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Review Article

Potential use of modulators of oxidative stress as add-on therapy in patients with anxiety disorders

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It is known that an increased oxidative stress is present in a wide range of diseases and, given the vulnerability of the central nervous system, its involvement has been in particular investigated in neurological and psychiatric diseases, including anxiety disorders. In this review we analyse the studies that have been conducted on the effects of oxidative stress modulators in anxiety, focusing on their possible clinical use. While preclinical studies have shown a clear anxiolytic-like effect of different oxidative stress modulators, less significant results have been obtained from clinical studies. After having reviewed the possible reasons for the discrepancy between preclinical and clinical data, we encourage further studies aimed at better investigating the utility of the modulation of oxidative stress in humans, as adjunctive therapy of the traditional integrated psychotherapeutic and pharmacological approach.

Keywords: Oxidative stress modulators, anxiety disorders, anxiety models, reactive oxygen species, reactive nitrogen species, lipid peroxidation.

Contents

1. Introduction

1.1. Anxiety disorders

Anxiety disorders are the most common class of psychiatric disorders [1, 2]. The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) divides them in: separation anxiety disorder, selective mutism, specific phobia, social phobia, panic disorder, agoraphobia, generalized anxiety disorder, anxiety disorder caused by use of substances, and anxiety disorder due to another medical condition [3].

Many epidemiological studies have shown that anxiety disorders typically start in childhood or in early adult age and tend to decrease in older age, with a median age of onset of 11 years [4], and in the absence of proper treatment they can become chronic. Anxiety disorders in Europe have a twelve months and a lifetime prevalence of 8,4% and 14,8%, respectively, with prevalence being approximately twice higher in women than in men [5]. They cause more loss of working days than other disorders of high social impact, with an economic burden of 41 billion Euros in the EU in 2004 [5]. Anxiety disorders are often in comorbidity with other psychiatric conditions, such as depressive disorders, drug and alcohol abuse, personality disorders, bipolar disorder [6] and also with chronic medical conditions, such as diabetes [7],chronic obstructive pulmonary disease [8], thyroid dysfunction [9] and coronary artery disease [10].

Amygdala and the insular cortex play a crucial role in the pathophysiology of anxiety [11]; these brain regions are activated in response to fear in healthy individuals, and are hyperactive during the processing of negative emotions in patients with anxiety disorder [11].

Although different theories have been proposed, the etiology of anxiety disorders is still unclear [12]. One theory suggests an association between the biochemical alterations caused by oxidative stress and anxiety, although a causal relationship between oxidative stress and anxiety has not been demonstrated, as yet.

1.2. Oxidative Stress

Oxidative stress is determined by the excessive production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) [13]. Their deleterious action on proteins, DNA and lipids causes cell damage.

1.2.1 Production and cellular effects of ROS

The main cellular source of ROS is the mitochondrial respiratory chain, which is composed by five multisubunit protein complexes, and utilizes nicotinamide adenine dinucleotide (NADH), flavin adenine dinucleotide (FADH2), coenzyme Q (CoQ), and cytochrome c (Cyt c)as electron donors [14]. Electrons are transferred one by one from the respiratory chain to O_2, leading to the formation of superoxide

anions (O_2^{--}), which may leave the respiratory chain and can generate additional ROS species by interacting with other molecules [15]. NADPH-oxidase (NOX), xanthine oxidase (XO), cyclooxygenase, lipoxygenase, epoxygenase, and cytochrome-P450 are additional sources of O_2^{--} [16]. O_2^{--} can be converted into hydrogen peroxide (H_2O_2) by copper-zinc superoxide dismutase (SOD1) in the cytosol and manganese superoxide dismutase (SOD2) in the mitochondria. The amount of O_2^{--} that is not inactivated undergoes protonation into hydroperoxyl (HO_2), which, in turn, causes lipid peroxidation. Hydrogen peroxide, which is also produced by peroxysomal enzymes is detoxified by catalase (CAT), glutathione peroxidase (GPx), and peroxiredoxins. Alternatively, hydrogen peroxide is converted into hydroxyl radicals ($OH^.$) in the presence of Fe^{2+} (Fenton reaction). Fe^{2+} can be generated from reduction of Fe^{2+} in the presence of O_2^{--}. The net reaction leading to the formation of $OH^.$ and hydroxyl anions (OH^-) is named Heber-Weiss reaction [14, 17]. Hydroxyl radicals are powerful oxidizing agents, that can damage proteins, lipids, sugars and DNA [16].

1.2.2 RNS and their cellular effects

The RNS, nitric oxide ($NO^.$) and peroxynitrite ($NOOO^-$), have gained popularity as intracellular and intercellular mediators, and may also cause cellular damage. Nitric oxide (NO) is synthesized from L-arginine by NO synthase (NOS) isoenzymes [18]. NO can compete with SOD for O_2^{--}, forming $NOOO^-$, which can nitrosylate tyrosine residues in proteins [19] leading to structural and functional protein changes [20]; $NOOO^-$ can also cause lipid peroxidation and apoptotic death [21, 22].

1.2.3 Lipid peroxidation and its products

Lipid peroxidation caused by ROS or RNS, represents a common mechanism of lipid damage. In this particular process, oxidants attack the lipids containing carbon-carbon double bonds, like polyunsaturated fatty acids (PUFAs), glycolipids, phospholipids and cholesterol

[23] . Lipid peroxidation is a chain reaction that once started continues until the formation of the final products. Lipid hydroperoxides are the main primary products of lipid peroxidation; alkens, ketones, and reactive carbonyl compounds are secondary products (RCCs) [23]. They include aldehydes, such as malondialdhyde (MDA) and 4-hydroxynonenal (4-HNE), and α-oxalaldehydes, such as glyoxal and methylglyoxal [24, 25]. These compounds may cause cytoxicity by promoting the formation of end glycation products or by damaging DNA, proteins, and membrane lipids [26].

2. Oxidative stress and anxiety in animal models

In preclinical studies, anxiety-like behaviour can be induced by exposing animals to environmental stressors, such as restraint, predator odor, and social defeat, or alternatively, by a paradigm of fear conditioning, in which a neutral stimulus is associated with an aversive stimulus (for example, a red light with a foot shock). The resulting anxiety-like behaviour can be evaluated by conflicts tests, such as the elevated plus maze (EPM), the light-dark box (LD), or the open field (OF) test, in which the natural propensity of rodents to explore in an open space is in conflict with the vulnerability to threats; the most anxious animals have a reduced tendency to exploration compared to the average behaviour of their strain [27].

2.1 Evaluation of anxiety-like behaviour in models of genetically induced oxidative stress

Lipid peroxidation products can be metabolized by enzymatic detoxification systems. For example, α-oxalaldehydes are detoxified by the glyoxalase system, composed by the enzymes glyoxalase 1(Glo1) and glyoxalase 2, and by reduced glutathione (GSH). Glyoxalase 1 has a broad substrate spectrum for oxoaldehydes and catalyses the reaction that leads to lactoylglutathione from the emithioacetal produced non enzymatically by the binding of an oxoaldehyde and a

reduced GSH molecule [28]. Glyoxalase 2, regenerates reduced GSH by the hydrolysis of lactoylglutathione into lactic acid [29]. Since the study of Hovatta et al (2005), mice with genetic manipulation of detoxifying enzymes have been widely used for the investigation of the correlation of oxidative stress and anxiety-like behaviour. Hovatta et al. examined the correlation between Glo1 and glutathione reductase 1 (Grs1) genes with anxiety-like behaviour. They found an increased expression of Glo1 in mouse strains with high levels of anxiety; in addition, lentiviral vector-induced overexpression of Glo1 and Grs1 in the cingulate cortex caused an increased anxiety-like behaviour, whereas a reduced anxiety was seen after Glio1 gene silencing [30]. In contrast, Krömer et al., using outbred CD1 mice, found an association between low level of anxiety and increased expression of Glo1 in the hypothalamus, amygdala and motor cortex [31], and in peripheral red blood cells [32].

A study by Williams et al. shows evidence that variability of Glo1 expression in mice is the result of a common duplication of a large genomic region including the Glo1 gene. Gene duplication is associated with increased Glo1 expression and anxiety-like behaviour both in inbred and outbred CD1 mice [33], in agreement with findings obtained by Hovatta et al. [30]. It has been proposed that methylglyoxal induced neuronal damage may be a critical determinant in the high- or low-anxiety behaviour seen in different studies. [34]

Using transgenic mice, Distler et al. found that Glo1caused an anxiolytic effect by reducing the formation of methylglyoxal, thereby activating $GABA_A$ receptors [35]. Moving from these findings, it has been proposed that Glo1 may represent a putative drug target in the treatment of anxiety disorders [36], although the precise role played by Glo1 in the pathophysiology of anxiety should be the subject of further investigation [35] .

2.2 Evaluation of anxiety-like behaviour in models of oxidative stress induced by chemical compounds

Other studies have employed rodent models of oxidative stress based on the use of oxidative stress inducers. Masood et al treated mice with buthionine sulfoximine (BSO), an oxidative stress inducer that depletes GSH, and found an increase in anxiety-like behaviour evaluated in the EPM and OF. Knowing that the NO-cGMP signalling influences anxiety-like behaviour, they also investigated if phosphodiesterase-2 (PDE-2) inhibitors could impact the association between anxiety-like behaviour and oxidative stress. They found that PDE inhibitors could restrain the anxiogenic effect of oxidative stress by increasing c-GMP signalling, while administration of diazepam was ineffective. They also found that the anxiety-like behaviour induced by BSO was antagonized by the NOX2 inhibitor apocynin [37]. Salim et al. found that subchronic but not acute treatment with BSO in rats increased anxiety-like behaviour, and this correlated with the presence of oxidative stress biomarkers in the serum, urine, amygdala, and locus coeruleus [38]. In a different study, the same Authors reported that BSO administration in rats decreased the expression of Glo1 and Gsr1 and induced the expression of ERK1/2 in the amygdala, cerebral cortex and hippocampus [39], in agreement with previous studies that showed the involvement of the ERK 1/2 pathway in anxiety [40, 41].

2.3 Evaluation of oxidative stress in models of innate and conditioned anxiety

Further evidence supporting the correlation between anxiety and oxidative stress was obtained with animal models of innate and conditioned anxiety in which different parameters of oxidative stress were evaluated. Hassan et al used two lines of rats, characterized by a different freezing response to fear conditioning, named "Carioca high- and low-conditioned freezing"(CHF and CLF). In the CHF line, the concentrations of free radicals and MDA were higher in the hippocampus, cerebellum, and cerebral cortex, CAT activity was decreased in hippocampus and cortex, and GPx activity

was decreased in all three structures, as compared to the CLF line [42].

A following study showed that administration of diphenyl diselenide, a compound endowed with antioxidant propriety, caused a reduction of freezing in anxious rats [43].

In rats, exposure to a predator odor caused an increased ROS production and SOD activity in the amygdala, and an increase in SOD activity only in the prefrontal cortex and hypothalamus [44]. In another study in which exposure to predator odor was associated to psychosocial stress, ROS levels were significantly elevated in the hippocampus and prefrontal cortex of stressed animals [45].

The role of RSN in anxiety was examined in a model of social isolation. Nitrite levels were increased in the hippocampus and cortex of stressed mice, and administration of NOS inhibitors reversed anxiety-like behaviour induced by social isolation [46]. Increased nitrosative stress was also found in restrain stress model of anxiety [47].

3. Oxidative stress in patients with anxiety and other psychiatric disorders

The Central Nervous System (CNS) is highly vulnerable to oxidative stress for the following reasons [48]: (i) the brain has a high oxygen consumption (about 20% of the assumed oxygen) [49]; (ii) neuronal membranes are rich in PUFA, which are susceptible to lipid peroxidation [50]; (iii) antioxidant defences are less efficient in the CNS than in other organs because of a lower expression of CAT, GSH-peroxidase, GSH and vitamin E [51]; and, (iv) intracellular iron accumulates in neurons of the aged brain [52].

The knowledge that the human brain is particularly vulnerable to oxidative stress stimulated the search for peripheral markers of oxidative in the blood of patients with psychiatric disorders, such as anxiety [49]. A clinical study evaluated oxidative stress in patients with generalized anxiety disorder (GAD), by measuring the total oxidative status (TOS), the total antioxidant status (TAS) and the ratio between TOS and TAS, which is the oxidative stress index (OSI). They found that TOS and OSI were significantly higher in patients affected by GAD than in healthy controls, while TAS was lower [53].

Increased levels of lipid peroxidation markers have also been found in patients with anxiety disorders [13]. Atmaca et al found increased MDA levels, and an increased activity of SOD, CAT, and GPx in patients with social phobia [54]. Increases in MDA levels, depletion of GSH, vitamins A and E, and increased SOD and GPx activity were found in patients suffering of panic attacks [55, 56]. In contrast, Hagan et al., in a cross-sectional study of 1,325 women, did not find an association between phobic anxiety and plasma fluorescent oxidation products (FLOPs), which are non-specific markers of global oxidative stress originating from the interaction of ROS with macromolecules [57]. In children with anxiety disorder Ceylan et al. found higher serum levels of lipid hydroperoxide (LOOH) as compared to healthy controls with no changes in the activity of the antioxidant enzymes, paraoxonase and arylesterase [58]. A similar increase in plasma LOOH levels was reported in adult patients affected by GAD. These patients also showed a reduced activity of paraoxonase [59].

Patients with depression, anxiety and alcohol abuse show increases in pro-inflammatory cytokines associated with an increased activity of transcription factors, such as nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kβ) and the cAMP response element-binding protein (CREB); these factors regulate the expression of inflammation related enzymes, like cyclooxygenase-2 (COX-2), NOS and NADPH-oxidase, which are involved in ROS production [13].

4. Oxidative stress modulators

Despite OS is only one of the multiple mechanisms involved in the pathophysiology of anxiety disorders,

OS modulation may provide a novel strategy for add-on treatment of anxiety, as highlighted by numerous observations.

4.1 Mechanisms of action of oxidative stress modulators

OS modulators act *via* different mechanisms that may operate simultaneously.

One of the best documented antioxidant mechanism consists in a direct free radical scavenging and metal chelation. This, for example, is characteristic of polyphenols, whose activity, however is, limited by their low central bioavailability [60].

An indirect antioxidant action can instead be exerted through the induction on the enzymatic antioxidant system. Induction of antioxidant enzymes follows the activation of the Nuclear factor (erythroid-derived 2)-like (Nrf2) pathway. Nrf2 is a transcription factor that drives the expression of several detoxification and antioxidant enzymes. Under basal conditions, Nrf2 is sequestered in the cytoplasm by Keap1, which acts as a substrate adaptor protein for the Cul3-containing E3 ligase that promotes Nrf2 ubiquitination and proteasome degradation [61]. Keap1 also acts as a sensor for oxidative and electrophile stress. Under OS conditions some critical cysteine residues of Keap-1 are covalently modified, causing a conformational change of the protein, which disrupts its interaction with Nrf2. As a result, Nrf2is not degraded and translocates into the nucleus, where it heterodimerizes with Maf proteins and binds to antioxidant sequence elements (AREs) sequences inducing the transcription of many drug-metabolizing and antioxidant enzymes. Nrf2 is highly expressed in the CNS and a defective activation of Nrf2 has been associated to chronic neurological diseases [62]. Data obtained in experimental animals suggest that the Nrf2 pathway plays a role in anxiety disorders. Intracerebroventricular injection of Nrf2 siRNA in adult Wistar rats increased anxiety-like behaviour associated with an increased Bax/Bcl ratio in the hippocampus, amygdala, and prefrontal cortex [63]. Nrf2 activation

has been shown to be a common mechanism of action of several antioxidants, such as curcuminoids, quercetin, epigallocatechin gallate, resveratrol, N-acetylysteine and ebselen. However, the risk-to-benefit ration should be carefully evaluated when using Nrf2 activators. For example, Nrf2 activation may promote tumour growth and cancer cell resistance to chemotherapy [64].

Another indirect antioxidant mechanism is the modulation of the activity of sirtuins, a family of seven proteins (SIRT1-7) that act as epigenetic regulators, and are involved in a wide range of cellular processes, including oxidative stress [65]. In particular, SIRT1 and SIRT3 are primarily involved in OS regulation [66]. SIRT1 has both cytoplasmic and nuclear localization and it mediates the deacetylation and activation of different transcription factors and co-activators of genes encoding for antioxidant proteins. Among its targets there are the transcription factors of the forkhead box class O (FoxO) family, which regulate the expression of SOD2 and the peroxisome proliferator-activated receptor co-activator 1-α (PGC-1α), which in turn regulates the expression of GPx1 and CAT. Furthermore, SIRT1 may deacetylate the p65 subunit of NF-kB, with ensuing suppression of inducible nitric oxide synthase (iNOS). SIRT3 is instead mainly localized in the mitochondria, where it deacetylates and activates SOD2 [66].

Because sirtuins have a widespread role in the regulation of cellular processes, the use of their activators and inhibitors may prove useful in different therapeutic areas. In anxiety disorders, although SIRT1 activation exerts antioxidant activity, it may increase anxiety, through other mechanisms such as monoamino oxidase A (MAO-A) activation [67]. This may help explain the contrasting data obtained with resveratrol, a SIRT1 activator, in the treatment of anxiety.

4.2 Polyphenols

4.2.1 Quercetin

One of first review about the neuroprotective properties of food plants, including quercetin, was written by

Aruoma and published in 2003 [68].Lu et al. found that quercetin reversed the neurotoxic effect of D-galactose in the mouse brain, maintaining Ca^{2+} homeostasis and increasing the transcript of the neural growth-associated protein GAP43. Quercetin also reduced anxiety-like behaviour in the OF and corrected the D-galactose-induced impairments in the "step-through" and Morris Water Maze tasks [69]. Afterwards, Kumar and Goyal studied the effects of quercetin on the biochemical and behavioural modifications induced by an immobilization acute stress in mice. Quercetin administration prior to exposure to stress reduced anxiety-like behaviour, lowered MDA and nitrite levels, and restored the activity of antioxidant enzymes [70]. In a similar experiment, Toumi et al. administered quercetin to pregnant rats prior to the exposure to predator odor. Quercetin pre-treatment reduced stress-induced activation of the hypothalamic-pituitary-adrenal (HPA) axis, attenuated oxidative stress in the dam brain, and decreased maternal anxiety [71].

Quercetin was also effective in reducing stress-induced depression and the associated increase in hypothalamic corticotropin-releasing factor (CRF) mRNA levels [72]. It has been hypothesized that quercetin restrain the anxyogenic and pro-depressant effects of CRF [73]. Accordingly, quercetin and the CRF receptor antagonist, antalarmin, produced synergistic anxyolitic and antidepressant effects perhaps through the modulation of OS [74].Quercetin administration was also shown to be protective against toxicity induced by heavy metals, such as aluminium [75] or cadmium [76]. Exposure to both agents caused a reduction in acetylcholinesterase (AChE) activity and ATP levels in the rat brain andan impairment in cognitive and behavioural tasks associated with an increased anxiety-like behavior. Quercetin treatment could reverse all these pathological changes. The effects of quercetin were ascribed to its ability to reduce OS, thus protective cell membranes and associates enzymes against oxidative damage.

Quercetin has been shown to rescue anxiety-like behaviour also in a model of neurotoxicity induced by polychlorinated biphenyls (PCBs) in adult rats. Quercetin also reduced PCB-induced ROS formation, hippocampal damage, and abnormalities in neurotransmitter levels [77].

Other studies have shown that the anxiolytic and pro-cognitive effects of quercetin are associated with changes in brain levels of GABA [78] and serotonin [79]. Quercetin, like other flavonoids, acts also as a Monoamino Oxidase A inhibitor [80–83].

4.2.2 Ginkgo biloba: EGb 761 and ginkgolides

Ginkgo biloba is a very ancient plant, and its fruits, seeds, and leafs have been widely used by traditional medicine [84]. Currently the extract EGb 761 of Ginkgo biloba leaves is primarily used to improve memory and other potential therapeutic uses are under investigation. The extract has numerous pharmacological activities mediated, *inter alia*, to terpenes and flavonoids.

Animal studies have shown that EGb 761 displays antidepressant/anxiolytic effects, described as anti-stress or stress-alleviating [85]. A first evidence for the anti-stress action of EGb 761 has been provided in 1990 by Porsolt, using the "learned helplessness" and other models of stress in rodents. Data showed that administration of EGb 761 was effective in reducing the deficit in avoidance response in the learned helplessness paradigm, and its efficacy was greater when administered prior to the induction of stress. Moreover, in the emotional hypophagia test, EGb 761 administration increased the amount of food consumed by rodents, showing an anxiolytic-like activity [86]. An anti-stress effect of EGb 761 has also been demonstrated in mice undergoing a learning discrimination task. Exposure to stress during the discriminative phase of learning had negative effects on learning and caused an increase in epinephrine, norepinephrine and corticosterone plasma levels. Treatment with EGb 761 for 20 days could reduce all these changes [87]. Amri et al. showed that treatment with EGb 761 or its components, ginkgolides A and B, reduced mRNA and protein levels of peripheral benzodiazepine receptors

(PBRs) in mitochondria of the adrenal gland. Because PBR regulates the transport of cholesterol across mitochondrial membranes, a lower PBR expression reduces the synthesis of corticosterone (corticosteron in rodents is the equivalent of cortisol in humans) and other adrenal hormones in the adrenal gland [88]. Moving from these findings, Marchilac et al. further investigated the effects of EGb 761 on the response of the hypothalamus-pituitary-adrenal (HPA) axis to stress. Mice were exposed to surgical stress, which caused the expected increase in CRH, ACTH, and corticosterone levels. Treatment with 100 mg/kg of EGb 761 50mg/kg restrained the activation of the HPA axis and also reduced the increase in circulating catecholamine levels induced by surgical stress [89].

A series of studies examined the effect of Ginkgo Biloba extracts on anxiety-like behaviour and social behaviour in rodents. Low doses of Zingicomb (ZC), a combination of extracts of Zingiber Officinalis and Ginkgo Biloba, behaved similarly to diazepam in produced anxiolytic-like effects in rats [90]. In contrast, systemic treatment with EGb 761 reduced social interaction in rats, which might be indicative of an anxiogenic activity of EGb 761. Of note, however, the anxiolytic action of diazepam was greater in animals pretreated with EGb 761. In addition, pretreatment with EGb 761 reversed the reduction of social interaction caused by β-carboline-3-carboxylic acid ethyl ester (β-CCE), which behaves as a partial inverse agonist at the benzodiazepine site of $GABA_A$ receptors. The complex interaction with diazepam and β-CCE suggests that EGb 761 modulates the activity of $GABA_A$ receptors [85, 91].

A subsequent study showed that bilobalide, a terpene lactone component of EGb 761, act as a competitive $GABA_A$ receptor antagonists, and this can be the basis of its vigilance-enhancing and antidepressant-like activities of EGb 761 [92].

Preclinical data laid the groundwork for the study of Ginkgo Biloba extracts in humans. Several randomized, placebo-controlled, double-blind, clinical trials have been conducted on the effects of EGb 761 on neuropsychiatric symptoms in patients with Alzheimer Disease (AD), vascular dementia (VaD), or mixed forms of dementia. In one study, patients were treated daily with either EGb 761 or placebo for at least 20 weeks, and changes in the Neuropsychiatric inventory (NPI) composite score and NPI caregivers distress score were considered as primary endpoints. Data showed that the NPI composite score improved after treatment with EGb 761, and EGb 761 was significantly superior to placebo in improving neuropsychiatric symptoms, associated with dementia, including anxiety [93].

The efficacy of EGb 761 was also examined in patients with primary anxiety disorders. In 2006, Woelk et al conducted a randomized, double-blind, placebo-controlled trial to evaluate the effect of the extract EGb 761 on generalized anxiety disorder(GAD) or adjustment disorder with anxious mood (ADWAM). The study included 107 patients, randomized to receive either EGb 761 or placebo for 4 weeks. The first endpoint of the study was the evaluation of anxiety through the Hamilton rating scale for anxiety (HAMA). Secondary endpoints were assessed through the list of complaints B-L', the Erlangen anxiety tension and aggression scale (EAAS), the clinical global impression and a global self-rating of perceived changes. The results showed that HAMA scores decreased significantly in patients treated with EGb 761 compared with patients treated with placebo and the effect was dose- dependent. The same result has occurred for secondary outcomes. The authors therefore suggest that Ginkgo Biloba extracts may be useful for the treatment of anxiety associated with cognitive impairment in the elderly as well as for the treatment of GAD in young people [94].

4.2.3 Curcumin

Curcumin (diferuloylmethane) is a phenolic compound extracted from the rhizome of the plant Curcuma Longa L. Several studies have demonstrated that curcumin has antioxidant, anti-inflammatory and antitumor properties

[95].

Some studies have found that curcumin is also endowed with anxiolytic activity.

Interestingly curcumin reverses anxiety-like behaviour caused by acute lead exposure [96] and acute immobilization stress [97]. Interestingly, acute stress was associated with lipid peroxidation, increased SOD activity, decreased CAT activity, and a compensatory increase in GPx activity. Curcumin treatment corrected all these abnormalities, raising the interesting possibility that antioxidants may be useful for the treatment of stress-induced anxiety.

The anxiolytic-like effect of curcumin in rats was also shown by Patel et al, who showed that curcumin has synergistic effects with amitriptyline in relieving depression and anxiety [98].

Curcumin was shown to enhance docosahexaenoic acid (DHA) synthesis in liver cells and to up-regulate the expression of DHA-synthesizing enzymes in the liver and brain when combined with dietary α-linolenic acid. Interestingly, this effect was related to the anxiolytic activity of curcumin [99]. Other studies suggest that the anxiolytic activity of curcumin cannot be exclusively explained by its antioxidant properties, but involves additional mechanisms, such as changes in monoamine levels in the CNS [100]. Accordingly, curcumin treatment could reverse changes in monoaminelevels induced by olfactory bulbectomy, which is a validated animal model of depression [101].

The antianxiety-like effect of curcumin has also been shown in mice. Gilhotra and Dhingra showed that curcumin, at a dose of 20 mg/kg, produced antianxiety-like effects in mice exposed to restraint stress. The action of curcumin was potentiated by pretreatment with the iNOS inhibitor, aminoguanidine, but not with the nNOS inhibitor, 7-nitroindazole [102]. This study moved from the evidence that curcumin relieved anxiety and attenuated oxidative damage induced by sleep deprivation in mice. These effects were prevented by the nitric oxide precursor, L-arginine, and were potentiated by the NOS pseudosubstrate, L-NAME [103].

The anxiolytic effect of curcumin was also investigated in humans. A randomized controlled clinical trial was conducted in the Nutrition Clinic at the Ghaem Hospital of Mashhad, Iran, in thirty obese patients. Patients were randomized to receive curcumin or placebo for 30 days, and then, after a wash-out period of 2 weeks, they were crossed-over to the alternative regimen for 30 more days. They were assessed for anxiety and depression with the Beck Anxiety Inventory (BAI) scale and Beck Depression Inventory (BDI) scale. This study showed that subjects supplemented with curcumin had lower scores on the BAI, but not on the BDI scale, suggesting a potential anxiolytic activity of curcumin in obese individuals. However, this was a sub-study of an investigation of the effects of curcuminoids on cardiovascular risk markers, and the presence of anxiety and depression was not considered as an inclusion criterium .In addition, no measurements of curcuminoid levels in the plasma were performed, and the sample size was small [104].

Despite the great amount of studies on its beneficial effects, it has been shown that curcumin can pertubate cellular membranes, giving rise to false protein binding signals and its therapeutic use is limited by its short half-life and poor biovailability [105]. Recently it has been identified both as a possible invalid metabolic panacea (IMP), molecule that seems to have curative proprieties but gives no results in clinical trials, and as a PAINS, pan-assay interference compound, a compound that shows activity in different assays by interfering with their readout. [106]

4.2.4 Epigallocatechin gallate (EGCG)

EGCG is the most represented and powerful antioxidant contained in green tea. Although it has no therapeutic indications, there are many preclinical studies that show its neuroprotective effect in animal models of neurodegenerative diseases [107]. For example, it may exert neuoprotective activity against ischemic injury by scavenging ROS and blocking lipid peroxidation [108],

and reduce excitotoxic neuronal death in *in vitro* models [109]. Recent observations suggest that EGCG regulate mechanisms of oxidative deamination, which generate protein carbonyl cross-reacting with IgM, and allow the scavenging of damaged molecules [110].

EGCG, like other flavonoids [111], could also have an effect on GABA$_A$ receptors, as shown by behavioural studies in mice [112]. Along this line, EGCG was able to reverse the anxiogenic-like effects of caffeine, as shown by a reduction the time spent by the animals in the open arms of the EPM [113].

A clinical trial (NCT00981292) has been conducted to evaluate the effect of EGCG on cognitive function and mood in healthy young adults, with negative results [114].

4.3 Vitamins

4.3.1 Vitamin C

Vitamin C or ascorbic acid (AA) is a water-soluble antioxidant capable of reducing reactive free radicals by donating electrons and terminating the lipid peroxidation chain reaction. After the transfer of an electron,vitamin C forms an ascorbyl radical, a free radical species that is relatively stable and therefore less harmful. Hence, it acts as a radical scavenger or quencher, by forming a less reactive radical species instead of a more reactive species. Vitamin C can also regenerate vitamin E (α-tocopherol) by reducing tocopheroxyl radicals [115, 116].

A randomized controlled clinical trial by Brody et al. assessed the efficacy of high doses of ascorbic acid on the response to an acute stress [117]. The 120 participants underwent the Trier Social Stress Test (TSST), a paradigm of psychosocial stress, composed of two phases, one of anticipation and one of testing, during which the subject had to make a free speech and perform mental arithmetic publicly. They were randomized to receive placebo or ascorbic acid for a period of 14 days. The results have shown that ascorbic acid mitigated the physiological responses of systolic acid mitigated the physiological responses of systolic and diastolic blood pressure and cortisol to stress. Furthermore, subjects in the ascorbic acid group showed less anxiety, as assessed by the Spielberg State Anxiety Scale, both before and after stress.

Subsequently, Mazloom et al conducted a randomized, single-blind, placebo-controlled clinical trial to investigate the efficacy of vitamin C and E supplementation on anxiety, depression and stress associated with type--2 diabetes. The trial involved 45 patients with type-2 diabetes, randomized to receive vitamin C, vitamin E or placebo for six weeks. The DASS-21 scale (Depression Anxiety Stress Scales 21-items) was used for patient evaluation. Patients receiving vitamin C showed reduced levels of anxiety, with no changes in depression [118].

The effect of vitamin C has been further investigated by de Oliveira, who conducted a double-blind, placebo-controlled, randomized clinical trials on 42 high school students. Treatment with vitamin C decreased anxiety, as, assessed by the Beck Anxiety Inventory, and this was correlated with plasma Vitamin C levels; in addition, there was a significant difference in the heart rate between the two groups [119]. This clinical trial further supported the anxiolytic effect of Vitamin C in humans.

4.3.2 Vitamin E

Vitamin E (α-tocopherol) is a lipid soluble antioxidant that also act terminating the lipid peroxidation chain reaction.

Both animal that human studies have shown that a chronic severe deficiency of vitamin E determines the onset of ataxia [120–122].This finding generated further studies on the effects of vitamin E deficiency [123].

A study by Goheil showed that, in α-Tocopherol Transfer Protein (α-TTP) knock-out mice, it also occurred a decrease in some behavioural parameters related to anxiety: mice moved shorter distances and spent less time in the open arms of the EPM and have less rearing behaviours. These results are consistent with an increased anxiety in α-TTP knock-out mice [124].

Another animal model of vitamin E deficiency are

knock-out mice for the gene coding for the Phospholipid Transfer Protein (PLTP). This protein, widely expressed in the brain, probably acts as a α-tocopherol transfer; its absence therefore correlates to low vitamin E concentrations in the brain. PLTP-knock-out mice showed an anxious-like behaviour in the EPM, indicated by a smaller number of entries and less time spent in the open arms [125].

These two experiments have thus shown that a congenital vitamin E deficiency is linked to an increased anxious-like behaviour in mice.

An experiment by Terada investigated whether vitamin E deficiency in juvenile or adult rat was similarly correlated to an anxious behaviour. A 4 weeks administration of a vitamin E-depleted diet had an anxiogenic effect, assessed through the EPM, both in juvenile and adult rats. Vitamin E deficiency was also associated with an increase in tissue and plasma lipid peroxidation, in both juvenile and adult rats. In addition, in vitamin E deficiency there was an increase of corticosterone concentrations under the EPM stress. Therefore, anxiety related to vitamin E deficiency seemed to be linked to increased oxidative stress and elevated corticosterone plasma concentrations [120].

However in other studies the α-tocopherol has been reported to be anxiogenic in Wistar rats at the EPM test [125, 126]. This could be due to a different action of vitamin E from the antioxidant effect.

4.4 Thiol agents

4.4.1 N-acetylcysteine (NAC)

N-acetyl cysteine (NAC) is a derivative of the amino acid cysteine [127], with initial main indication as an antidote to paracetamol intoxication, as a mucolytic agent in chronic obstructive pulmonary disease and cystic fibrosis and as a renal protector in contrast-induced nephropathy [128]. Over the past decade it has also been paid much attention to the use of NAC in the treatment of neuropsychiatric disorders [129]. Among the actions exerted by NAC in these disorders, there is the antioxidant one. NAC has a weak direct antioxidant effect due to the presence of a free thiol group capable of interacting with the electrophilic group of ROS [130, 131]. Its principal antioxidant mechanism of action, however, is the indirect one, as precursor of GSH, one of the most important intracellular antioxidant agents. GSH is a tripeptide formed from glutamic acid, cysteine and glycine, synthesized in the cell cytoplasm, in a manner dependent on the cellular availability of cysteine and on the activity of the enzyme glutamate cysteine ligase (GCL) [132]; NAC can easily cross the cellular membrane and inside the cell it is deacetylated to cysteine, used for the synthesis of GSH [130]. The intracellular entry of the NAC at the level of the central nervous system is mainly linked to the amino acid transporter C1 (ECAAC1) and, in the astrocytes, also to the cystine/glutamate (Xc-) antiporters; [133] through the latter, NAC derived cysteine can lead to an increased glutamate release from the astrocytes, that permits the activation of the Gi-coupled metabotropic glutamate receptors, with subsequent influence also on dopamine release [133, 134].

Furthermore, some studies have shown that NAC is able to reduce neuroinflammation probably inhibiting microglia and the production of proinflammatory cytokines and oxidative species by the same. [135]

The discovery of the influence of NAC on oxidative stress, neuroinflammation and monoamine transmission has increased the interest to its use in the treatment of neuropsychiatric disorders, as all these mechanisms are involved in their pathogenesis. In recent years, several clinical trials have been conducted on these disorders using NAC as adjunctive treatment and the results were generally positive, although the data are still limited. Among the psychiatric disorders examined there are addiction, schizophrenia, bipolar disorders, obsessive compulsive and related disorders, as trichotillomania, skin-picking and nails biting [131]; after a trial on patients with substance abuse and post traumatic stress disorder (NCT02499029) [136], another trial on post-traumatic stress disorders is currently recruiting patients (NCT01664260) [137].

With regard to the topic of interest of this article, Strawn and Saldaña reported the case of a 17 year boy with GAD and social phobia, affecting the normal proper functioning of the person, only partially responding to treatment with high doses of sertraline; the addition to therapy of NAC 1200 mg twice a day was followed by significant improvement of both psychic and somatic anxiety symptoms [138] . A preclinical study on zebrafish has shown that NAC prevented the anxiety-like behaviours induced by an acute stressor and increased the time spent by the animals in the lit side in the LD test [139]. A recent study has shown an anxiolytic-like effect of NAC in mice in the light-dark box and the open field test [140]. These preclinical findings lay the groundwork for the study of NAC in patients affected by anxiety disorders.

4.5 Metals

4.5.1 Selenium

Selenium is a trace element present in food, essential in human diet. Although selenium deficiency is rare, its decreased blood concentration has been associated for many years with different pathological conditions [141]; thus, numerous studies have been conducted to evaluate the preventive or therapeutic effect of selenium replacement therapies [142].

Selenium has antioxidant property, both by itself and through its metabolites, but their short half life limits the possibility of quantifying their therapeutic effectiveness [143]. It can act as a cofactor for some antioxidant enzymes, such as GPx and to take advantage of this property different organoselenium compounds have been recently developed [144]; these include diphenil-diselenide, which has been shown to reduce anxiety-like behaviour in EPM and in contextual fear condition in rats treated with monosodium glutamate [145].

A clinical study on selenium supplementation in HIV/AIDS affected patients has shown that, after 12 months, treated patients reported less anxiety compared to the control group, suggesting that it could be useful in these patients to alleviate the anxiety associated with the disease [146].

4.6 Synthetic antioxidants

4.6.1 Edaravone

Edaravone is an antioxidant molecule developed in Japan and commercialized in Japan and India. It acts as a direct antioxidant by scavenging ROS and it is used for its anti-cytokine and neuroprotective action in amyotrophic lateral sclerosis. It is considered an orphan drug [147] and in a phase II study has been demonstrated a slower decline in patient treated with edaravone [148], although its efficacy has not been completely demonstrated, according to a recent study [149]. Edaravone has been also used in cardiovascular disease and in the prevention of atherosclerosis for its capacity to reduce lipid peroxidation and monocyte adhesion to vascular endothelium.

Edaravone has been tested in a model of anxiety in rats induced by the administration of bacterial lipopolysaccharide; its administration could revert the anxiety-like response in the EPM. [150]

In a model of chronic restrain stress, edaravone could ameliorate the oxidative stress response measured by enzyme activity and glutathione concentration in hippocampus and prefrontal cortex and it could also reverse the anxiety-like behaviour in the EPM [151], suggesting an effect exerted essentially by neuroprotection.

4.6.2 MitoQ

MitoQ is the most characterized mitochondrial acting compound and is composed by a quinone linked to a triphenylphosphonium (TTP) with an alkyl chain [152]. This compound exerts its effect passing the mitochondrial membrane, due to the TTP moiety, which permits an accumulation in the mitochondria [152]. After passing the membrane the quinone moiety is converted by the succinate dehydrogenase in the active antioxidant form of ubiquinol, which detoxifies ROS yielding H-ion and it is than converted in ubiquinone. Succinate dehydrogenase converts the ubiquinone in

ubiquinol, permitting the recycling of the antioxidant and blocking the lipid peroxidaxion [153]. Furthermore, the reduced form ubiquitinone is slowly oxidized by the complex III, switching the reaction to succinate dehydrogenase and increasing the antioxidant efficacy [154].

MitoQ has been tested in animal models of neurological diseases such as Hungtington Disease [155] and in a phase 2 trial on Parkinson disease [156].

In a recent study by Nussbaumer et al MitoQ has been shown to reduce anxiety-like behaviour in genetically modified HAB-mice but not in LAB-mice, suggesting a selectivity for anxiety disorder [157]. Although these results are interesting, the effectiveness of MitoQ should be analysed in other anxiety models, for example using non inbred mice, and the response difference between HAB and LAB mice should be clarified on a greater sample.

4.6.3 Minocycline

Minocycline is a second generation tetracycline derivative, introduced as an antimicrobial agent in 1967 [158, 159], which can also be considered belonging to the class of phenolic antioxidants, for the presence in its molecule of a mulch-substituted phenolic ring, similar to that of a-tocopherol [160]. Minocycline can pass the BEE and exerts neuroprotective effect by different mechanisms, beyond the antioxidant one [161]. It has an anti-apoptotic effect, either in a caspase-dependent or in a caspase-independent manner: it reduces the release of pro-apoptotic factors from the mitochondria and increases the release of anti-apoptotic factors. It reduces inflammation, for example by suppression of microglial activation, inhibition of pro-inflammatory cytokines and inhibition of MMPs. Furthermore, minocycline can modulate the glutamatergic transmission, probably acting on the kinurenine pathway and thus reducing the production of the NMDA agonist QUIN; it can also act indirectly on the monoamine system [162].

Given its targets, minocycline has been studied for its possible use in psychiatric disorders. In 1996, Levine wrote a case report that suggested the possible antidepressant effect of minocycline in a patient with catatonic schizophrenia [163]. Then the antidepressant effect of minocycline has been studied though animal models as the Forced Swimming Test (FST) [164]. It has also been proven to be effective in improving cognitive deficits in a murine model of schizophrenia, achieved by administration of phencyclidine [165].

Furthermore, animal studies have also suggested that minocycline can act on anxiety symptoms [166].

Neigh et al have shown that pre-treatment with minocycline was able to reduce the anxiety-like behaviour and the neuronal damage in mice undergoing 8 min of cardiac arrest/cardiopulmonary resuscitation (CA/CPR). Since the anxiety-like behaviour correlated with the microglial activation, it seemed that the anxiolytic action of minocycline was linked to modulation of inflammation resulting from cerebral ischemia [167].

Kovesdi et al investigated the effect of minocycline treatment on the neurobehavioural modifications induced by mild traumatic brain injury in rats. The animals were subjected to behavioural tests before the injury and after (8 and 45 days); select markers of inflammation, and of vascular, neuronal or glial damage were measured in serum and brain regions linked to memory and anxiety, 52 days after the injury.. Minocycline treatment prevented the anxiety increase at the EPM 45 day after the injury, but not 8 days after, and normalized serum and tissue levels of the examined markers [168].

Jarrett investigated whether minocycline, thanks to its ability to suppress the microglial activation, could prevent the alterations in immune function and anxiety-like behaviour, resulting from the administration of a Repeated Social Defeat (RSD) stress in C57bl/6 mice. The administration of minocycline, beginning two days before the stress, was found to prevent: the increase in macrophage trafficking to the brain and in circulating leukocytes, to reduce the activation of circulating monocytes and granulocytes and to prevent the

induction of an anxiety-like behaviour by the RDS stress. The authors therefore suggest that minocycline can improve stress-induced anxiety by acting on cell activation and redistribution of these immune cells [169] .

Majidi et al treated neonatal mice with lipopolysaccharide to assess whether minocycline developmental treatment could reduce the abnormalities in adulthood, induced by an early-life insult. Minocycline was found to have positive effects on anxiety and depressive-like symptoms, HPA hyperactivity and increased levels of proinflammatory cytokines in the hippocampus, due to the early-life stress [170].

In contrast with the results of the studies described above, a recent study by Vogt doesn't confirm the protective effect of minocycline against anxiety. Vogt et al conducted tests of depression and anxiety, such as the FSP, DLB and OF in adult C57bl/6 mice. In mice treated with minocycline 20-40 mg/kg i.p, it did no occur a change in depressive- and anxious- like behaviours, unlike what happened with the administration of imipramine or diazepam. However, the authors do not exclude that minocycline a higher dosage could have an effects in the behavioural tests and a clinical effect on mood disorder as add-on therapy [171].

These animal studies have been followed by different clinical trials on the potential use of minocycline for the pharmacological treatment of psychiatric disorders.

Utari et al provide a first survey on the effects of minocycline on children with X-fragile syndrome. The study took into account the 50 patients that were treated with minocycline for at least 2 weeks, with a mean treatment duration of 3.5 months at a dosage of 20-200 mg/day. The results of treatment were evaluated through questionnaires administrated to parents that used Likert scale to assess the patients' changes in different areas. Among the areas, in which parents noticed a greater improvement there were anxiety, social communication, attention and language [159].

In a randomized placebo-controlled double-blind clinical trial, minocycline exerted a beneficial effect on negative symptoms of early schizophrenia, but this trial presents several limitations, including the small number of patients, that don't allow to clearly establish its effectiveness [172].

Additional clinical trials on the possible effectiveness of minocycline in schizophrenia and depression are underway and some have as a secondary outcome the evaluation of its effect on anxiety symptoms [173, 174].

4.7 Endogenous antioxidants

4.7.1 Melatonin

Melatonin is produced by the pineal gland and is involved in circadian rhythm and cell signalling. It has a recognized antioxidant effect by stimulating the synthesis of GSH and its metabolites acetyl formyl kinuramine and acetyl kinuramine have a ROS scavenging activity [175]. The prevalent therapeutic use of this molecule is the sleep disturbances that often are in comorbidity with anxiety disorders [176]. Melatonin has shown an ameliorating rating of the Hamilton anxiety scale score, in patients with sleep disturbances and breast cancer [177], but anxiety was assessed as an outcome in patients with psychiatric illness depending by clinical conditions. In the methanalysis by Hansen et al, authors consider melatonin effective as a premedication in pre and post operative anxiety and it is considered safe and effective in paediatric patient undergoing medical procedures [178].

A phase 3 trial is ongoing for the valuation of melatonin as an add on medication in patient with anxiety and depression in comorbidity with breast cancer.

In preclinical studies, melatonin has been observed to reduce oxidative stress in coadministration with buspirone in a model of contention stress [179].

In a model of anxiety induced by intraperitoneal injection of E. coli lipopolysaccharides, the anxiety-like behaviour, evaluated by EPM, was reduced by the administration of luzindole, an antagonist of the melatonin receptor [180], although this model of anxiety

induction is more similar to concomitant anxiety in other clinical condition. It has been also observed that intra-amygdaloid al melatonin injection in pineleactomized rats improves the time spent in the open arm in the EPM test [181]; melatonin can also revert behavioural alterations induced by diisonyl-phthalate, by enhancing oxidative stress [182].

To our knowledge there are no data about melatonin in more specific animal models and on patients with anxiety non associated to other medical conditions.

Agomelatine instead, an antidepressant based on melatonin function, has a confirmed effectiveness versus placebo in a randomized controlled trial on patient with GAD [183]; recently it has been shown that agomelatine can increase SOD activity in rats striatum and catalase activity in cerebellum but no influence has been reported on lipid peroxidation and brain carbonylation [184].

5. *Conclusions*

Increased levels of oxidative stress have been associated with a wide range of CNS disorders, including anxiety disorders. An anxiolytic activity of of oxidative stress modulators has been widely demonstrated in rodents, whereas data obtained in humans are less clear. A number of factors should be considered when comparing preclinical and clinical data. For example, most of the animal models of anxiety do not recapitulate the complexity of anxiety disorders in humans, i.e., the same model has not construct, face, and pharmacological validity at the same time. Anxiety in humans is strongly influenced by the personal history (particularly early life experiences) and social context [185], whereas most of preclinical data were obtained in normal rats or mice exposed to a conflicting situation (e.g., light-dark box or elevated plus maze) or exposed to natural threats (in which fear response can be considered as defensive, and, therefore, "physiological"). According to some Authors oxidative stress and anxiety in humans might be associated

without a clear cause-effect relationship [186].Perhaps several clinical studies with antioxidants did not produce positive results because there other factors contributing to the pathogenesis of anxiety are insensitive to antioxidants [187].Another possibility is that ROS in humans may be physiologically important [187], and the broad-spectrum antioxidant drugs used in clinical studies do not distinguish between physiological and pathological actions of ROS and other oxidative species. Other limiting factors in human studies are the route of administration and the doses and time of administration of antioxidants, which may not reach the clinically effective concentrations in the CNS [187].It is always difficult to find a right dose of an antioxidant in humans, which maintains an optimal risk-to-benefit ratio by restraining the toxic effects of ROS without interfering with their physiological activity [188].Last, but not least, the inclusion criteria in clinical studies did not take into account the presence of specific biomarkers of oxidative stress [187].

Despite these potential limitations of human studies, the evidence of efficacy of some oxidative stress modulators in rodents is encouraging and further stimulate translational research.

Interestingly, some putative oxidative stress modulators produce anxiolytic effect *via* mechanisms other than a classical antioxidant mechanism. NAC, for example, activates the cysteine-glutamate antiporter leading not only to an increase in intracellular cysteine (and, therefore, to GSH synthesis), but also to an increase in extracellular glutamate. This in turn activates type-2 metabotropic glutamate receptors in the preterminal region of the axon, thereby reducing glutamate release from nerve terminals [189]. Of note, mGlu2 receptors are targets for the treatment of anxiety and drug addiction [190, 191].

We believe that the development of an oxidative stress-modulating therapy in anxiety disorders could be beneficial as an add-on therapy integrating conventional psychotherapeutic and pharmacological approaches. The effectiveness of

an additional therapeutic strategy could be useful given the limitations of the current therapies, for example the latency time of selective serotonin reuptake inhibitors(SSRIs) and the potential of abuse of benzodiazepines.

Abbreviations:

AChE: Acetylcholinesterase

α-TTP: α-Tocopherol Transfer Protein

BAI: Beck Anxiety Inventory scale

β-CCE: Ethyl-β-Carboline-3-Carboxyl

BDI: Beck Depression Inventory scale

BSO: Buthionine sulphozimine

CAT: Catalase

DHA: Docosahexaenoic acid

EGCG: Epigallocatechin gallate

EPM: Elevated Plus Maze

Glo1: Glyoxalase 1

GPx: Glutathione peroxidase

GSH: Glutathione

Gsr1: Glutathione reductase 1

H_2O_2: hydrogen peroxide

HAMA: Hamilton Rating scale for Anxiety

LD: Light Dark

LOOH: Lipid hydroperoxide;

MDA: Malonyldialdheide;

NAC: N-acetylysteine

NADPH: Nicotinamide Adenine Dinucleotide Phosphate

NO: nitric oxide

NOS: NO Synthase

NOX: NADPH-Oxidase

O_2^-: Superoxide anion

OH^-: Hydroxyl radical

Nrf2: Nuclear factor (erythroid-derived 2)-like

OF: Open Field

OSI: Oxidative Stress Index

$ONOO^-$: Peroxynitrite anion

PCBs: Polychlorinated-biphenyls

PLTP: Phospholipid Transfer Protein

PUFAs: Polyunsaturated Fatty Acids

RNS: Reactive Nitrogen Species

ROS: Reactive Oxygen Species

RSD: Repeated Social Defeat

SOD: Superoxide Dismutase

TAS: Total Antioxidant Status

TOS: Total Oxidant Status.

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