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Effects of Oral Vitamin C Supplementation on Anxiety in Students: A Double-Blind, Randomized, Placebo-Controlled Trial

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ABSTRACT

Vitamin C (ascorbic acid) is a well-known antioxidant that is involved in anxiety, stress, depression, fatigue and mood state in humans. Studies have suggested that oxidative stress may trigger neuropsychological disorders. Antioxidants may play an important therapeutic role in combating the damage caused by oxidative stress in individuals that suffer from anxiety. In this context, it was hypothesized that oral vitamin C supplementation would reduce anxiety. However, few up to date studies have evaluated the consequences of oral vitamin C supplementation on anxiety in humans. The present study examined the effects of oral vitamin C supplements in 42 high school students, in a randomized, double-blind, placebo-controlled trial. The students were given either vitamin C (500 mg day⁻¹) or placebo. Plasma concentrations of vitamin C and blood pressure were measured before the intervention and then one day after the intervention. Anxiety levels were evaluated for each student before and after 14 days following supplementation with the Beck Anxiety Inventory. Results showed that vitamin C reduced anxiety levels and led to higher plasma vitamin C concentration compared to the placebo. The mean heart rates were also significantly different between vitamin C group and placebo control group. Present study results not only provide evidence that vitamin C plays an important therapeutic role for anxiety but also point a possible use for antioxidants in the prevention or reduction of anxiety. This suggests that a diet rich in vitamin C may be an effective adjunct to medical and psychological treatment of anxiety and improve academic performance.

Key words: Vitamin C, anxiety, high school students, oxidative stress, beck anxiety inventory

INTRODUCTION

Vitamin C (ascorbic acid) is an antioxidant involved in anxiety, memory, fatigue and mood state studies. Some animals, including humans, cannot synthesize ascorbic acid due to lacking the enzyme, L-glulonolactone oxidase (Naidu, 2003). Many studies have shown that vitamin C is related to anxious behavior and psychology triggered by stressful situations. Treatment for 14 days with high doses of vitamin C (1.000 mg three times per day) decreased blood pressure, lowered cortisol and reduced subjective responses associated with acute psychological stress (Brody *et al.*, 2002).

Vitamin C plasma concentration levels have been found to be inversely associated with systolic and diastolic blood pressure in cross-sectional studies (Ness *et al.*, 1997; Bates *et al.*, 1998). Mazloom *et al.* (2013) evaluated the effects of two antioxidants (vitamins C and E) on anxiety, depression and stress in type 2 diabetic patients. The vitamin C group showed a significant decrease in anxiety scores compared with vitamin E and placebo, however, no significant differences were found between groups for depression scores or stress.

More recently, another clinical study conducted by Aburawi *et al.* (2014) investigated the effect of vitamin C as a treatment for depression and its associated action with

antidepressants, such as paroxetine, fluoxetine, clomipramine, fluvoxamine and the combination of olanzapine and clomipramine. The authors concluded that the combination of vitamin C with antidepressants resulted in a better therapeutic response for depression. Similarly, Amr *et al.* (2013) also demonstrated the efficacy of adding vitamin C to fluoxetine as an adjunct for treatment of major depressive disorder in pediatric patients. Moreover, numerous studies of animal models of depression have shown the antidepressant effects of vitamin C (Binfare *et al.*, 2009; Moretti *et al.*, 2011, 2012a, b, 2013, 2014).

Vitamin C supplementation has yielded inconsistent results for the treatment of fatigue in humans. However, a clinical trial conducted by Suh *et al.* (2012) obtained positive results. Intravenous administration of vitamin C decreased fatigue in office workers at two hours and fatigue levels remained lower for one day. Workers in the experimental condition also exhibited higher plasma vitamin C levels and reduced oxidative stress compared to the placebo group. For workers in this study, vitamin C supplementation proved to be a safe and effective method to reduce fatigue. Furthermore, studies have indicated a high prevalence of hypovitaminosis C and D in acute-care hospitals. Administering Vitamin C improves mood and reduces distress in hospitalized patients (Evans-Olders *et al.*, 2010; Wang *et al.*, 2013; Zhang *et al.*, 2011).

Anxiety is a response to a threat that is unknown, vague or internal which can alter physiological signals (Gautam *et al.*, 2012). Therefore, anxiety typically serves an adaptive function and prepares an individual for potential danger, alerting a person to be prepared to an imminent threat (Gautam *et al.*, 2012; Weinberger, 2001). However, when the anxiety is extremely high and persistent, it can become pathological and meet criteria for disorder (Weinberger, 2001). For many individuals, anxiety is also associated with secondary problems, such as a lack of self-confidence or for students, academic difficulties (Guney *et al.*, 2014). Moreover, for students, other symptoms may be expressed in the classroom and contribute to low academic performance, such as: panic, fear of failing examinations, feeling nervous and incapable of accomplishing tasks and racing heartbeat (Vitasari *et al.*, 2011). High levels of anxiety can impair working memory and increase distractibility in students (Aronen *et al.*, 2005; Cassady and Johnson, 2002). Other research has shown that students with increased anxiety levels during their end-of-semester examinations tend to obtain lower marks (Hamzah, 2007). Anxiety was the main predictor of academic performance among students (McCarty, 2007) and increasing anxiety levels can be associated with lower academic performance (Mazzone *et al.*, 2007; Sena *et al.*, 2007).

Oxidative stress can contribute to the pathophysiology of anxiety disorders (Guney *et al.*, 2014; Ranjana *et al.*, 2012). Present demonstrated that there are associations between total oxidant/antioxidant levels and anxiety disorders in children and adolescents (Guney *et al.*, 2014). This suggests that oxidative stress may be harmful in children and adolescents with anxiety disorders. In this sense, the high oxygen

consumption that occurs in the brain and its lipid-rich constitution (Halliwell, 2006; Ng *et al.*, 2008) may contribute to oxidative stress and this can promote or trigger psychiatric disorders (Bouayed *et al.*, 2009; Hovatta *et al.*, 2010). Among other factors such as genetics, neurochemistry, neurobiology and psychology, oxidative stress may be an important factor for the etiopathogenesis of anxiety disorders (Guney *et al.*, 2014). Other studies have also found a link between oxidative stress and Obsessive-Compulsive Disorder (OCD) and Panic Disorder (PD), further indicating that oxidative metabolism can affect the regulation of anxiety (Kuloglu *et al.*, 2002a, b). To combat such neurochemical changes, biological systems are equipped with antioxidant defenses. Therefore, antioxidant supplementation may play an important therapeutic role to combat oxidative stress in individuals that suffer from anxiety (Gautam *et al.*, 2012).

The aim of this study was to assess whether an important antioxidant like vitamin C exerts an anxiolytic-like effect in high school students. Anxiety levels of the students were evaluated by a validated instrument known as the Beck Anxiety Inventory which allowed to investigate the potential therapeutic role of vitamin C on anxiety-related human cognitive behavior.

MATERIALS AND METHODS

Participants: High school students of both sexes from Ceilândia, Distrito Federal, Brazil were invited to participate in this study. All participants were told the details of the study and signed forms indicating their informed consent. The experimental protocol and assessments of anxiety are in accordance with the Declaration of Helsinki and Good Clinical Practice guide and had been approved by the Ethics Committee on Human Research, Faculty of Health Sciences, University of Brasília, under the number, 022/12.

Inclusion and exclusion criteria: All students in good physical health were included. There was no history of smoking among participants. Students were excluded if they were pregnant, regularly taking vitamin C supplements or prescription drugs, regularly taking restricted medications (psychiatric patients), or if they had a history of illness including: psychiatric conditions, diabetes, hypertension, heart problems, lung, predisposition to kidney disease, as well as conditions related to malnutrition: rickets, low body weight for their age and mental problems.

Experimental procedures: Forty-two students were recruited and randomly assigned (n = 21 for each group) to receive either vitamin C or placebo. There were no drop outs. Participants took either placebo capsules or 500 mg vitamin C supplement capsules every day for 14 days. Only the nutritionist responsible for monitoring and distribution of capsules was, aware of the capsules' composition. Students were evaluated by a multidisciplinary team with expertise in biochemistry, nutrition, psychology and neuroscience. The team conducted interviews with all students. Students were informed of the purpose and procedures of the trial. The first

day of the experiment began at 8:00 AM with the reception of students in the laboratory, where they received a kit from a nutritionist containing either placebo or vitamin C capsules. Vitamin C and placebo capsules were obtained from Pharmacy Medicines, Brasilia, Distrito Federal, Brazil (Pharmacotechniques). Placebo capsules were identical in appearance to vitamin C capsules (both in green and white colors). Next, students' systolic and diastolic blood pressure and heart rate were measured and blood samples were collected (5 mL). All students had been informed that the collection of blood samples required eight hours of fasting and that blood collection would start at 9:00 AM. The procedures were conducted by the staff of the Central Public Health Laboratory of the Federal District (LACEN-DF), using recommendations from the State Department of Health of the Federal District, Brazil. Blood collection ended at 10:00 AM and all samples were prepared to be forwarded to LACEN-DF for analysis. After blood collection, the students and the team were offered breakfast. Students were then assessed by the team psychologist in a random order with the BAI, a neuropsychological test of anxiety. These tests on day 1 constituted the baseline measures. These experimental procedures were repeated on 15 day (post-treatment), following the supplementation.

Blood pressure measurements: Before the blood samples were collected, students' systolic and diastolic blood pressure and heart rate were recorded with an automatic self-inflating portable sphygmomanometer (Powerpack MS-918). The procedure was repeated on 15th day (post-treatment).

Blood sample and laboratory procedures: Plasma ascorbic acid concentration was initially based on the oxidation of ascorbic acid by diketogulonic and dehydroascorbic acids. These products react with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazone. This compound, after reacting with sulfuric acid (H_2SO_4), forms a product with an absorption band that can be measured at 520 nm. In this study, the ascorbic acid concentration in plasma was assessed following the protocol proposed by Bessey (1960) with methodology similar to previous studies (Marim *et al.*, 2012; Garlipp-Picchi *et al.*, 2013). Venous blood samples (5 mL) were collected from all students with use of hypodermic needle. A 100 mL solution containing 2,4-dinitrophenylhydrazine (2%), thiourea (5%) and copper sulfate (0.6%), DTC was prepared. Immediately following this, a 0.4 mL sample (serum) was removed and added to 1.6 mL of 5% Trichloroacetic Acid (TCA) mix for 30 sec and centrifuged at 2.500 rpm for 10 min. Then, 0.2 mL of DTC reagent was added to 0.6 mL of supernatant and the solution was subsequently shaken for 30 sec. This procedure was performed in triplicate. In order to complete the standard solution, 1 mL of 65% H_2SO_4 was added and the samples were shaken for 30 sec. After 30 min, in protection from light, the reading was taken in a spectrophotometer (SpectraMax M5, Molecular Devices) at 520 nm. The ascorbic acid concentration in the plasma was calculated using a calibration curve.

Anxiety behavior test: The Beck Anxiety Inventory (BAI) was designed by Beck, Brown, Steer and Epstein in 1986 (Beck *et al.*, 1988; Cunha, 2001). Twenty-one items were designed to reflect the somatic, affective and cognitive symptoms characteristic of anxiety. This inventory was built to avoid confusion with depression symptoms. Scores between 8 and 15 are interpreted as mild anxiety, between 16 and 25 as moderate anxiety and between 25 and 63 as severe anxiety.

Statistical analysis: The program software Graph Pad Prism was used for building figures for this experiment. Statistical analyses were conducted using IBM SPSS version 20.0 for Windows (IBM Corp. NY, USA). Data are expressed as Means±Standard Deviation (SD). To assess the normality of the variable distributions, Kolmogorov-Smirnov tests was conducted. When results were normally distributed, differences between groups were assessed using parametric Student's t-tests. When the results were not normally distributed, non-parametric Wilcoxon signed-rank tests and Mann-Whitney U test were used. Categorical variables were analyzed using Chi-square tests. Significance levels were set at $p < 0.05$.

RESULTS

The demographic characteristics of the students are summarized in Table 1. The mean age of the vitamin C group was 30.43 ± 14.35 years and the mean age of the placebo control group was 24.24 ± 11.07 years. A student's t-test indicated that there was no difference between the vitamin C group compared the placebo control group ($p = 0.125$). There was no difference across gender ($p = 1.000$). Assessments of height, weight and Body Mass Index (BMI) revealed no significant differences between the vitamin C compared the placebo; in short, the two groups no differ from one another regarding the descriptive characteristics.

The mean scores of anxiety, plasma vitamin C concentration, systolic blood pressure, diastolic blood pressure and heart rate are shown in Table 2, both at baseline and after experimental intervention (post-treatment). A Wilcoxon signed-rank test revealed that there was a significant decrease among vitamin C group post-treatment in comparison to baseline treatment ($p = 0.010$). As shown in Fig. 1, anxiety scores after the intervention were significantly lower for the vitamin C than the placebo control group ($p = 0.010$), indicating that oral vitamin C supplements improved the anxiety levels of students. Plasma vitamin C concentration was greater in the vitamin C group than the placebo group following the intervention period ($p = 0.001$). Likewise, there

Table 1: Demographic characteristics of the study participants⁽¹⁾ group (n = 21)

Characteristics	Vitamin C	Placebo control	p-value
Age (years)	30.43±14.35	24.24±11.07	0.125 ⁽²⁾
Sex N (female/male)	21 (16/5)	21 (16/5)	1.000 ⁽³⁾
Height (m)	1.62±0.09	1.63±0.09	0.930 ⁽⁴⁾
Weight (kg)	61.43±14.46	59.00±11.59	0.624 ⁽⁴⁾
BMI ($kg\ m^{-2}$)	23.32±4.77	22.27±3.76	0.513 ⁽⁴⁾

N: No. of participants, BMI: Body Mass Index, ¹All values are Means±SDS, ²Student's t-test, ³Chi-square test and ⁴Mann-Whitney U test

Table 2: Anxiety scores baseline and post-treatment either vitamin C supplementation or placebo, biochemical and physiological parameters or profiles⁽¹⁾

Variables	Vitamin C group (n = 21)		Placebo control group (n = 21)		p-value ⁽²⁾
	Baseline	Post	Baseline	Post	
BAI scores	22.33±10.35	16.86±10.19	25.43±10.56	24.95±13.11	0.010
PCVC (mg dL ⁻¹)	1.09±0.52	1.85±0.46	1.19±0.38	1.31±0.53	0.001
SBP (mm Hg)	121.70±23.84	119.00±13.16	114.30±11.85	114.20±9.41	0.933
DBP (mm Hg)	76.10±13.03	75.70±8.49	73.20±10.57	72.00±9.50	0.698
HR (bpm)	79.20±11.98	74.70±9.36	81.50±13.85	81.00±12.90	0.032

BAI: Beck anxiety inventory, PCVC: Plasma concentrations of vitamin C, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, HR: Heart rate, Post, refers to after the 14-day treatment, ¹All values are Means±SDS, ²Wilcoxon signed-rank test

Table 3: Dietary recall from the students⁽¹⁾

Variables	Vitamin C (n = 21)		Placebo (n = 21)		Total (n = 42)		p ⁽²⁾
	N	(%)	N	(%)	N	(%)	
Name of meals							
Breakfast	15	19.23	19	21.83	34	20.60	0.569
Morning snack	11	14.10	7	8.04	18	10.90	
Lunch	18	23.07	20	22.98	38	23.03	
Afternoon snack	16	20.51	14	16.09	30	18.18	
Dinner	15	19.23	19	21.83	34	20.60	
Supper	3	3.84	8	9.19	11	6.67	
Subtotal	78	100.00	87	100.00	165	100.00	
Food							
Salads	16	57.14	19	65.51	35	61.40	0.807
Fruits	7	25.00	6	20.68	13	22.80	
Greens	5	17.85	4	13.79	9	15.78	
Sub total	28	100.00	29	100.00	57	100.00	
Beverages							
Soft drinks	10	71.42	15	75.00	25	73.52	0.816
Juices	4	28.57	5	25.00	9	26.47	
Total	120	100.00	136	100.00	256	100.00	

⁽¹⁾All values are expressed as averages. N: No. of participants, %: Frequency, ⁽²⁾Chi-square test

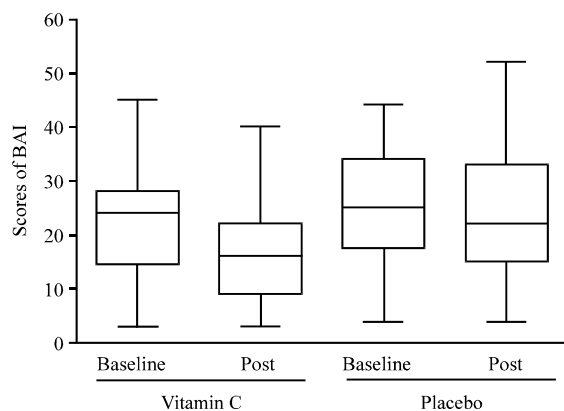


Fig. 1: Effects of vitamin C or placebo on anxiety assessed by BAI, Box plots of student anxiety scores in the vitamin C (n = 21) and placebo (n = 21) groups. Baseline and post-treatment anxiety assessed by BAI in both groups. (p = 0.010) were calculated by using Wilcoxon's signed-rank test. Post, refers to after the 14-day treatment

was a significant difference in the mean heart rate of the vitamin C group compared to placebo control group (p = 0.032). However, there was no significant difference between the groups vitamin C and placebo either pre-or post-treatment in mean systolic blood pressure (p = 0.933) and

diastolic blood pressure (p = 0.698). These results suggest that the experimental vitamin C group had better physiological response compared with placebo control group, only on the mean heart rate, indicate that vitamin C improved this parameter that is involved with anxiety symptoms.

Additionally, in terms of nutrition students were assessed using dietary recall data are available in Table 3. In general, students were considered euthrophic. There was no difference between the groups vitamin C and placebo on the meals (p = 0.569); food (p = 0.807) and beverages (p = 0.816), indicating that the groups are homogeneous in relation the nutritional aspects.

DISCUSSION

The present study evaluated the effects of oral vitamin C supplementation on anxiety in high school students. Vitamin C showed an anxiolytic-like effect, as indicated by the reduction of BAI anxiety scores. In addition, vitamin C also decreased heart rate compared to placebo. These results are in agreement with a previous study that investigated the effects of six weeks of vitamin C (1.000 mg per day) and vitamin E (400 IU per day) supplementation on anxiety levels, depression and stress, in type-2 diabetic patients. The results of this study showed that vitamin C significantly reduced anxiety scores compared to vitamin E and placebo. In contrast, vitamin E significantly increased anxiety scores. Similarly, this

study found that vitamin C significantly decreased stress levels compared to the placebo group. In this sense, vitamin C had improved anxiety levels in diabetic patients by reducing oxidative damage in the brain which had been causing nervous system impairment (Mazloom *et al.*, 2013).

The data found from this search are convergent in another study that examined the effect of an intervention designed to reduce anxiety and improve academic performance in engineering students (Vitasari *et al.*, 2011). This study used breaths per-minute (bpm) to measure anxiety. Each student received six two-hour-long sessions of treatment consisting in breathing retreatment, relaxation and in studying coping skills. The results indicated that all participants had reduced anxiety and probably as a consequence, improved academic performance. This style of intervention, therefore, was considered an effective approach to reducing anxiety among students. Taken together these results suggest that oral vitamin C supplementation can reduce levels of anxiety among high school students and may improve academic performance. Likewise, vitamin C may be a possible adjunct treatment for anxiety.

Anxiety and depression are common, stress-induced, psychiatric disorders (Gautam *et al.*, 2012). Deficiencies of vitamin C can trigger depressive symptoms. Low levels of ascorbic acid have been associated with depressive symptoms and higher mortality rates in older people (Hamer *et al.*, 2011). Additionally, Amr *et al.* (2013) demonstrated that vitamin C improved the efficiency of fluoxetine to treat depression and, given the lack of substantial adverse effects in a pediatric patients diagnosed with major depressive disorder, can be considered an attractive therapeutic adjuvant. The authors highlighted the need for more large-scale clinical trials to assess the therapeutic efficacy of vitamin C for the treatment of depression and its action as adjuvant treatment associate to antidepressant medications (Amr *et al.*, 2013). Epidemiological studies have shown that early-onset anxiety disorders can contribute as triggers for development of depression and other mood disorders arising later in life (Beesdo *et al.*, 2007; Duffy *et al.*, 2013). Likewise, anxiety disorders and mood disorders are associated with pathogenic mechanisms involved with the oxidative pathway (Guney *et al.*, 2014). For these authors vitamin C supplementation can act as an antioxidant leading to biochemical and behavioral changes, reducing anxiety, similarly in mechanism to its effects on depressive symptoms, fatigue and mood state. Thus, data collected here support these hypotheses.

In terms of nutrition, there is an additional demand on the body in a condition of stress, such as increase in adrenal production and mobilization of vitamins and minerals that accelerate metabolism of carbohydrates, proteins and fats, occurring production of energy to normalize stress situation (Gautam *et al.*, 2012). There are abnormalities that can alter the function of the Hypothalamic-Pituitary-Adrenal (HPA) axis which is involved with stress responses and anxiety disorder and implicated in emotional response (Masood *et al.*,

2008; Mathew *et al.*, 2008). For example, chronic stress exposure has been shown to trigger oxidative damage, activating the HPA axis (Aschbacher *et al.*, 2013). Another study found that high anxiety levels significantly increase oxidative stress (Rammal *et al.*, 2008). Moreover, oxidative stress is an excessive production of free radicals and failure of the antioxidant defense mechanism (McCord, 1993). The deficit of antioxidants can decrease the protection against Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) which are highly reactive and toxic causing damage to proteins, lipids, carbohydrates, DNA and mitochondria (Maes *et al.*, 2011; Sindhi *et al.*, 2013). Antioxidants neutralize the effects of ROS and exert action preventing several diseases (Sindhi *et al.*, 2013). These authors suggests that the supplementation of antioxidant compounds may be a new strategy for prevention or reduction of anxiety levels but also that individuals with anxiety disorders may be deficient in antioxidants, with indications of increased oxidative stress.

The results of this study showed that after the intervention, the vitamin C group had a decreased heart rate compared to the placebo group. The neurotransmitter γ -aminobutyric acid (GABA) is involved in cardiovascular regulation. Intracerebroventricular administration of GABA agonists decreases arterial blood pressure and heart rate and vitamin C stimulates 3H-GABA binding (Grigor'ev and Neokesariiskii, 1986). Another study found an increase in blood pressure and heart rate after microinjection of glutamate into the paraventricular nucleus. The N-Methyl-D-Aspartate (NMDA) receptor antagonist DL-2-amino-5-phosphonovaleric acid (AP-5) blocked these responses (Li *et al.*, 2006). In contrast, a high level of traumatic anxiety indicated by elevated heart rate triggers increase catecholamine release (Aburawi *et al.*, 2014). Ascorbic acid can modulate catecholaminergic activity and decrease stress reactions (Aburawi *et al.*, 2014). This hypothesis is based on several findings. Ascorbic acid has been described as a regulating factor of Na^+/K^+ -ATPase through the modulation of catecholamines. It also acts on neurotransmitter turnover in the central nervous system (Wiglusz *et al.*, 1983), demonstrated by a study in which OCD was treated with vitamin C (Jorm *et al.*, 2004). Likewise, Vitamin C may reduce anxiety and relieve stress either by stimulated GABA binding and block NMDA-gated channel function (Rebec and Pierce, 1994), or act through activation of dopaminergic and glutamatergic systems (Aburawi *et al.*, 2014). Reduced anxiety levels can be associated with decreased heart rates which are indicative of decreased catecholamine release. As previously suggested ascorbic acid can modulate catecholaminergic activity and consequently may be responsible for this lowered heart rate found in ours study.

Bruno *et al.* (2012) investigated the effect of acute vitamin C administration on muscle sympathetic activity and cardiac sympathovagal balance in hypertensive patients, vitamin C was able to reduce cardiovascular adrenergic drive in hypertensive patients which indicates that oxidative stress may

contribute to sympathetic activation in hypertension. Therefore, this study suggests that antioxidants may be able to restore vagal control of heart rate. Additionally, high-doses of vitamin C have been shown to reduce systolic and diastolic blood pressure, subjective stress and anxious responses to an acute interpersonal psychological stressor and, following the stress, vitamin C promoted faster recovery of salivary cortisol (Brody *et al.*, 2002). However, the results of current study do not suggest any significant differences in blood pressure (systolic and diastolic) between the vitamin C group and the placebo group, although the participants in this study had no history of hypertension. Vitamin C significantly lowered blood pressure in hypertensive patients but not in normotensive individuals (Bruno *et al.*, 2012) which were similar to the results found in the present study, since that vitamin C was administered to normotensive high school students.

In addition the data presented here are in accordance with the results found by Brody *et al.* (2002) which showed that plasma vitamin C levels increased significantly for the vitamin C group (from a means of 1.55-2.65 mg dL⁻¹) but not placebo group (from a mean of 1.36-1.40 mg dL⁻¹). Moreover, treatment with vitamin C increased blood plasma levels of vitamin C, suggesting that vitamin C deficiency is involved with psychological abnormalities (Chang *et al.*, 2007; Kinsman and Hood, 1971). Additionally, another study found that there is an increase in plasma vitamin C concentration by about 50% after vitamin C supplementation at a dose of 500 mg day⁻¹ for eight weeks (Khassaf *et al.*, 2003) and also the average half-life of vitamin C in an adult is about 10-20 days (Naidu, 2003). Thus, these results are similar to the current study (Table 2).

In future research, further study should be done to evaluate changes in serum oxidative stress parameters in patients with anxiety or other neuropsychiatric disorders and investigate the relationship between vitamin C supplementation, oxidative stress and antioxidants in the treatment of these conditions. The supplemental intake of nutrients such as vitamin C is likely to have an impact on eating habits for prolonged periods, with the intensity of the effect being modulated by the frequency and different degrees of promoting health, mood and well-being, as well as controlling anxiety levels of individuals differentially.

CONCLUSION

This study not only adds to the evidence that vitamin C plays an important therapeutic role on anxiety but also points to the possible use of antioxidants in the prevention and reduction of anxiety levels. The authors recommend the implementation of nutritional programs in high schools that include healthy foods rich in micronutrients such as the antioxidant, vitamin C. It is also recommend the use of vitamin C as an adjunct treatment for anxiety and for improving academic performance among students. In conclusion, this study suggests that a diet rich in vitamin C can help to reduce anxiety levels and possibly increase academic performance among anxious students.

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REFERENCES

- Aburawi, S.M., F.A. Ghambirlou, A.A. Attumi, R.A. Altubuly and A.A. Kara, 2014. Effect of ascorbic acid on mental depression drug therapy: Clinical study. *J. Psychol. Psychother.*, Vol. 4.
- Amr, M., A. El-Mogy, T. Shams, K. Vieira and S.E. Lakhan, 2013. Efficacy of vitamin C as an adjunct to fluoxetine therapy in pediatric major depressive disorder: A randomized, double-blind, placebo-controlled pilot study. *Nutr. J.*, Vol. 12. 10.1186/1475-2891-12-31
- Aronen, E.T., V. Vuontela, M.R. Steenari, J. Salmi and S. Carlson, 2005. Working memory, psychiatric symptoms and academic performance at school. *Neurobiol. Learn. Memory*, 83: 33-42.
- Aschbacher, K., A. O'Donovan, O.M. Wolkowitz, F.S. Dhabhar, Y. Su and E. Epel, 2013. Good stress, bad stress and oxidative stress: Insights from anticipatory cortisol reactivity. *Psychoneuroendocrinology*, 38: 1698-1708.
- Bates, C.J., C.M. Walmsley, A. Prentice and S. Finch, 1998. Does vitamin C reduce blood pressure? Results of a large study of people aged 65 or older. *J. Hypertens.*, 16: 925-932.
- Beck, A.T., N. Epstein, G. Brown and R.A. Steer, 1988. An inventory for measuring clinical anxiety: Psychometric properties. *J. Consult. Clin. Psychol.*, 56: 893-897.
- Beesdo, K., A. Bittner, D.S. Pine, M.B. Stein, M. Hofler, R. Lieb and H.U. Wittchen, 2007. Incidence of social anxiety disorder and the consistent risk for secondary depression in the first three decades of life. *Arch. Gen. Psychiatry*, 64: 903-912.
- Bessey, O.A., 1960. Ascorbic Acid: Microchemical Methods. In: *Vitamin Methods*, Wright, L.D., H.R. Skeggs and P. Gybrgy (Eds.). Academic Press, New York, pp: 303.
- Binfare, R.W., A.O. Rosa, K.R. Lobato, A.R.S. Santos and A.L.S. Rodrigues, 2009. Ascorbic acid administration produces an antidepressant-like effect: Evidence for the involvement of monoaminergic neurotransmission. *Prog. Neuro-Psychopharmacol. Biol. Psychiatry*, 33: 530-540.
- Bouayed, J., H. Rammal and R. Soulimani, 2009. Oxidative stress and anxiety: Relationship and cellular pathways. *Oxidat. Med. Cell. Longev.*, 2: 63-67.
- Brody, S., R. Preut, K. Schommer and T.H. Schurmeyer, 2002. A randomized controlled trial of high dose ascorbic acid for reduction of blood pressure, cortisol and subjective responses to psychological stress. *Psychopharmacol.*, 159: 319-324.

- Bruno, R.M., E. Daghini, L. Ghiadoni, I. Sudano and I. Rugani *et al.*, 2012. Effect of acute administration of vitamin C on muscle sympathetic activity, cardiac sympathovagal balance and baroreflex sensitivity in hypertensive patients. *Am. J. Clin. Nutr.*, 96: 302-308.
- Cassady, J.C. and R.E. Johnson, 2002. Cognitive test anxiety and academic performance. *Contemp. Educ. Psychol.*, 27: 270-295.
- Chang, C.W., M.J. Chen, T.E. Wang, W.H. Chang, C.C. Lin and C.Y. Liu, 2007. Scurvy in a patient with depression. *Dig. Dis. Sci.*, 52: 1259-1261.
- Cunha, J.A., 2001. [Beck Scale Manual Portuguese Version: Translation and Adaptation]. Casa do Psicologo, Sao Paulo, Brazil, (In Portuguese).
- Duffy, A., J. Horrocks, S. Doucette, C. Keown-Stoneman, S. McCloskey and P. Grof, 2013. Childhood anxiety: An early predictor of mood disorders in offspring of bipolar parents. *J. Affect. Disorders*, 150: 363-369.
- Evans-Olders, R., S. Eintracht and L.J. Hoffer, 2010. Metabolic origin of hypovitaminosis C in acutely hospitalized patients. *Nutrition*, 26: 1070-1074.
- Garlipp-Picchi, M., R. Deminice, P.P. Ovidio and A.A. Jordao, 2013. Effects of ascorbic acid on oxidative stress biomarkers of elite swimmers. *Revista Brasileira Medicina Esporte*, 19: 394-398.
- Gautam, M., M. Agrawal, M. Gautam, P. Sharma, A.S. Gautam and S. Gautam, 2012. Role of antioxidants in generalised anxiety disorder and depression. *Indian J. Psychiatry*, 54: 244-247.
- Grigor'ev, I.P. and A.A. Neokesariiskii, 1986. [Effect of ascorbic acid on the binding of 3H-GABA and 3H-glutamic acid to synaptosomes of the rat cerebral cortex]. *Biulleten Eksperimental'noi Biologii Meditsiny*, 102: 288-289, (In Russian).
- Guney, E., M.F. Ceylan, A. Tektas, M. Alisik and M. Ergin *et al.*, 2014. Oxidative stress in children and adolescents with anxiety disorders. *J. Affect. Disorders*, 156: 62-66.
- Halliwell, B., 2006. Oxidative stress and neurodegeneration: Where are we now? *J. Neurochem.*, 97: 1634-1658.
- Hamer, M., C.J. Bates and G.D. Mishra, 2011. Depression, physical function and risk of mortality: National diet and nutrition survey in adults older than 65 years. *Am. J. Geriatr. Psychiatry*, 19: 72-78.
- Hamzah, M.H., 2007. Language anxiety among first year Malay students of the international Islamic college: An investigation of 12 skills, sources of anxiety and L2 performance. Master's Thesis, International Islamic University of Malaysia, Malaysia.
- Hovatta, I., J. Juhila and J. Donner, 2010. Oxidative stress in anxiety and comorbid disorders. *Neurosci. Res.*, 68: 261-275.
- Jorm, A.F., H. Christensen, K.M. Griffiths, R.A. Parslow, B. Rodgers and K.A. Blewitt, 2004. Effectiveness of complementary and self-help treatments for anxiety disorders. *Med. J. Aust.*, 181: S29-S46.
- Khassaf, M., A. McArdle, C. Esanu, A. Vasilaki and F. McArdle *et al.*, 2003. Effect of vitamin C supplements on antioxidant defence and stress proteins in human lymphocytes and skeletal muscle. *J. Physiol.*, 549: 645-652.
- Kinsman, R.A. and J. Hood, 1971. Some behavioral effects of ascorbic acid deficiency. *Am. J. Clin. Nutr.*, 24: 455-464.
- Kuloglu, M., M. Atmaca, E. Tezcan, O. Gecici, H. Tunckol and B. Ustundag, 2002a. Antioxidant enzyme activities and malondialdehyde levels in patients with obsessive-compulsive disorder. *Neuropsychobiology*, 46: 27-32.
- Kuloglu, M., M. Atmaca, E. Tezcan, B. Ustundag and S. Bulut, 2002b. Antioxidant enzyme and malondialdehyde levels in patients with panic disorder. *Neuropsychobiology*, 46: 186-189.
- Li, Y.F., K.L. Jackson, J.E. Stern, B. Rabeler and K.P. Patel, 2006. Interaction between glutamate and GABA systems in the integration of sympathetic outflow by the paraventricular nucleus of the hypothalamus. *Am. J. Physiol. Heart Circ. Physiol.*, 291: H2847-H2856.
- Maes, M., P. Galecki, Y.S. Chang and M. Berk, 2011. A review on the Oxidative and Nitrosative Stress (O&NS) pathways in major depression and their possible contribution to the (neuro) degenerative processes in that illness. *Progr. Neuro-Psychopharmacol. Biol. Psychiatry*, 35: 676-692.
- Marim, R.G., A.S.D. Gusmao, R.E.P. Castanho, R. Deminice and A.L.S. Therezo *et al.*, 2012. Effects of vitamin C supplementation on acute phase Chagas disease in experimentally infected mice with *Trypanosoma cruzi* QM1 strain. *Revista Instituto Medicina Tropical Sao Paulo*, 54: 319-323.
- Masood, A., A. Nadeem, S.J. Mustafa and J.M. O'Donnell, 2008. Reversal of oxidative stress-induced anxiety by inhibition of phosphodiesterase-2 in mice. *J. Pharmacol. Exp. Therapeut.*, 326: 369-379.
- Mathew, S.J., R.B. Price and D.S. Charney, 2008. Recent advances in the neurobiology of anxiety disorders: Implications for novel therapeutics. *Am. J. Med. Genet. Part C: Semin. Med. Genet.*, 148C: 89-98.
- Mazloom, Z., M. Ekramzadeh and N. Hejazi, 2013. Efficacy of supplementary vitamins C and E on anxiety, depression and stress in type 2 diabetic patients: A randomized, single-blind, placebo-controlled trial. *Pak. J. Biol. Sci.*, 16: 1597-1600.
- Mazzone, L., F. Ducci, M.C. Scoto, E. Passaniti, V.G. D'Arrigo and B. Vitiello, 2007. The role of anxiety symptoms in school performance in a community sample of children and adolescents. *BMC Public Health*, Vol. 7. 10.1186/1471-2458-7-347
- McCord, J.M., 1993. Human disease, free radicals and the oxidant/antioxidant balance. *Clin. Biochem.*, 26: 351-357.
- McCraty, R., 2007. When anxiety causes your brain to jam, use your heart. Heart Math Research Center, Institute of Heart Math, Boulder Creek, CA., USA.

- Moretti, M., A.E.D. Freitas, J. Budni, S.C.P. Fernandes, G.D.O. Balen and A.L.S. Rodrigues, 2011. Involvement of nitric oxide-cGMP pathway in the antidepressant-like effect of ascorbic acid in the tail suspension test. *Behav. Brain Res.*, 225: 328-333.
- Moretti, M., J. Budni, C.M. Ribeiro and A.L.S. Rodrigues, 2012a. Involvement of different types of potassium channels in the antidepressant-like effect of ascorbic acid in the mouse tail suspension test. *Eur. J. Pharmacol.*, 687: 21-27.
- Moretti, M., A. Colla, G. de Oliveira Balen, D.B. dos Santos and J. Budni *et al.*, 2012b. Ascorbic acid treatment, similarly to fluoxetine, reverses depressive-like behavior and brain oxidative damage induced by chronic unpredictable stress. *J. Psychiatr. Res.*, 46: 331-340.
- Moretti, M., J. Budni, D.B. dos Santos, A. Antunes and J.F. Daufenbach *et al.*, 2013. Protective effects of ascorbic acid on behavior and oxidative status of restraint-stressed mice. *J. Mol. Neurosci.*, 49: 68-79.
- Moretti, M., J. Budni, A.E. Freitas, P.B. Rosa and A.L.S. Rodrigues, 2014. Antidepressant-like effect of ascorbic acid is associated with the modulation of mammalian target of rapamycin pathway. *J. Psychiatr. Res.*, 48: 16-24.
- Naidu, A.K., 2003. Vitamin C in human health and disease is still a mystery: An overview. *Nutr. J.*, Vol. 2, 10.1186/1475-2891-2-7
- Ness, A.R., D. Chee and P. Elliott, 1997. Vitamin C and blood pressure: An overview. *J. Hum. Hypertens.*, 11: 343-350.
- Ng, F., M. Berk, O. Dean and A.I. Bush, 2008. Oxidative stress in psychiatric disorders: Evidence base and therapeutic implications. *Int. J. Neuropsychopharmacol.*, 11: 851-876.
- Rammal, H., J. Bouayed, C. Younos and R. Soulimani, 2008. The impact of high anxiety level on the oxidative status of mouse peripheral blood lymphocytes, granulocytes and monocytes. *Eur. J. Pharmacol.*, 589: 173-175.
- Ranjana, S.K., R. Negi, D. Pande, S. Khanna and H.D. Khanna, 2012. Markers of oxidative stress in generalized anxiety psychiatric disorder: Therapeutic implications. *J. Stress Physiol. Biochem.*, 8: 32-38.
- Rebec, G.V. and R.C. Pierce, 1994. A vitamin as neuromodulator: Ascorbate release into the extracellular fluid of the brain regulates dopaminergic and glutamatergic transmission. *Prog. Neurobiol.*, 43: 537-565.
- Sena, J.D.W., P.A. Lowe and S.W. Lee, 2007. Significant predictors of test anxiety among students with and without learning disabilities. *J. Learn. Disabil.*, 40: 360-376.
- Sindhi, V., V. Gupta, K. Sharma, S. Bhatnagar, R. Kumari and N. Dhaka, 2013. Potential applications of antioxidants: A review. *J. Pharm. Res.*, 7: 828-835.
- Suh, S.Y., W.K. Bae, H.Y. Ahn, S.E. Choi, G.C. Jung and C.H. Yeom, 2012. Intravenous Vitamin C administration reduces fatigue in office workers: A double-blind randomized controlled trial. *Nutr. J.*, Vol. 11 10.1186/1475-2891-11-7.
- Vitasari, P., M.N.A. Wahab, T. Herawan, A. Othman and S.K. Sinnadurai, 2011. A pilot study of pre-post anxiety treatment to improve academic performance for engineering students. *Proc. Soc. Behav. Sci.*, 15: 3826-3830.
- Wang, Y., X.J. Liu, L. Robitaille, S. Eintracht, E. MacNamara and L.J. Hoffer, 2013. Effects of vitamin C and vitamin D administration on mood and distress in acutely hospitalized patients. *Am. J. Clin. Nutr.*, 98: 705-711.
- Weinberger, D.R., 2001. Anxiety at the frontier of molecular medicine. *New Engl. J. Med.*, 344: 1247-1249.
- Wiglusz, Z., A. Nasal, B. Damasiewicz and A. Radwanska, 1983. Possible role of ascorbic acid in catecholamines-stimulated Na⁺-K⁺-adenosine triphosphatase activity of the central nervous tissues. *Pol. J. Pharmacol. Pharm.*, 36: 313-321.
- Zhang, M., L. Robitaille, S. Eintracht and L.J. Hoffer, 2011. Vitamin C provision improves mood in acutely hospitalized patients. *Nutrition*, 27: 530-533.