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-gulonolactone 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). Mazloog 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 (Binare 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 (McCraty, 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 demonstrate 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 Brasilia, 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 (Pharmacotechnics). 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 (Powerspack 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 (H₂SO₄), 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 (3%) 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% H₂SO₄ 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 GraphPad 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>
<thead>
<tr>
<th>Characteristics</th>
<th>Vitamin C</th>
<th>Placebo control</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.4±14.35</td>
<td>24.2±11.07</td>
<td>0.125</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>21 (16/5)</td>
<td>21 (16/5)</td>
<td>1.000</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.62±0.09</td>
<td>1.63±0.09</td>
<td>0.930</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>61.4±14.46</td>
<td>59.0±11.59</td>
<td>0.624</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3±4.72</td>
<td>22.2±3.76</td>
<td>0.513</td>
</tr>
</tbody>
</table>

N: No. of participants. BMI: Body Mass Index. All values are Means±SD; Student’s t-test; Chi-square test and Mann-Whitney U test.
Table 2: Anxiety scores baseline and post-treatment either vitamin C supplementation or placebo, biochemical and physiological parameters or profiles\(^{(5)}\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Vitamin C group (n = 21)</th>
<th>Placebo control group (n = 21)</th>
<th>p-value(^{(5)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Post</td>
<td>Baseline</td>
</tr>
<tr>
<td>BAI scores</td>
<td>22.33±10.35</td>
<td>16.86±10.19</td>
<td>25.43±10.56</td>
</tr>
<tr>
<td>PCVC (mg dL(^{-1}))</td>
<td>1.09±0.52</td>
<td>1.85±0.46</td>
<td>1.19±0.38</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>121.70±23.84</td>
<td>119.00±13.16</td>
<td>114.30±11.85</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>76.10±13.03</td>
<td>75.70±8.49</td>
<td>73.20±10.57</td>
</tr>
<tr>
<td>HR (bpm)</td>
<td>79.20±11.98</td>
<td>74.70±9.56</td>
<td>81.50±13.85</td>
</tr>
</tbody>
</table>

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, \(^1\)All values are Means±SDS, \(^2\)Wilcoxon signed-rank test.

Table 3: Dietary recall from the students\(^{(5)}\)

<table>
<thead>
<tr>
<th>Variables</th>
<th>Vitamin C (n = 21)</th>
<th>Placebo (n = 21)</th>
<th>Total (n = 42)</th>
<th>p(^{(5)})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N (%)</td>
<td>N (%)</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Name of meals</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Breakfast</strong></td>
<td>15</td>
<td>19</td>
<td>34</td>
<td>0.569</td>
</tr>
<tr>
<td><strong>Morning snack</strong></td>
<td>11</td>
<td>7</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td><strong>Lunch</strong></td>
<td>18</td>
<td>20</td>
<td>38</td>
<td>0.230</td>
</tr>
<tr>
<td><strong>Afternoon snack</strong></td>
<td>16</td>
<td>14</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td><strong>Dinner</strong></td>
<td>15</td>
<td>19</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td><strong>Supper</strong></td>
<td>3</td>
<td>8</td>
<td>11</td>
<td>0.672</td>
</tr>
<tr>
<td><strong>Subtotal</strong></td>
<td>78</td>
<td>100.00</td>
<td>165</td>
<td></td>
</tr>
<tr>
<td><strong>Food</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Salads</strong></td>
<td>16</td>
<td>19</td>
<td>35</td>
<td>0.614</td>
</tr>
<tr>
<td><strong>Fruits</strong></td>
<td>7</td>
<td>6</td>
<td>13</td>
<td>0.228</td>
</tr>
<tr>
<td><strong>Greens</strong></td>
<td>5</td>
<td>4</td>
<td>9</td>
<td>0.157</td>
</tr>
<tr>
<td><strong>Sub total</strong></td>
<td>28</td>
<td>100.00</td>
<td>57</td>
<td>0.807</td>
</tr>
<tr>
<td><strong>Beverages</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Soft drinks</strong></td>
<td>10</td>
<td>15</td>
<td>25</td>
<td>0.735</td>
</tr>
<tr>
<td><strong>Juices</strong></td>
<td>4</td>
<td>5</td>
<td>9</td>
<td>0.816</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>120</td>
<td>136</td>
<td>256</td>
<td></td>
</tr>
</tbody>
</table>

\(^{(5)}\)All values are expressed as averages. N: No. of participants, %: Frequency, \(^{(5)}\)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 euphoric. 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 retraining, 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 anxiety levels among high school students and 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 (Gunev 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 (Ramlal 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 Neokesariuskii, 1986). Another study found an increased 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 as a 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 1.61-1.40 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 (Chung 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 recommended 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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