea on the reduction of heart rate and blood pressure in the subgroup analysis for study participants with pre-hypertension after a period of 28 consecutive days. Note: HR, Heart rate (beats per minute); SBP, Systolic blood pressure (mmHg); DBP, Diastolic blood pressure (mmHg). * indicates a significant difference at the level of $p < 0.05$

3.4. The relaxation effects of GO tea extracts

Throughout the study period, the physical examination revealed normal in research participants without physical discomforts or abnormal laboratory data. Intriguingly, it is surprising that GO rapidly and effectively increases alpha-brain wave score after 30 minutes and four weeks as shown in Figure 2 ($p < 0.05$). In accordance with prior research, GO tea induces relaxation in a rapid and

effective way [8]. In this study, GO tea contains abundant GABA and amino acid that could induce relaxation. It is worth noting that the α -brain wave score related to relaxation was immediately increased by 1.8 times at 30 minutes after drinking GO tea. Further, the α -brain wave score was increased by 2.3 times after 28 days of continuous GO tea consumption.

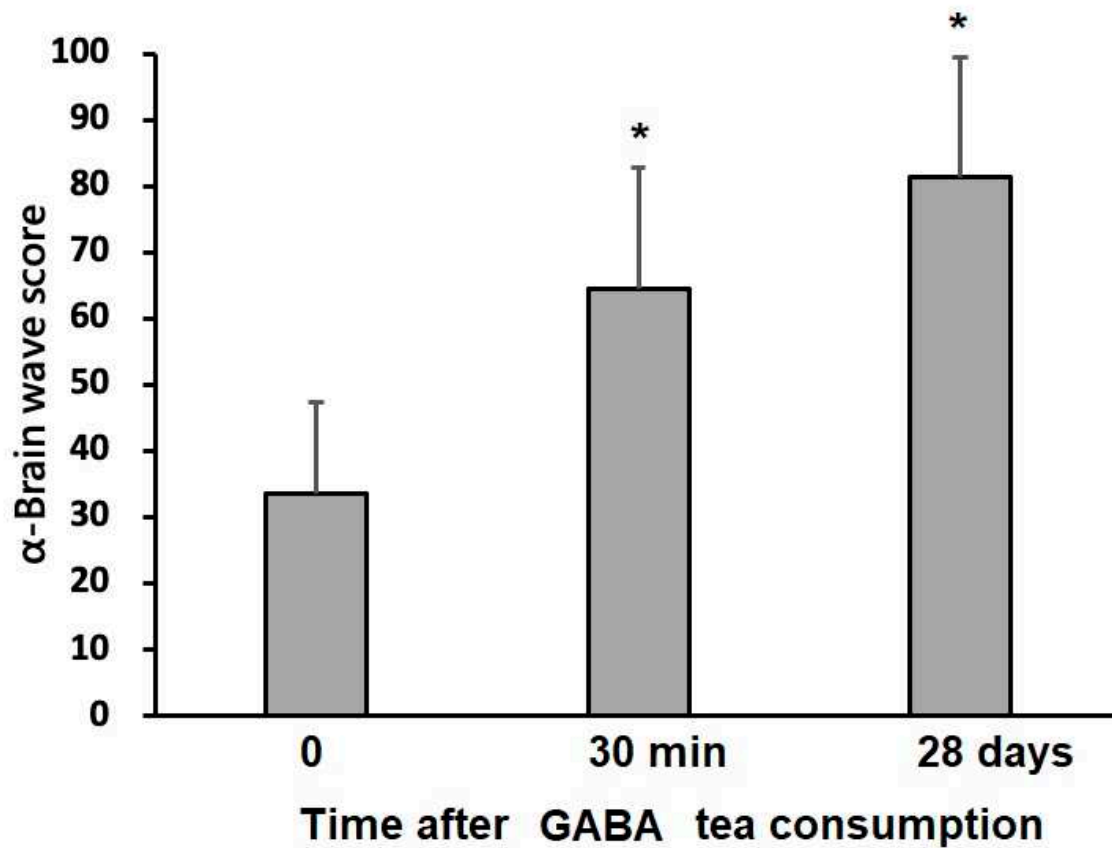


Figure 1. Effect of drinking GABA oolong tea on the α -brain wave score. * indicates a significant difference at the level of $p < 0.05$.

3.5. The QOL improvement of GO tea extracts

QOL questionnaires were further performed in all study subjects, including pre-test and post-test between a period of 28 days. Eight dimensions are shown as follows: euphoria, relaxation feeling, benefit to the sleep, less headache, less muscle tension, less physical discomforts, benefit to the concentration and benefit to the QOL, respectively (all p values < 0.05) (Table 6). A higher QOL score indicates higher satisfaction after 28 days of continuous GO tea consumption. We found that daily drinking of GO for 28 consecutive days benefits the subjects' stress relief that all QOL indices were positively improved (Table 6).

Table 6. Change of 8-item QOL questionnaire score before and after 28 consecutive days of GABA oolong tea consumption.

item	Treatment	
	before	after
Euphoria	3.4	4.3*
Relaxation feelings	3.3	4.2*
Better sleep	3.2	4.2*

Less headache	3.0	3.8*
Less muscle tension	3.0	4.1*
Less physical discomforts	3.1	4.0*
Improvement of concentration	2.9	4.3*
Helpful for subjective enjoyment of life	2.9	4.1*

Each item is scored on a 5-point Likert scale. * indicates a significant difference at the level of $p < 0.05$.

4. Discussion

Herein we develop a new manufacturing technology to produce a Taiwanese oolong tea product with high GABA content, which is termed as GO tea. This study aimed to analyze the bioactive phytochemicals in GO tea via HPLC colorimetric assay in comparison with four Taiwan famous tea products. Through using an interventional prospective cohort study design, the encouraging results achieved in the current research that GO tea could relieve mental stress, reduce blood pressure and improve QOL in the middle-aged population. Toward a better understanding of the underlying mechanism, GO tea contains numerous bio-active constituents including polyphenols, catechins and amino acids (e.g., GABA) that synergistically act to improve stress-induced autonomic imbalance (increasing alpha-brain waves and reducing blood pressure/ heart rate), contributing to QOL and human health (Figure 3). Several important findings in this work deserve further discussion.

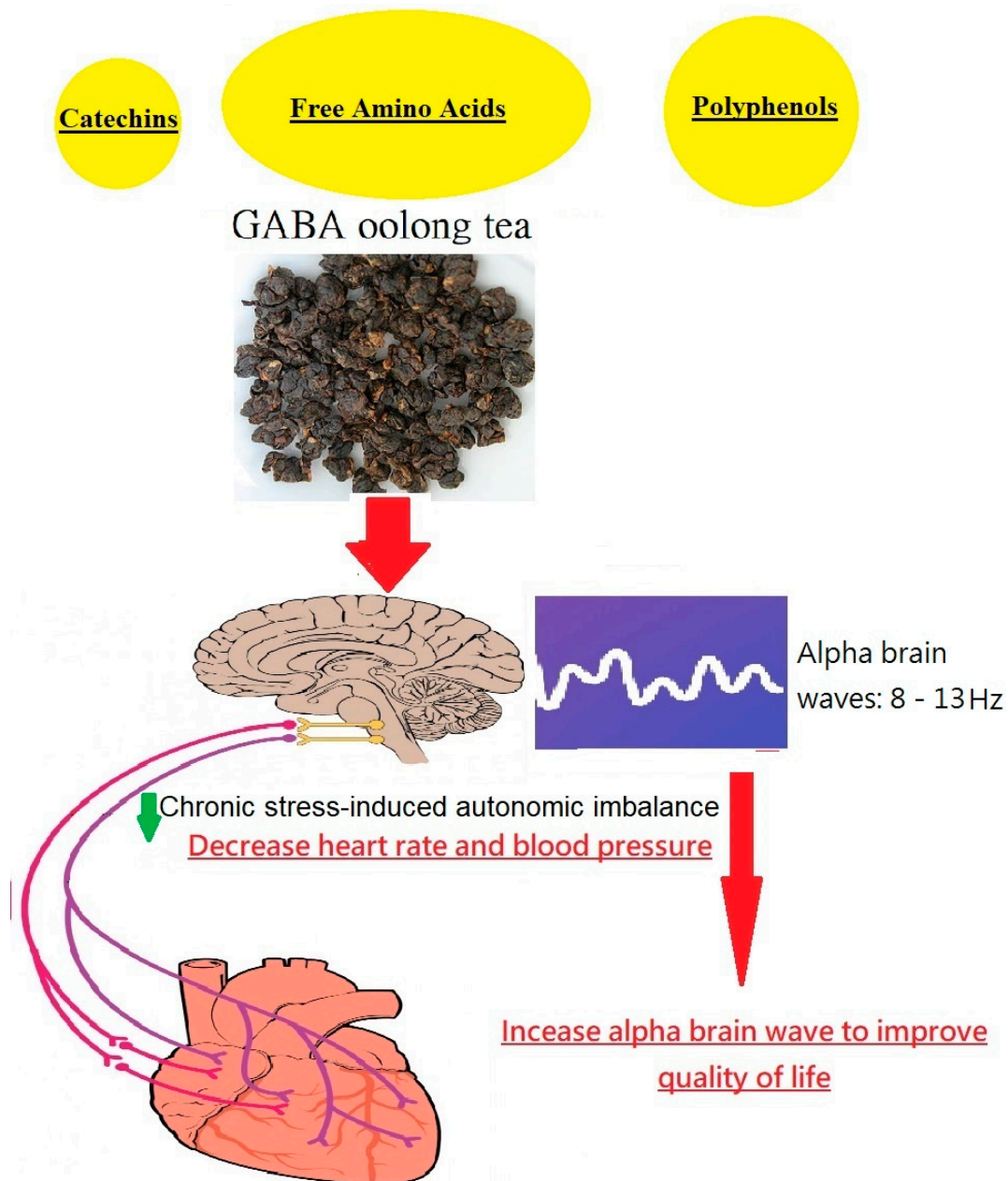


Figure 3. Gaphical abstract of beneficial effects of GABA oolong tea on human health.

4.1. Optimization of pH condition in the industrial production of GO teas

Lactic acid bacteria (LAB) are pivotal organisms concerning the fermentation of diverse food processing that functions as GABA producers [18, 19]. Myriads of raw foods and materials contain abundant glutamate that could be metabolized by LAB to enhance their tolerance against acidic conditions. As a result, such GABA-producing LAB have been utilized via isolation from a wide range of fermented foods including cheese, kimchi, soybean or various fermented Asian fish products. To investigate it further, we analyzed pH values in various tea products. As expected, the GO tea exerts more acidic pH (5.07) than other teas (p value < 0.05). Our results were in accordance with the previous study that an optimal pH condition (pH 4.5–5.5) could improve GABA production [18, 19]. Our findings provide advantageous results for enhancing GABA production strategies and optimal conditions in the food processing and industrial application.

4.2. The major bioactive phytochemicals in GO teas: Pharmaceutical roles of GABA

As shown in Table 2, GO tea appeared to contain less polyphenols and catechins than other Taiwan tea products. In comparison, we found that there is the most abundant level of free amino acid in the GO tea ($p < 0.05$). Although four Taiwan famous tea products were found to contain trace GABA (below 1mg/g) in HPLC method, only GO contained 187 mg/g GABA in our prior analysis. GABA plays a critical role in the pathogenesis of hypertension, which is associated with targeting angiotensin-converting enzyme regulation [3]. Moreover, central GABA is able to suppress blood pressure and slow heart rate through the activation of GABA receptor [20, 21]. When it comes to diverse pharmaceutical properties of GABA, Ngo et al. comprehensively review and illustrate GABA exerts therapeutic effects on a myriad of diseases, particularly in hypertension, diabetes, insomnia, anxiety, hypothyroidism, cancer, kidney injury, alcohol-related hepatotoxicity, infection, allergic, oxidative, inflammatory, atherosclerotic and neurological disorders, etc. [5]. In light of the versatile beneficial effects of GABA, the biotechnological techniques for high GABA-producing strategy have been applied in food industry. Although the preliminary results were encouraging in clinical applications, the further testing and validation concerning the safety and efficacy of daily GABA consumption are necessary in large-scale randomized, placebo-controlled trials.

4.3. The effects of GO tea on stress relief and QOL improvement

The information regarding changes of mental stress obtained from EEG signals remains scarce. Thus, we used the NeuroSky MindWave device to measure alpha brain waves in accordance with prior research [22, 23]. The alpha electromagnetic waves ranging between 8 and 13 Hz in frequency and between 30 and 50 μ V in amplitude are clearly enhanced in closed eyes periods that serve as an index of relaxation [22, 23]. In our study protocol, the α -brain wave score was measured before and after drinking tea infusion at different time points (0, 30 minutes and 28 days). The baseline the α -brain wave score was 35.0 ± 17.3 . Intriguingly, it is surprising that GO rapidly increases alpha-brain wave score after 30 minutes ($p < 0.05$). As expected, GO tea induces relaxation in a rapid and effective way [8]. Above effect relies on abundant contents of GABA and amino acids in GO tea that could induce relaxation (Table 2). Notably, the α -brain wave score related to relaxation was immediately increased by 1.8 times at 30 minutes after drinking GO tea and 2.3 times after 28 days of continuous GO tea consumption. The potent relaxation effect results in the significant improvement of QOL (Table 6). A higher QOL score suggests higher satisfaction after 28 days of continuous GO tea consumption, including euphoria, relaxation feeling, better sleep, less headache, less muscle tension, less physical discomforts, helpful for my concentration and subjective enjoyment of life. We found that daily drinking GO for 28 consecutive days benefits the subjects' feelings of relaxation that all QOL indexes were positively improved (all p values < 0.05). The potential mechanism and synergistic effects of GO tea contributing to the human health were illustrated in Figure 3.

5. Conclusions

Our study confirmed that GO tea contained not only polyphenols but also significantly higher levels of amino acids including GABA, contributing to whole body relaxation and anti-hypertensive effect. Among study subjects with pre-hypertension, daily drinking GO tea could reduce heart rates, systolic and diastolic blood pressure, respectively. Not only alpha-brain wave but also QOL was effectively improved. Evidence from our human study recommends that GO tea may potentially serve as a natural source of alternative therapy to lower mental stress-related blood pressure elevation and benefit QOL. Therapeutic effects of GO tea are versatile that the large-scale clinical placebo-controlled trials are in urgent need.

Author Contributions: C.-C.L. and J.-F.C. were responsible for the study concept and design, interpretation of data and writing of the manuscript. C.-Y. H., S.-C. C. and L.-F. C. assisted in electroencephalographic measure, biochemical analysis and experimental support. Y.-C. C. and T.-H. H. implemented the manuscript revisions. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: All data used to support the findings of this study are available from the corresponding author, Chang, J.-F.; upon reasonable request.

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Conflicts of Interest: The authors declare no conflict of interest.

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