



eISSN: 2321-323X
pISSN: 2395-0781

Review article

Ascorbic acid: A therapeutic viability in anxiety disorders

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Abstract

Anxiety, a subjective feeling of unease, discomfort, apprehension or fearful concern is the most common psychiatric illness seen in patients irrespective of nations, societies and religions. Although it is a normal, emotional, reasonable and expected response to real or potential danger however, if the symptoms of anxiety are prolonged, irrational, disproportionate and/or severe and occur in the absence of stressful events then, these are called Anxiety Disorders which are accompanied by a host of autonomic and somatic manifestations. Studies have suggested that oxidative stress triggers and play an important role in patho-biology of anxiety and antioxidants have shown beneficial results in preclinical and clinical studies. Vitamin C (ascorbic acid) is a well-known antioxidant that is involved in anxiety, stress, depression, and fatigue and mood state in humans. Vitamin C is required for growth and repair of tissues, including collagen and synthesis of norepinephrine and serotonin. It also elevates moods, reduces stress and reduces anxiety. The biochemical functions of vitamin C include stimulation of certain enzymes, collagen biosynthesis, hormonal activation, detoxification of histamine, phagocytic functions of leukocytes, and formation of nitrosamine and proline hydroxylation amongst others. A deficiency of vitamin C reduces production of neurotransmitters associated with anxiety while Vitamin C infusions increase blood vessel smooth muscle communications among small capillaries and increase body's ability to transport blood through brain. Although exact mechanism is yet to be elucidated, yet its involvement in catecholamine, serotonin and neuropeptide synthesis, inhibition of peroxidation of membrane phospholipids and as a scavenger of free radicals in the brain may be a suitable explanation for its anxiolytic mechanism.

Keywords: Anxiety, ascorbic acid, GABA, neuropeptide, oxidative stress, serotonin.

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Anxiety

The environment we are living in is physically, mentally, emotionally, socially and morally dynamic and challenging. Although, we possess effective mechanisms to meet every day stress yet normal adaptive mechanisms can be over-activated and, thus, become maladaptive. A common outcome of such over-activation is anxiety and insomnia [1]. A brief episode of anxiety caused by a stressful event such as that of public speaking is a normal reaction to immediate stress and in fact is a motivation to do better. But when anxiety becomes irrational, persistent and excessive, it is pathological and often manifests into anxiety disorders. There are several types of anxiety disorders including panic disorder, post-traumatic stress disorder, and obsessive-compulsive disorder and generalized anxiety disorders [2]. Anxiety disorders are among the most common mental, emotional and behavioral problems [3]; affecting one-eighth of the total population worldwide and up to 15% of all

people suffers during their life from an anxiety disorder [4]. This suggests anxiety, more chronic than affective or substance abuse disorders [5]. Anxiety, or learned fear, is not necessarily harmful to everyday life rather, is a natural ability that may have arose to evade unnecessary dangers. However, excessive anxiety is debilitating or disadvantageous for life as it reduces behavioral activities necessary for adaptation. Moreover, anxiety can be a core symptom of various mental/ behavioral disorders, such as major depressive disorders, obsessive compulsive disorders, panic disorder, adaptive disorder, post traumatic stress disorder, social withdrawal disorder and various phobias [6]. Treatment of anxiety disorders and consequences of the disease cause high costs and are connected with severe social problems [7]. One in four patients with generalized anxiety disorder is not in a position to meet its daily life requirements [8].

Palanza (2001) suggests that human anxiety disorders can be considered disorders of defense as they involve the inappropriate activation of defensive behavior arising from the erroneous assessment of danger [9]. Anxiety and fear are most common, normal and obvious reactions to a stressful stimuli yet anxiety is a future-oriented mood state that is characterized by apprehension around the inability to predict or control upcoming events while fear is an immediate emotional reaction to current danger that is characterized by strong escapist action tendencies and often an increase in the sympathetic nervous system. While anxiety and fear responses are normal and adaptive to certain degrees, in humans anxiety becomes maladaptive when the anxiety and fear response becomes excessive and leads to related behavioral disturbances [10].

In the search for effective anxiolytic agents, chlordiazepoxide was synthesized in 1957 as the first benzodiazepine by Sternberg. The benzodiazepines, such as chlordiazepoxide and diazepam, were the first primary anti-anxiety agents. Until the mid-90s benzodiazepines were the most commonly prescribed anxiolytics. Despite known sedative effects and addictive potential, they are safe drugs for the short-term treatment of anxiety [11]. At present, anxiety disorders are commonly and effectively treated with anxiolytic medications including Selective Serotonin Reuptake Inhibitors (SSRIs) and benzodiazepines. SSRIs work by preventing the reuptake of serotonin from the synapse, allowing the neurotransmitter to remain in the area of activity for longer. Similarly, benzodiazepines stimulate GABA receptors, mimicking the calming effects of the neurotransmitter and thus reducing anxiety. Benzodiazepines are the major class of compounds used in anxiety and they have remained the most commonly prescribed treatment for anxiety.

While anxiolytic medication is useful in the treatment of anxiety; many negative side effects can be experienced. These side effects can include: addiction, depression, suicide, seizures, sexual dysfunction, headaches and more. Furthermore, the brain often becomes accustomed to these anxiolytic medications resulting in the medication losing its effectiveness and higher doses or different drugs being required [12]. This list of unwanted side effects has prompted considerable research in order to evaluate new compounds with less undesirable side effects.

Vitamin C: Its function at physiological echelon

Vitamin C is one of the important soluble vitamins but humans cannot synthesize ascorbic acid due to the absence of the enzyme L-gulonolactone oxidase, whereas most mammals (e.g., rat and mouse) endogenously produce vitamin C in the liver. Ascorbic acid, or vitamin C, is a six-carbon compound similar in structure to glucose. It exists in two active forms: the reduced form known as ascorbic acid and the oxidized form, dehydroascorbic acid. The molecular structure contains two ionizable enolic hydrogen atoms that give the compound its acidic character [13].

Vitamin C is absorbed from the gastrointestinal tract in the form of ascorbic acid, while dehydroascorbic acid is reduced to ascorbic acid for gastrointestinal absorption. Most ascorbic acid is excreted by the kidneys, but a limited amount is metabolized in the body. Ascorbic acid is oxidized to dehydroascorbic acid, which can undergo irreversible hydrolysis to 2, 3-diketo-L-gulonic acid, with decarboxylation to CO₂ and components of the pentose phosphate cycle or oxalic acid plus threonic acid.

Ascorbic acid has many physiological functions and antioxidant effects. It can also regenerate other antioxidants such as α -tocopheroxyl, urate and β -carotene radical cation from their radical species [14]. This role includes its necessity for the formation of collagen protein found in skin, connective tissue, cartilage and bone and is essential for wound healing, affects immune responses, helps maintain strength in blood vessels, helps protect the body against infections, bacterial toxins, viruses, influences formation of hemoglobin, absorption of iron from intestinal tract, deposition of iron in liver tissue and assists in the secretion of hormones from adrenals. A deficiency of vitamin C may result in symptoms such as pink or hemorrhagic skin follicles, hemorrhages in the eye, inflamed gums, joint pains, excessive hair loss, easy bruising and bleeding gums [15].

Vitamin C has been shown to decrease neophobia and measures of fear in Japanese quail and broiler chickens, attenuate anxiogenic effects of prolonged exposure to loud noise in mice [16], and decrease several forms of anxiety related behavior in rats [17]. In humans too, ascorbic acid has been found to reduce salivary cortisol, blood pressure and subjective anxiety responses to a psychological stress or [18]. Vitamin C beside a broad spectrum radical scavenger also acts as a neuromodulator within the brain, modulating dopamine and glutamate-mediated neurotransmission [19].

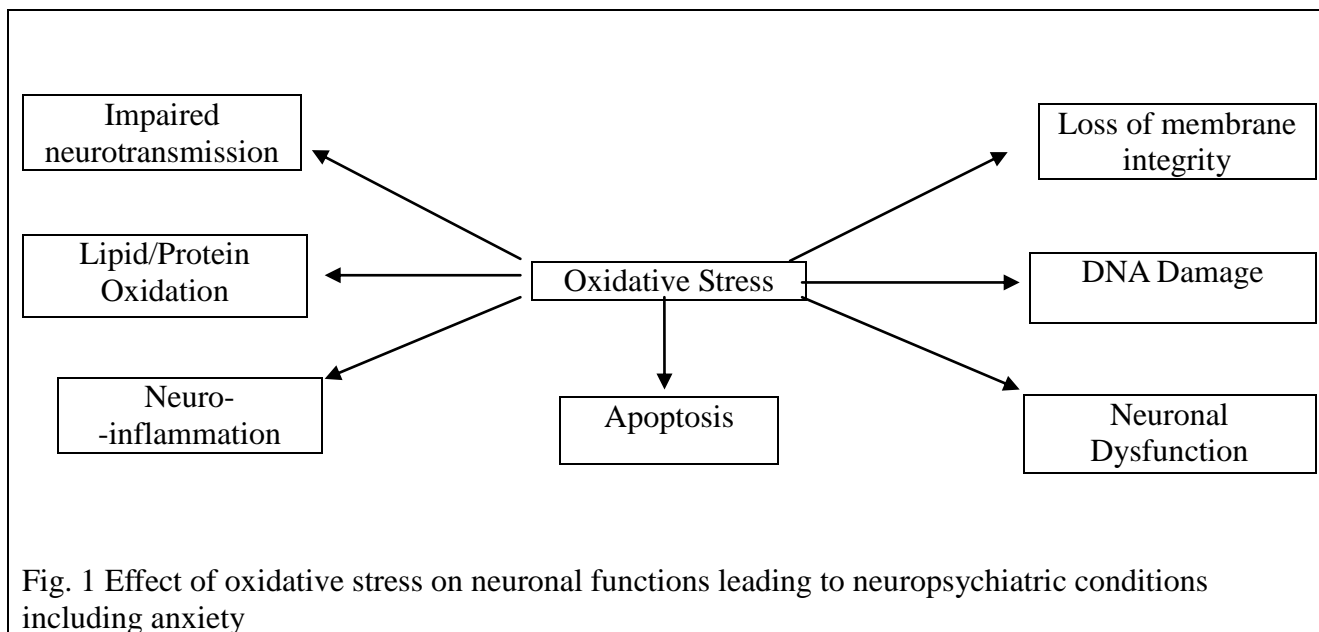
Function of oxidative stress

Oxidative phosphorylation takes place in the mitochondria and is a major source of ATP in aerobic organisms. As a by-product, it produces free radicals, including reactive oxygen species [ROS], reactive nitrogen species [RNS], carbon-centered and sulfur-centered radicals. Free radicals are atoms or groups of atoms with an unpaired number of electrons, which are highly reactive substances that results in chain reactions forming a free radical in each step. The process of oxygen reduction to water generates ROS as intermediates that can cause damage. The primary ROS generated in humans are hydrogen peroxide [H₂O₂], superoxide radical and hydroxyl radical [OH·]. The superoxide radical is generated during auto-oxidation of hemoglobin and photolysis.

Reactive oxygen species can be produced from both endogenous and exogenous sources. Endogenous sources include mitochondria, cytochrome P450 metabolism, peroxisomes, and inflammatory cell activation. The literature has shown that isolated mitochondria can generate

approximately 2-3 nmol of superoxide per minute per milligram of protein. Other cellular sources of superoxide radical generation include xanthine oxidase (XO), which catalyzes the reaction of hypoxanthine to xanthine and xanthine to uric acid, consequently generating superoxide anions and hydrogen peroxide. Neutrophils, eosinophils, and macrophages are other potential sources of cellular reactive species production. The role of cytochrome P450, microsomes and peroxisomes is also well-documented. Notably, superoxide anions can further interact with other

molecules to generate secondary ROS either directly or through enzyme- or metal-catalyzed reactions. Similarly, the auto-oxidation of small molecules, including hemoglobin and myoglobin, mitochondrial components and oxidative enzymes (e.g., xanthine oxidase (XO), nicotinamide adenine dinucleotide phosphate [NADP-H₂]⁺ oxidase] and cyclooxygenases), and the oxidation of unsaturated fatty acids are also reported to produce ROS [20].



In general, any abnormal increase in an oxidative stress promoting substance, often called pro-oxidants, is mitigated by an antioxidant response and thus the pro-oxidant/antioxidant balance is critical. When this balance is disturbed, oxidative and nitrosative stress is initiated as a result of overproduction of ROS and/or insufficiency of the antioxidant defense mechanisms. In the balanced redox status, ROS are beneficial for normal physiological functions and protect the cell from infections by destroying invading pathogens, function as second messengers in the regulation of cardiac and vascular cell functioning and are involved in intracellular regulation of calcium concentration, protein phosphorylation and/or dephosphorylation. Excessive ROS, however, may have detrimental effects and can disturb the maintenance of normal adenine and pyridine nucleotide status, which can affect the viability of DNA, introduce mutation and modify gene expression.

The human brain consumes approximately 20% of basal oxygen during metabolic processes, making the central nervous system very sensitive to oxidative stress. Oxygen metabolism results in the production of oxygen ions and various free radicals. The radicals derived from oxygen represent the most important class of such species generated in living systems and may damage normal cellular compartments, resulting in compromised function.

Therefore, it is not surprising that oxidative stress is implicated in several disorders of the brain including neurodegenerative disorders, psychiatric ailments and anxiety [20].

Oxidative stress, anxiety and vitamin c

Various psychodynamic, psychoanalytic, behavioral, cognitive, genetic and biological theories have been proposed to explain the etiology and path physiology of anxiety disorders [5]. One of the well accepted explanation for anxiety is involvement of oxidative stress.

The nervous system has tremendous reservoirs of polyunsaturated and saturated fatty acids that are extremely susceptible to the escalating effects of oxidative stress. The loss of membrane integrity, protein damage, neuronal dysfunction, lipid and protein oxidation and DNA damage are some key examples of the consequences of oxidative stress. Antioxidant enzymes, such as superoxide dismutase (SOD), catalase, glutathione peroxidase and non-enzymatic antioxidants, including vitamin C, vitamin E, carotenoids, thiol antioxidants (e.g., glutathione, thioredoxin, and lipoic acid), natural flavonoids, melatonin (i.e., a hormonal product of the pineal gland), and other compounds constituting a defense mechanism that prevents the escalating effects of ROS.

Oxidative stress has been strongly implicated in the pathophysiology of anxiety disorders. Some animal studies have linked genes glyoxalase 1 (Glo1) and glutathione reductase 1 (GR), both of which protect against oxidative stress, with anxiety in mice [21]. It was found that the over expression of Glo1 and GR in the cingulate cortex was linked to increased anxiety behaviors in mice, while the inhibition of the Glo1 gene reduced anxiety behaviors.

It has also been shown that patients with Generalised Anxiety Disorder (GAD) and depression have significantly lower plasma levels of vitamins and chronic treatment with vitamin C leads to significant reduction in anxiety and depression scores as observed along with a significant increase in the blood levels of vitamin C [22]. When taken together with the antioxidant changes reported in some psychiatric disorders, the above may suggest a link between vitamin C, oxidative stress and such disorders.

Besides that, a number of studies have demonstrated the potential antioxidant effects of antidepressant medications. Bilici et al. (2001) [23] found that patients who were majorly depressed had higher levels of antioxidant enzyme activities and lipid peroxidation compared to those of healthy controls. After 3 months treatment with selective serotonin reuptake inhibitors (SSRIs), antioxidant enzyme activities and lipid peroxidation levels were significantly decreased to normal levels. Hovatta et al., (2005) [21] demonstrated a close correlation between brain expression of genes of the antioxidative defense system (glutathione reductase 1 and glyoxalase1) and anxiety-related phenotypes across all mouse strains. They further found that the activity of the antioxidative enzymes of glutathione reductase 1 and glyoxalase 1 is highest in the most anxious strain and lowest in the least anxious strains. A link between oxidative stress and emotional stress is not surprising per se; since it is well accepted that oxidative damage in the brain may cause an impairment of the nervous system. It was found that local over expression of glutathione reductase 1 and glyoxalase 1 in the cingulated cortex of the murine brain results in an increase of anxiety-like behaviour, while inhibition of glyoxalase 1 expression produces low-anxiety. Thus a causal link between the antioxidative status of the brain and anxiety-related behaviour supposing that glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice is proposed. However, in vivo, antioxidant genes (e.g. superoxide dismutase, glutathione peroxidase and glutathione reductase) are normally over expressed in response to an uncontrolled production of ROS.

Also, when the production of ROS prevails over the brain defense systems, the lipid-rich constitution of brain may favor lipid peroxidation, constituting a free radical chain reaction that may result in decrease in membrane fluidity and damage in membrane proteins inactivating receptors, enzymes and ion channels, even disrupting membrane integrity resulting eventually in cell death. In addition to oxidative damage of neuronal membrane lipids and proteins, oxidation of other sensitive components such as nucleic acids and neurotransmitters can occur. As a result,

OS can alter neurotransmission, neuronal function and overall brain activity [24].

Vitamin C readily scavenges reactive oxygen and nitrogen species, like superoxide and hydroperoxyl radicals, aqueous peroxy radicals, singlet Oxygen, ozone, peroxy nitrite, nitrogen dioxide, nitroxideradicals, and hypochlorous acid, thus effectively protecting other substrates from oxidative damage [25].

Anxiety and oxidative stress

There are several researchers who investigated and found vitamin c an anxiolytic because of its antioxidant attributes [22, 26, 27 and 28] yet the exact mechanism is awaited to be explored. In recent studies it is observed that the expression of two antioxidant enzymes involved in the oxidative stress pathway and implicated in anxiety-like behaviors. Glyoxalase [GLO]-1 and glutathione reductase [GSR]-1, were reduced in the hippocampus, amygdala and the cortex of pro-oxidant buthionine-[S, R] - sulfoximine [BSO] treated rats, which was prevented with antioxidant grape powder treatment. Perhaps, failing antioxidant defense contributed by reduced expression of GLO1 and GSR1 leads to anxiety-like behavior in these animals [29, 30].

The mouse GLO1 gene encodes the 21 kDa, 184-amino-acid enzyme glyoxalase I (GLO1). It is found as a dimer in the cytosol of cells. Its physiological function is to detoxify dicarbonyl metabolites, mostly methylglyoxal (MG), glyoxal and other low-molecular-weight acyclic -oxoaldehydes. Hovatta and colleagues [21] also identified the link between anxiety-like behavior and oxidative stress in their behavioral study of inbred mice strains. They found that in the cingulated cortex of the brain, altered expression of glutathione reductase 1 and glyoxalase 1, genes that have a significant role in the antioxidant system, resulted also in changes of anxiety-like behaviour, in a manner that over expression of these genes increased anxiety-like behaviour, whereas inhibition of glyoxalase 1 by siRNA resulted in decreased anxiety. Furthermore, it was reported that the anxiety level was correlated with malondialdehyde, superoxide dismutase and glutathione peroxidase in patients with social phobia [31].

Vitamin C and neurochemicals: Possible involvement in anxiety

The neuroanatomic circuits that support fear and anxiety behavior are modulated by a variety of neurochemicals. Vitamin C also synthesizes neuropeptides. The agents like Adenosine, GABA, KF-1 an ubiquitin ligase, Atrial natriuretic peptide, LYNX2 a prototoxin genes, melatonin and neurosteroids are anxiolytics. Cholecystokinin, CRF, Estrogen, Arginine vasopressin, Glutamatergic transmission, Glucagonlike peptide-1, melanin concentrating hormone, Norepinephrine, progesterone, PKCe, serotonin and Tachykinins are Anxiogenic. Whereas Cannabinoids, Neuropeptide Y and Galanin have dual action i.e. anxiogenic as well as anxiolytic [6].

Vitamin C is also required for the activity of peptidylglycine alpha-amidating mono-oxygenase. This enzyme catalysis the rate-limiting step in the biosynthesis of neuropeptides. Vitamin C is also believed to be a neuromodulator of both glutamate and dopamine mediated neurotransmission [19]. Dopamine and glutamate have both been found to be implicated in the release of ascorbate and in turn appear to be regulated by this release [32]. It suggests that ascorbic acid in particular brain regions may act as a type of signalling molecule, regulating the postsynaptic efficacy of these neurotransmitters. Behavioural, electrophysical, and pharmacological evidence for ascorbic acid-related modulation of neuronal function currently exists.

Role of anxiety, HPA axis and vitamin C

The concept of stress is based on the observation that different kinds of physical or psychological conditions that threaten the organism's homeostasis elicit the same set of bodily changes, the so-called 'general adaptation syndrome' (Selye H, 1936). The most characteristic stress response is the release of the adrenocorticotrophic hormone (ACTH) and corticoids (cortisol in humans and cortisone in rats) into the blood stream as a result of activation of the HPA axis. The stimuli or situations that elicit the general adaptation syndrome are called stressors and the organism response is the stress reaction. In addition to the HPA axis, acute stress also activates the sympathetic division of the neurovegetative nervous system as part of the fight/flight reaction, or 'emergency response'. As a result, noradrenaline is released from peripheral sympathetic nerve fibers in different tissues, and adrenaline (also some noradrenaline), from the adrenal medulla into the blood stream. Stressors may be physical, such as tissue damage or extreme changes in temperature, but may also be psychological. As to the latter, reported results have consistently shown that the HPA axis and the sympathetic nervous system are activated by novelty or cues that signal the delivery of punishment or the withholding of an expected reward (frustration), thus generating anticipatory anxiety [34].

The neural circuits that mediate the neuroendocrine responses to psychological stressors include the cortical activation of the basolateral nucleus of the amygdala, which in turn activates its central nucleus. The message is then conveyed to hypothalamic neurons through different pathways: a direct one, an indirect one, through the bed nucleus of the stria terminalis, and still another one, through brainstem serotonin (5-HT) and catecholamine-containing neurons. Neurons of the hypothalamic paraventricular nucleus secrete the corticotrophic releasing hormone (CRH) into the portal circulation of the pituitary gland. In the anterior pituitary, CRH stimulates ACTH-secreting cells that release ACTH into the blood stream. ACTH acts on the adrenal cortex promoting cortisol release into the blood stream. In addition to ACTH, prolactin is consistently released from the anterior pituitary in stressful conditions [35]. Both the HPA axis and the sympatho-adrenal axis are

activated by anticipatory anxiety. In acute anxiety, the activation of the HPA axis is adaptive, since, among other things, corticoids seem to reduce perceived fear by impairing memory retrieval of emotionally arousing information. In chronic anxiety, however, long-term activation of the HPA axis may become harmful, since corticoids hamper resilience mechanisms in the hippocampus [36].

Preclinical evidence has also shown that 5-HT 1A receptors in the hippocampus facilitate development of tolerance to chronic stress. Corticoids decrease the sensitivity of hippocampal 5-HT 1A receptors, impairing the coping mechanism. Accordingly, in the major depressive disorder, the feedback regulation of cortisol blood level is damaged, resulting in constant high levels of circulating cortisol. This would desensitize the hippocampal 5-HT 1A receptors, perpetuating the clinical condition. A key mechanism of action of antidepressant agents is to enhance the efficacy of 5-HT neurotransmission, which occurs following chronic treatment [37].

As vitamin C is highly concentrated in the adrenal glands, with concentrations as high 10 mmol/L, vitamin C and stress have long been associated. Furthermore, an increase in the concentration of vitamin C in human plasma has been related to corticotrophin [38]. In animals, ACTH also causes the loss of vitamin C from the adrenal glands [39]. While direct evidence is limited to support the association between stress and vitamin C, a study by Padayatty et al. (2007) found that stimulation increased adrenal vein vitamin C concentrations, suggesting that adrenal vitamin C secretion in humans is an integral part of the stress response.

Systemic administration of ascorbic acid has also been shown to change neuronal firing rates during single unit recording, which may be due to the modulation of the N-methyl-D-aspartate (NMDA) receptor function [40]. The modulation of the above neurotransmitters by a readily available vitamin, ascorbic acid, has important clinical implications as dopamine and glutamate play a critical role in severe neurological and psychiatric disorders such as Parkinson's disease, schizophrenia, and anxiety disorders. Vitamin c has shown potent anxiolytic properties and the best suited explanation is antioxidant potential beside that it has number of effects on the brain including protection against oxidative damage, involvement in catecholamine synthesis and possible neuromodulation of neural transmission via glutamine, dopamine, acetylcholine and GABA along with associated behavioral responses [41,42]. Vitamin C is needed to convert tryptophan, amino acid present in the animal proteins in the diet, into serotonin, major neurotransmitter of the brain involved in anxiety and target of anxiolytic drugs. Since synthesis of serotonin, dopamine and nor epinephrine requires vitamin C, it is to be expected that their synthesis would be impaired if vitamin C is deficient. It is an established fact that serotonin, dopamine and nor-epinephrine play important roles in maintaining mood. Deficiency of nor- epinephrine can cause clinical depression and poor memory, and

deficiency of serotonin can produce a depressant effect [43]. Overall it can be safely concluded that vitamin C is beneficial in maintenance of mental health [44].

Conclusion

Vitamin C is implicated in the functioning of many systems within the human body including enzyme activation, synthesis of serotonin, neuropeptides, oxidative stress reduction and has even been associated with modulation of neurotransmitters dopamine and glutamate release. As oxidative stress has been implicated in the pathophysiology of a number of psychiatric disorders including anxiety, vitamin C may have potential merit in the treatment of anxiety and stress related disorders.

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