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Original Article

The Effect of Short-term Green Tea Supplementation on Serum Cortisol and Serotonin Levels in Young Anxious Women

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ABSTRACT

Background and Objectives: In recent years, the use of dietary supplements to combat anxiety has increased. Therefore, the present study aimed to assess the effect of short-term green tea supplementation on serum cortisol and serotonin levels in young anxious women.

Materials and Methods: In this randomized clinical trial, 20 young anxious women were randomly assigned to either a control or a green-tea supplementation group. Participants in the supplementation group consumed 1000 mg of green tea daily for one week. Heart rate, systolic blood pressure, rate-pressure product, serum cortisol and serotonin levels were assessed before and after the intervention. Data were analyzed using paired and independent t-tests at a significance level of 0.05.

Results: The findings indicated no significant differences between the control and supplementation groups for changes in heart rate, systolic blood pressure and cortisol levels ($P = 0.09$, $P = 0.15$ and $P = 0.96$, respectively). However, green tea supplementation significantly decreased the rate-pressure product and significantly increased serum serotonin levels ($P = 0.05$, $P < 0.0001$, respectively).

Conclusions: Overall, short-term green tea supplementation seemed to contribute to anxiety management in young anxious women by decreasing the rate-pressure product and increasing serum serotonin levels.

Keywords: Green tea, Cortisol, Serotonin, Anxiety

Highlights

- Short-term daily consumption of 1000 mg of green tea resulted in a significant increase in serum serotonin levels in young anxious women.
- Green tea supplementation seemed to contribute to anxiety management in young anxious women by decreasing the rate-pressure product.
- Short-term consumption of green tea did not have a significant effect on cortisol levels. However, the downward trend in cortisol concentrations suggests a potential role for green tea in stress modulation.

Introduction

Anxiety is a psychological condition characterized by a state of anticipatory fear (1). Based on the latest estimates, approximately 60 million individuals in Europe suffer from anxiety disorders. Epidemiological studies indicate that in the United States, the lifetime prevalence of any anxiety disorder is approximately one-third, surpassing that of any other diagnostic category. Anxiety is a significant risk factor for diminished quality of life, increased

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mortality rates and various physical health complications, particularly cardiovascular diseases (CVD). Survey data from the United States suggest that women are twice as likely as men to develop panic disorder or phobias throughout their lifetime. Although gender-based differences in anxiety disorders are relatively subtle, evidence indicates a increasing prevalence of anxiety in women. The exacerbation of anxiety symptoms, whether emotional (e.g., fear and restlessness) or physiological (e.g., palpitations and tremors), represents a common hallmark within all anxiety disorders (1,2). Physiological perspective on anxiety from a physiological standpoint, research indicates that anxiety activates the sympathetic nervous system, affecting several physiological markers such as heart rate and blood pressure, leading to an increase in the two parameters (3). This phenomenon may increase myocardial oxygen demand, with the rate-pressure product (RPP) as one of its key indicators (4,5). Additionally, the assessment of other physiological markers, including specific hormones such as cortisol, serotonin and endorphins, serves as an effective approach for diagnosing anxiety-linked disorders (2). From these, cortisol is a hormone that regulates metabolism, preparing organs to deal with various conditions by enhancing the activity of other stress-linked hormones such as adrenaline and noradrenaline. Cortisol is widely documented as a key biomarker of stress and due to high levels of stress, the human body is frequently exposed to increased cortisol levels (2,6). On the contrary, neurotransmitters and mediators such as serotonin and endorphins are regarded as biomarkers of well-being and happiness, playing a crucial role in emotional regulation and mental health. Serotonin (5-hydroxytryptamine) was first discovered in 1948 in blood platelets and its presence in the brain was identified in later years. Then, extensive research has been carried out on its role in psychiatric disorders. Serotonin is associated with several psychological functions, one of which is anxiety-linked disorders (7). Based on the high prevalence of anxiety in today's society, particularly in women, researchers continuously search for effective strategies for its prevention and management. In recent years, the effect of various dietary and herbal supplements on anxiety has received significant attention in the health research field. From these, green tea supplementation has emerged as a widely used herbal remedy for promoting relaxation and improving mental well-being. Recent studies have investigated the effects of green tea on mental disorders, including anxiety and stress. Conger et al. (2019) reported that even a small intake of green tea could include a positive psychological effect. For example, drinking hot green tea has been shown to enhance mood (8). Green tea is derived from the leaves and shoots of the *Camellia sinensis* plant and contains high quantities of catechins, minerals and various vitamins. Catechins are potent antioxidants, while flavonoids, theanine and aromatic compounds are key constituents of green tea. Recent studies indicate that theanine, found in green tea, can decrease stress by inhibiting cortical nerve excitation in humans or by affecting neurotransmitter secretion and function in the central nervous system (CNS) (9-12). Epidemiological studies have indicated a connection between green tea consumption and a decrease in psychological distress. Research on the nutrients in green tea, especially flavonoids such as epigallocatechin gallate (EGCG) and L-theanine, has yielded varied results. Components of green tea modulate acute brain oscillatory activity. The L-theanine, present in green tea, includes anxiolytic effects, increasing alpha brain-wave activity in humans and preventing an increase in blood pressure. The EGCG, another constituent of green tea, enhances alpha and beta wave fluctuations. However, in another study, glutamate (Glu) of green tea included no effect on stress in rats (13-17). Based on the contradictory results regarding the effects of green tea on anxiety and increasing prevalence of anxiety, particularly in women, further research is critical. Based on existing reviews, only a few studies have investigated effects of green tea supplementation on anxiety-linked hormonal markers in women, with a very limited data available in this area. Therefore, the present study aimed to investigate effects of short-term green tea supplementation on serum cortisol and serotonin levels, as well as other physiological markers, in young anxious women.

Materials and Methods

The present study was a randomized clinical trial carried out in a pretest-posttest format within a control group. The study population consisted of young anxious women aged 18–22 y. Participants were screened and

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selected using Spielberger anxiety inventory, which included questions on trait and state anxieties. Based on the anxiety scores achieved as the primary inclusion criteria and considering the nature and objectives of the study, 20 young anxious women were voluntarily selected and randomly assigned to two groups of A) control group (ten participants) and B) green-tea supplementation group (ten participants). All procedures of the study were carried out in accordance with the ethical principles of the Medical Ethics Committee, Qazvin University of Medical Sciences, Qazvin, Iran (IR.QUMS.REC.1403.031).

The participants in the supplementation group consumed the specified quantities of green tea supplementation daily. The supplement consisted of green-tea herbal tablets, which were purchased in bulk from Dineh, Iran. Each tablet contained 500 mg of green-tea leaf powder and 50 mg of total polyphenols. Participants in the supplementation group consumed two tablets daily for 1 w, resulting in a daily green tea dose of 1000 mg (18). The timing and manner of supplement consumption were carefully controlled by the researcher.

Before and after the supplementation time, several physical and physiological indicators of the participants, including height, weight, body mass index (BMI), heart rate, systolic and diastolic blood pressures, rate-pressure product and cortisol and serum serotonin levels, were assessed. Additionally, the menstrual cycle of the participants was carefully controlled through self-report, with the assessments during the luteal phase (nearly Days 17–25). To achieve blood pressure and heart rate values, participants were asked to visit the medical venue at the scheduled time. Heart rate and blood pressure were assessed using digital upper-arm blood pressure monitor (Beurer BM96, Germany). The procedure was as follows: the participant sat in a completely relaxed position on a chair for 10-15 min before the assessment. The device was then attached to the left wrist based on standard procedures, with the arm positioned at heart level. The device was activated and the heart rate and systolic and diastolic blood pressure were recorded. The myocardial oxygen demand was calculated by multiplying the systolic blood pressure by the heart rate.

Then, 3 ml of blood (without anticoagulant) was collected from the antecubital vein (orange) using 5-ml syringes. The sample was then set at room temperature (RT) (22–25°C) for 30 min to clot. Then, the serum was separated using centrifuge and cortisol levels were assessed using Architect cortisol kit E-Abbott 8D15, Germany, while serotonin levels were assessed using ELISA kit, Zellbio, Germany. The two hormone levels were quantified using auto-analyzer and reported in international units (IU) per liter. After data collection, normality of data distribution was assessed using Shapiro-Wilk test. Data were analyzed using paired and independent t-tests. Statistical analyses were carried out using SPSS v.22 (IBM, USA), with a significance level set at 0.05 ($P < 0.05$).

Results

Table 1 presents the participants' demographic characteristics for mean and standard deviation (SD). The collected data, based on the Shapiro-Wilk test, showed a normal distribution ($P < 0.05$).

Results of the paired t-test indicated no significant differences between the pre and post-test values for heart rate, systolic blood pressure, rate-pressure product and serum cortisol in the control and green-tea supplementation groups (Table 2). However, a significant difference was observed between the pre and post-test values for serum serotonin in the green-tea supplementation group, with serum serotonin levels significantly increased in this group (Table 2). Furthermore, the findings showed no significant differences between the control and green-tea supplementation groups for the range of changes in heart rate, systolic blood pressure and cortisol (Table 3). However, green tea supplementation led to a significant decrease in the range of changes in rate-pressure product and a significant increase in the range of changes in serum serotonin (Table 3).

Table 1. Participants' demographic characteristics (mean ±SD) by group

Variable	Control Group	Supplement Group	P
Age (years)	20.6 ± 1.89	20.3 ± 1.25	0.99
Height (cm)	162.1 ± 7.2	160.9 ± 7.54	0.98
Body Weight (kg)	59.75 ± 12.54	62.25 ± 13.86	0.67
Body Mass Index (BMI)	23.04 ± 4.15	24.6 ± 5.19	0.71
Body Fat Percentage (%)	27.44 ± 5.77	28.43 ± 6.07	0.86
Anxiety Score	52.70 ± 8.9	54.75 ± 5.58	0.73

SD, Standard deviation

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Table 2. Variables assessed in the control and green-tea supplement groups during pre and post-tests

Variable	Group	Stage	Δ (pre-post)	t	P
Heart Rate (beats/min)	Control	Pre-test	78.5 ± 2.8	-0.19	0.84
		Post-test	78.6 ± 9.7		
	Supplement	Pre-test	82.6 ± 6.02	1.7	0.86
		Post-test	80.9 ± 9.5		
Systolic Blood Pressure (mmHg)	Control	Pre-test	111.01 ± 9.6	-1.96	0.081
		Post-test	114.3 ± 6		
	Supplement	Pre-test	111.4 ± 9.3	0.6	0.77
		Post-test	110.5 ± 8.5		
Double Product (Heart Rate * Systolic Pressure)	Control	Pre-test	8733.9 ± 1297.8	-1.82	0.10
		Post-test	8996.1 ± 1124.4		
	Supplement	Pre-test	9222.8 ± 1328.4	1.24	0.24
		Post-test	8979.4 ± 988.9		
Serum Cortisol (µg/dL)	Control	Pre-test	8.5 ± 3.09	0.33	0.74
		Post-test	8.4 ± 2.7		
	Supplement	Pre-test	9.27 ± 2.77	0.22	0.82
		Post-test	9.14 ± 2.78		
Serum Serotonin (ng/mL)	Control	Pre-test	139.4 ± 4.58	1.11	0.29
		Post-test	138.3 ± 3.1		
	Supplement	Pre-test	132.6 ± 18.43	-12.4	0.07*
		Post-test	145.89 ± 21.89		

*Significant pre- and post-test difference in the supplement group (P < 0.01)

Table 3. Changing ranges of variables in the control and green-tea supplement groups

Variable	Mean difference	t	P
Heart Rate (beats/min)	1.8 ± 0.2	1.77	0.13
Systolic Blood Pressure (mmHg)	3.9 ± 0.6	1.49	0.15
Double Product (Heart Rate * Systolic Pressure)#	506.9 ± 87.2	6.14	0.05
Serum Cortisol (µg/dL)	0.03 ± 0.06	0.04	0.96
Serum Serotonin (ng/mL)#	-13.5 ± 1.15	-11.7	0.0001

#Significant difference with the control group (P < 0.01)

Discussion

Anxiety is a common issue in modern life that can include negative effects on individuals' quality of life. One of the natural and effective methods to decrease anxiety is the use of natural dietary supplements. Therefore, the present study was carried out to investigate the effects of green tea supplementation on serum cortisol and serotonin levels, as well as physiological markers, in young anxious women. The results of this study indicated that daily consumption of 1000 mg of green tea for 1 w significantly decreased the range of changes in the double product and significantly increased the range of changes in serum serotonin, compared to that consumption of a placebo did. Although no significant changes were observed in heart rate, systolic blood pressure and serum cortisol in young anxious women following green tea consumption, these markers decreased after 1 w of supplementation with green tea. In a study by Wu et al. (2022), the effect of green tea

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on the prevention of high blood pressure in mice on high-salt diets was assessed. The findings of this study showed that green tea supplements played an important role in preventing the increase in blood pressure caused by a high-salt diet. Particularly, these supplements activate the phosphoinositide 3-kinase/protein kinase B signaling pathway, which modulates the gene expression linked to blood pressure regulation. Additionally, green tea supplementation showed significant potentials in improving tissue damages in vital organs such as heart, liver and kidneys (19). Similarly, Ta'ati and colleagues (2021) reported that 3 w of green tea consumption resulted in a decrease in blood pressure and heart rate in sedentary middle-aged women. Similar to the present study, Nogueira et al. (2017) reported that daily consumption of 500 mg of green tea supplementation for 1 w did not result in significant differences in systolic blood pressure; however, the consumption for 4 w led to significant decreases (20,21).

Green tea is rich in antioxidant polyphenols such as catechins and flavonols and green tea extract includes a vasodilatory effect, the two of which can be beneficial for cardiovascular health. These physiological effects of green tea can affect CVDa, including hypertension, and may lead to a decrease in systolic blood pressure by up to 5 mm Hg. In animal studies, green tea supplementation and EGCG, the major catechin in green tea, have been reported to prevent increases in blood pressure. To enhance the effectiveness of green tea supplementation and its extract on blood pressure and provide strong recommendations for adults, high-quality, randomized controlled trials with large sample sizes are essential (22).

The present study showed that the consumption of 1000 mg of green tea per day for 1 w did not include a significant effect on serum cortisol levels, although a decrease in cortisol was further pronounced in the supplement group. These findings differed from those of other studies that have reported a positive effect of green tea on decreasing cortisol levels. Almohdi et al. (2022) reported that the consumption of six cups of green tea for 6 w resulted in a significant decrease in cortisol levels and stress (23).

Researchers believe that the catechins and polyphenols in green tea, especially in the decaffeinated variety, play a key role in decreasing cortisol levels and improving stress. These antioxidant and anti-inflammatory compounds help regulate the nervous system's activity and decrease inflammatory responses in the body (24). Studies suggest that EGCG can inhibit the activity of 11,β-hydroxysteroid dehydrogenase type 1 (11,β-HSD1) at the cellular level; thereby, stopping the conversion of cortisone to cortisol. By inhibiting this enzyme, EGCG enables green tea to indirectly decrease the active cortisol level (25). A study carried out by Silva et al. (2025) demonstrated that L-theanine can affect responses to acute stress. This research assessed its effects on tissue cortisol levels, oxidative stress markers and behavior in zebrafish. The findings indicated that L-theanine at a concentration of 45 mg l^{-1}, prevented the increase of cortisol levels (26). However, no significant effect on cortisol levels was observed in this study, which might be attributed to the short duration of consumption and the lower dose of green tea, compared to previous studies. Additionally, individual differences in response to the active compounds in green tea could be a factor contributing to these varying results.

In contrast to cortisol, results of the present study indicated that green tea consumption significantly increased serum serotonin levels in young anxious women. Similar to the current study, Ikram and colleagues (2017) investigated the effects of green tea on behavior and neurochemical profiles in rats exposed to chronic stress. The results showed that although food intake decreased in the two groups of rats (green-tea and water groups), the anxiety-linked effects of stress decreased in the rats that consumed green tea. Additionally, serotonin levels in these animals shifted to normal. Overall, this study suggests that the beneficial effects of green tea in managing stress are linked to changes in serotonin metabolism (27). Moreover, a study carried out by Mirza et al. (2013) demonstrated that 5 w of green tea consumption led to an increase in serotonin and a decrease in anxiety symptoms in rats (28). Another study investigating the acute effects of green tea extract (GTE) showed that even a single dose could increase serotonin levels in plasma. These findings, focusing on the short-term effects of green tea consumption, indicated its potential ability to regulate various metabolite levels (29). The L-theanine, an active compound in green tea, plays a significant role in decreasing stress by relieving symptoms of depression and stress linked to psychosocial conditions, improving stress hormone imbalances such as cortisol, decreasing caffeine effects on serotonin and regulating serotonin and glutamate system, which suppresses excessive glutamate excitation, hence improving brain function (24,30). Additionally, a study showed that EGCG included significant effects on serotonin levels. In the group treated with EGCG, serotonin levels in the hippocampus (a brain region linked to mood) increased, while serotonin levels in the gut decreased. These changes suggested that EGCG might help regulate serotonin levels in the brain and gut, exerting antidepressant effects through this neurotransmitter regulation (31). In a study by Seo et al. (2017), which assessed fermented green tea (FGT) consumption, increases in serotonin and improved energy metabolism were observed (32). However, findings of some previous studies were not similar to the findings of the current study. For example, studies by Elseweidy et al. (2009) and Zhang et al. (2021) showed that consuming green tea extract for 10 w and green tea for 12 w resulted in decreased serotonin levels (33,34). These differences might

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be due to variations in the concentration of active compounds or insufficient bioavailability of these compounds in the supplement or they might be resulted from individual differences. Moreover, the lack of significant within-group and between-group differences in certain variables might be attributed to the relatively small sample size of the present study. However, definitive conclusions need further precise studies.

Conclusion

The results of this study indicated that the daily supplementation of 1000 mg of green tea for 1 w led to a significant increase in serum serotonin levels in young women with anxiety, whereas no statistically significant changes were observed in cortisol levels. Nevertheless, a downward trend in cortisol concentrations was recorded in the supplementation group, suggesting a potential role of green tea in stress modulation. Due to its high content of bioactive compounds including catechins and polyphenols, green tea might include beneficial effects on cardiovascular health. Similar to other studies, findings of this investigation underscored the necessity of large-scale, long-term randomized controlled trials to clarify the underlying physiological mechanisms of green tea effects and establish optimal dose and duration parameters for its consumption.

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