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Evaluation of total oxidative status in adult attention deficit hyperactivity disorder and its diagnostic implications

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ABSTRACT

Adult Attention Deficit Hyperactivity Disorder (A-ADHD) is one of the psychiatric disorders which awareness is growing. The exact causes of A-ADHD are still unknown. In addition to neurochemical and neuroanatomic disorders, genetic and environmental factors are discussed in its etiology. In our study, we aimed to evaluate the oxidative status of A-ADHD patients and investigate whether oxidative metabolites can be used as diagnostic tools or not in A-ADHD. Blood samples were taken from enrolled 50 A-ADHD patients and 31 controls in appropriate way and Total Antioxidative Status (TAS), Total Oxidative Status (TOS), and Oxidative Stress Index (OSI) were studied in Harran University Biochemistry Labs. Results were compared between groups and ROC curve was drawn in order to evaluate diagnostic performances. Patients' TAS, TOS and OSI were significantly higher than controls. There was not a significant difference between comorbid cases and only A-ADHD patients in terms of measured values. A-ADHD can be predicted for TOS over 9.8575 μmol H_2O_2 Eqv./L level with 86% positive predictive value and %100 negative predictive value. In A-ADHD, oxidative balance is impaired. High antioxidant levels may be compensatory against the oxidant increase. Oxidative parameters may be used in A-ADHD diagnosis.

1. Introduction

35 years ago, preliminary studies of Adult Attention Deficit Hyperactivity Disorder (A-ADHD) were written (Wood et al., 1976). The phenomenon was well described in children but only recent studies have shown its impact across life span (Wilens et al., 2002). Debates whether it exists or not, may be justified because nomenclature of the disorder has several flaws. The diagnostic criteria were made according to the childhood and focusing rather than "attention deficit" is the major problem (Doyle, 2006). However, several improvements have been made for the description of A-ADHD (Gunay et al., 2006; Wender, 1995). It is clear that not all but some of the adults develop adaptive behaviors against symptoms and the course of the disease changes but its effect on functionality still persists (Faraone et al., 2000; Wilens and Dodson, 2004). Why some people recover after childhood and others not is another issue to be studied.

Numerous researches have been conducted regarding neurobiology of pediatric ADHD but A-ADHD studies are relatively few. Several neurochemical and genetic mechanisms are believed to be involved in A-ADHD although the etiology remains unclear (Bulut et al., 2007; Faraone, 2004).

While aerobic life depends on oxygen, sometimes it may be hazardous for living beings which is known as "oxygen paradox"(Davies, 1995). During oxygen involved oxidation–reduction reactions for life energy, several "harmful wastes" called oxidants are produced. Oxidants are removed from the body by antioxidant defense mechanisms. The imbalance of oxidative metabolism is called oxidative stress (Valko et al., 2006). The association between oxidative stress and psychiatric disorders such as schizophrenia, bipolar disorder, depression and anxiety disorders were well studied before (Andreazza et al., 2008; Herken et al., 2007; Selek et al., 2008a,b).

Few studies focused on oxidative stress of either pediatric or adult ADHD (Bulut et al., 2007; Ceylan et al., 2010). We have previously reported that oxidant nitric oxide levels were high and antioxidant superoxide dismutase levels were low in A-ADHD (Selek et al., 2008c). However, a total status of oxidative

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metabolism has not been evaluated, yet. Plasma concentrations of antioxidants can already be measured separately in the laboratory, but these measurements are time-consuming, labor-intensive and costly. The number of different antioxidants in plasma, serum, urine, or other biological samples makes it difficult to measure each antioxidant separately. Since antioxidative effects of antioxidant components of plasma are additive, the measurement of total antioxidative status (TAS) and total oxidative status (TOS) can only reflect the antioxidative status of plasma whose measurement methods were developed by Harran Biochemistry Labs (Erel, 2004, 2005). On the other hand, there may be shifts in TAS and TOS. Thus, Erel also hypothesized that current oxidative status can be stated with oxidative stress index (OSI) which can be figured out by TAS/TOS (Erel, 2005). The general relation between those parameters can be seen in Fig. 1. Therefore, for exploring a specific relationship between oxidative metabolism and suggested diseases, Erel's parameters are useful.

In this study we aim to explore the total oxidative and anti-oxidative status of A-ADHD and investigate whether oxidative metabolites can be used as diagnostic tools or not in A-ADHD.

2. Methods

2.1. Patients and controls

54 A-ADHD patients between 18 and 45 years of age, diagnosed according to Turgay's Turkish version of Adult ADD/ADHD DSM IV – Based Diagnostic Screening and Rating Scale by two psychiatrists (H.A.S. and S.S.) in the Psychiatry Department of Gaziantep University Hospital were involved. Since the patients were initially diagnosed as A-ADHD, they were free from stimulant and A-ADHD medication. The scale is DSM IV based and it was developed by Turgay for diagnosis and severity evaluation (Gunay et al., 2006). Exclusion criteria were as follows: tardive dyskinesia related to neuroleptics, presence of severe organic condition, use of any antioxidant agent (i.e. vitamins E and C), presence of epilepsy and severe neurologic disorder which were previously found to be associated with oxidative status, presence of infectious disease, excessive obesity and insufficient sampling. Those patients with psychiatric comorbidity were applied Clinical Global Impression–Severity Scale and participants with below score of 2 ("borderline mentally ill") were accepted for the study (Guy, 1976). 4 patients were excluded due to insufficient sampling.

The control group is formed of 37 healthy subjects who were chosen among the doctors and hospital staff. These were free of any medication for at least 6 weeks prior to blood sampling. None of the control subjects were alcohol drinker, heavy smoker, or had ever taken psychotropic drugs. They had no history or family history of psychiatric disorder.

In order to match sex and age, 6 of the female controls were removed randomly by a random number generator. All subjects gave their written informed consent which had been approved by the local ethics committee in accordance with the Declaration of Helsinki.

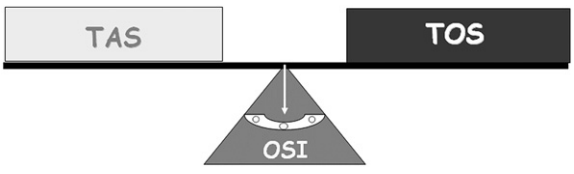


Fig. 1. The relation between oxidative stress markers; total oxidative status (TOS), total antioxidative status (TAS) and oxidative stress index (OSI).

2.2. Sampling

Venous blood samples from left forearm vein were collected into 5 ml vacutainer tubes at 7–8 a.m. after overnight fasting once. The blood samples were centrifuged at 2000 rpm for 10 min to obtain sera. Samples were stored frozen at −40 °C before analysis. The biochemical analyses were made after all the blood samples were collected.

2.3. Measurement of the total oxidative status of plasma (TOS)

The total oxidative status of the plasma was measured using a novel automated colorimetric measurement method for TOS (Erel, 2005). In this method Oxidants present in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Eqv./L).

2.4. Measurement of the total antioxidative status of plasma (TAS)

The total antioxidative status of the plasma was measured using a novel automated colorimetric measurement method for TAS (Erel, 2005). In this method the hydroxyl radical, the most potent biological radical, is produced by the Fenton reaction, and reacts with the colorless substrate o-dianisidine to produce the dianisyl radical, which is bright yellowish-brown in color. Upon the addition of a plasma sample, the oxidative reactions initiated by the hydroxyl radicals present in the reaction mix are suppressed by the antioxidant components of the plasma, preventing the color change and thereby providing an effective measure of the total antioxidative status of the plasma. The assay results are expressed as mmol Trolox Eqv./L, and the precision of this assay is excellent (Cao and Prior, 1998).

2.5. Determination of oxidative stress index (OSI)

The ratio of TOS to TAS was accepted as the oxidative stress index (OSI). For calculation, the resulting unit of TAS was changed to mmol/L, and the OSI value was calculated according to the following formula: OSI (arbitrary unit) = TOS (îmol H_2O_2 Eqv./L)/TAS (mmol Trolox Eqv./L) (Harma et al., 2006).

For a detailed understanding of measurements, readers should go over Erel's articles (Erel, 2004, 2005).

2.6. Apparatus

A Cecil 3000 spectrophotometer with a temperature controlled cuvette holder (Cecil) and an Aeroset automated analyzer (Abbott) were used (Erel, 2004). The relationship between parameters is shown in Fig. 1.

2.7. Statistical analysis

SPSS® for Windows 13.0 statistical program was used for statistics. Graphs were drawn by Statistica 7.0. Parametric statistical analysis was used if the conditions were satisfied. The significance of differences between groups was estimated by *T* test. Chi-square test was used when comparing proportions. Multiple comparisons were made by ANOVA. Differences were accepted as significant when $p < 0.05$. Bivariate comparisons were examined via Pearson

Table 1
Some of the sociodemographic and clinical variables of the participants.

	Patients	Controls	Values
Age (mean years ± SD)	24.7 ± 7.5	27.9 ± 7.9	$T = -1.816$, $p = 0.073$
Sex (male/female)	35/15	20/11	$X^2 = 0.264$, $p = 0.607$
ADHD subtypes (*N*)			
Attention deficit	17		
Hyperactivity/impulsivity	8		
Combined	19		
Not otherwise specified	6		
Comorbidities (*N*)			
Depression	4		
Anxiety disorders	22		
Personality disorders	3		
Others	3		

correlation coefficients and values were corrected for ties. Receiving operator characteristics (ROC) curve was plotted in order to find the cutoff point. Positive predictive and negative predictive values were figured out for diagnostic performance of oxidative parameters.

3. Results

Some of the sociodemographic and clinical variables are tabulated in Table 1. Fig. 2 shows TAS, TOS and OSI levels of groups.

There were significant differences between two groups of all parameters and TAS was 1,3; TOS was 4,2 and OSI was 3,2 times higher in patients than controls ($T = 14.087$, $p < 0.001$; $T = 7.422$, $p < 0.001$ and $T = 6.449$, $p < 0.001$, respectively). There was not any significant difference among subtypes of ADHD (TAS: $F = 0.430$, $p = 0.732$; TOS: $F = 0.210$, $p = 0.889$ and OSİ: $F = 0.260$, $p = 0.854$). There was not any significant difference between comorbid patients and only ADHD patients of TAS, TOS and OSI ($t = 0.255$, $p = 0.800$; $t = -0.31$, $p = 0.975$ and $t = -0.049$, $p = 0.961$, respectively). There was not a correlation between oxidative parameters and disease severity (scale score) and number of fulfilled diagnostic criteria. However, there was a positive correlation between age and TOS & OSI ($r0 = 0.48$, $p < 0.001$, $N = 50$ and $r0 = 0.47$, $p = 0.001$, $N = 50$, respectively). The correlation was still significant when ADHD not otherwise specified case were removed (TOS: $r0 = 0.46$, $p = 0.002$, $N = 44$ and OSI: $r0 = 0.45$, $p = 0.002$, $N = 44$). On the other hand there was not a significant correlation of age in controls.

ROC curve was plotted, and cutoff point was drawn from the curve for TAS, 2.1855 μmol Trolox Eqv./L for TOS 9.8575 μmol H_2O_2 Eqv./L and for OSI 6.0216 for diagnostic measures (Fig. 3). Higher values were signed as disease state. For those cutoff points, positive predictive value (PPV) and negative predictive values (NPV) of TAS were 80% and 100%, TOS were 86% and 100%, and OSI were70% and 100%.

4. Discussion

Our first finding is higher TOS levels in patients. In our previous studies we have found elevated specific oxidants such as Nitric

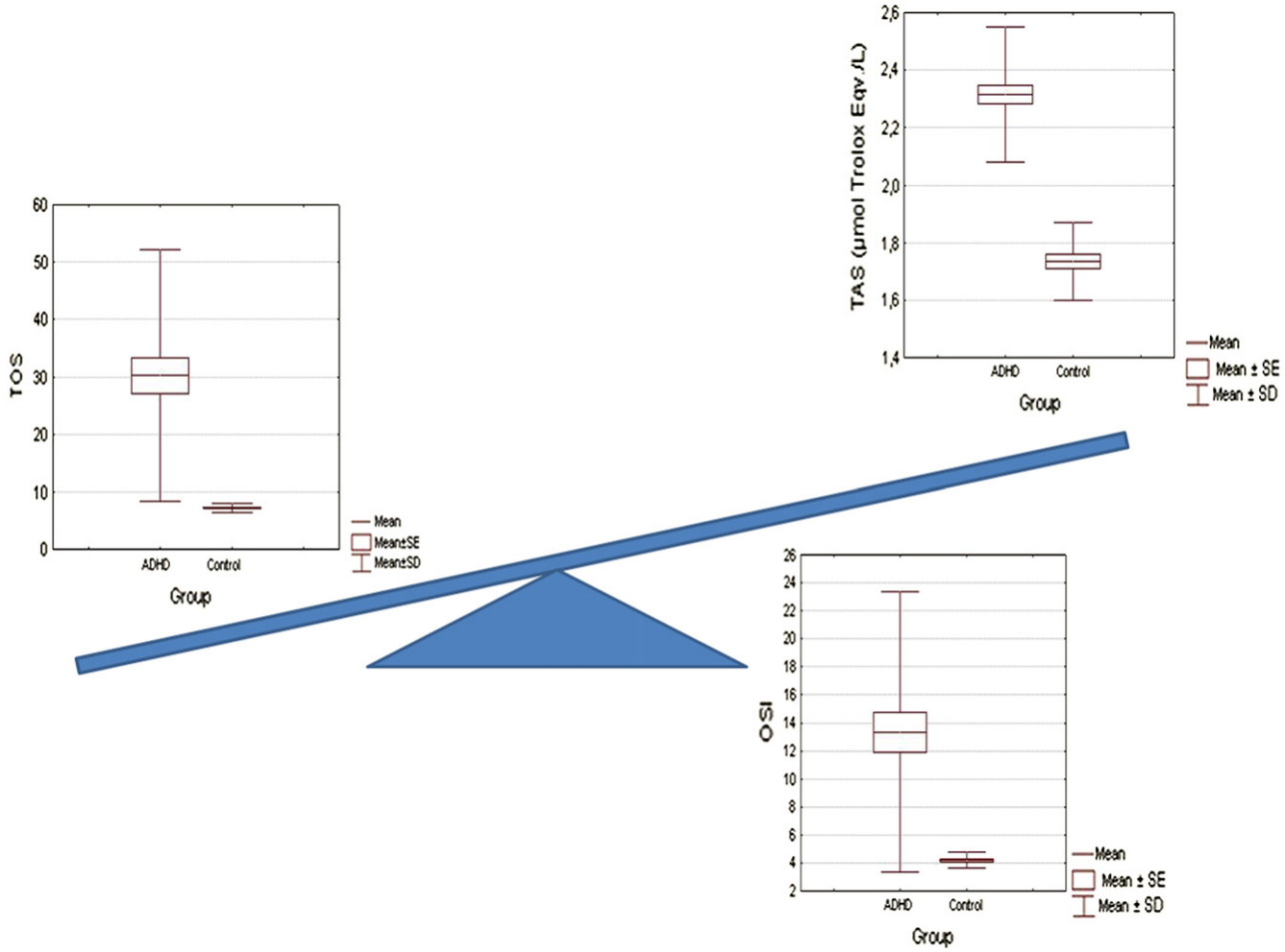
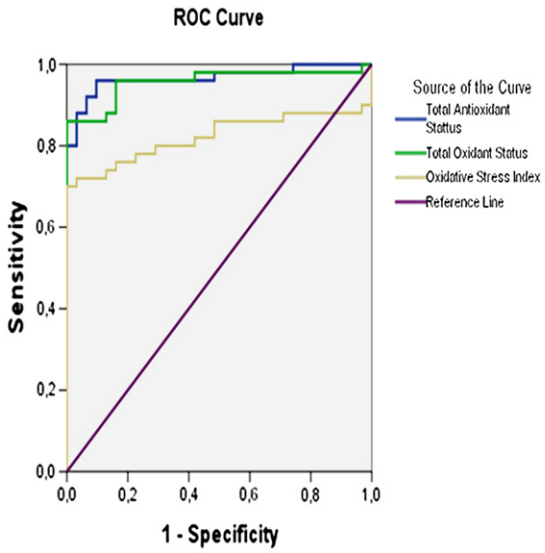


Fig. 2. Boxplot of total oxidative status (TOS), total antioxidative status (TAS) and oxidative stress index (OSI) of patients and controls.



Test Variable	Area	P Value	%95 Confidence Interval	
			Lower Bound	Upper Bound
TAS	0.966	p<0.001	0.929	1.004
TOS	0.957	p<0.001	0.912	1.002
OSI	0.822	p<0.001	0.726	0.918

Fig. 3. ROC curves and other parameters of total oxidative status (TOS), total antioxidative status (TAS) and oxidative stress index (OSI).

oxide and Malone dealdehyde (Bulut et al., 2007; Ceylan et al., 2010; Selek et al., 2008c). Our current finding is congruent with previous results. There was not difference between only A-ADHD patients and comorbid patients suggesting the oxidative impairment may be attributed to the whole disease entity. Although TOS was found to be increased in several other psychiatric disorders, the remarkable increase (4.2 times) in A-ADHD suggest a more strong association between A-ADHD and oxidative stress (Andreazza et al., 2008; Ersoy et al., 2008).

Our second finding is positive correlation between age and TOS in patients but not in controls. There are several studies showing that oxidative status is also impaired in pediatric ADHD (Ceylan et al., 2010). According to our findings oxidants appear to be increased with disease duration, since ADHD is a childhood onset disease.

Our third finding is increased TAS levels. Antioxidants may be increased due to their balancing attempts so called as “rebound phenomenon”. In fact, elevated antioxidants in other psychiatric disorders are interpreted as compensative increases (Savas et al., 2006). Since the OSI, showing general balance of oxidative stress is also increased; the reactive increase appears to be insufficient to balance.

Our fourth finding is increased OSI. The index shows the oxidative imbalance in patients.

Dopamine, which is believed to be involved in etiology of ADHD, is very susceptible to autooxidation when antioxidant defense is weak (Viggiano et al., 2004). Therefore, oxidative stress may be involved in dopaminergic pathways in A-ADHD. However, the exact relationship between oxidative stress and A-ADHD remains unclear. In another preliminary study, Pycnogenol which has antioxidative properties administration to the ADHD patients decreased the symptoms and withdraw of the medication caused relapse of the symptoms (Trebatická et al., 2006). Fixing the oxidative impairment in ADHD may lead to further treatment regimens.

Our last finding is the diagnostic performance of oxidative parameters. A blood test for A-ADHD is still not valid. TOS levels above 9.8575 μmol H_2O_2 Eqv./L were highly predictive for the disease state (PPV = 86%) and negative predictive value was also high (100%). This is the first attempt of combining oxidative research results and diagnostic test development attempts in A-ADHD. Thus, our results should be regarded as preliminary until they are replicated.

There are several limitations regarding our study. Samples size, comorbidities, and heterogeneity of treatment modalities. Because it is unethical to cut the patients treatments, we did not suspend the treatments. However, there was not a significant difference between comorbid patients and only A-ADHD. Since serum levels were measured, these results will offer an insight about global alterations of oxidative stress in A-ADHD and may not reflect the brain levels.

In conclusion, oxidative metabolism is found to be impaired in A-ADHD and may be related with disease duration. Oxidative parameters may be useful diagnostic tools for the disorder.

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None.

Contributors

Author Selek and Bulut designed the study. Kalenderoglu, Bulut and Selek collected the samples. Ocak made the biochemical analysis. Selek made the statistical analysis. Savas edited the writing of the manuscript. All of the authors finally revised the paper and gave consent for publication.

Conflict of interest

The authors declare no conflict of interest.

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None.

References

Andreazza AC, Kauer-Sant'Anna M, Frey BN, Bond DJ, Kapczinski F, Young LT, et al. Oxidative stress markers in bipolar disorder: a meta-analysis. Journal of Affective Disorders 2008;111(2–3):135–44.

Bulut M, Selek S, Gergerlioglu HS, Savas HA, Yilmaz HR, Yuce M, et al. Malondialdehyde levels in adult attention-deficit hyperactivity disorder. Journal of Psychiatry & Neuroscience 2007;32(6):435–8.

Cao G, Prior RL. Comparison of different analytical methods for assessing total antioxidant capacity of human serum. Clinical Chemistry 1998;44(6):1309.

Ceylan M, Sener S, Bayraktar AC, Kavutcu M. Oxidative imbalance in child and adolescent patients with attention-deficit/hyperactivity disorder. Progress in

Neuropsychopharmacology and Biological Psychiatry 2010;34(8):1491–4. doi: 10.1016/j.pnpbp.2010.08.010.
Davies K. Oxidative stress: the paradox of aerobic life; 1995.
Doyle BB. Understanding and treating adults with attention deficit hyperactivity disorder. 1st ed. Arlington: American Psychiatric Publishing; 2006.
Erel O. A novel automated method to measure total antioxidant response against potent free radical reactions. Clinical Biochemistry 2004;37(2):112–9.
Erel O. A new automated colorimetric method for measuring total oxidant status. Clinical Biochemistry 2005;38(12):1103–11.
Ersoy MA, Selek S, Celik H, Erel O, Kaya MC, Savas HA, et al. Role of oxidative and antioxidative parameters in etiopathogenesis and prognosis of panic disorder. International Journal of Neuroscience 2008;118(7):1025–37. doi:10.1080/00207450701769026.
Faraone SV. Genetics of adult attention-deficit/hyperactivity disorder. The Psychiatric Clinics of North America 2004;27(2):303.
Faraone SV, Biederman J, Spencer T, Wilens T, Seidman LJ, Mick E, et al. Attention-deficit/hyperactivity disorder in adults: an overview. Biological Psychiatry 2000;48(1):9–20.
Gunay S, Savran C, Aksoy UM, Maner F, Turgay A, Yargic I. The norm study, transliteral equivalence, validity, reliability of adult hyperactivity scale in Turkish adult population. Psychiatry in Turkey 2006;8:98–107.
Guy W. ECDEU assessment manual for psychopharmacology-revised (DHEW Publ No ADM 76-338). Rockville, MD: National Institute of Mental Health; 1976.
Harma MI, Harma M, Erel O. Measuring plasma oxidative stress biomarkers in sport medicine. European Journal of Applied Physiology 2006;97(4):505.
Herken H, Gurel A, Selek S, Armutcu F, Ozen ME, Bulut M, et al. Adenosine deaminase, nitric oxide, superoxide dismutase, and xanthine oxidase in patients with major depression: impact of antidepressant treatment. Archives of Medical Research 2007;38(2):247–52. doi:10.1016/j.arcmed.2006.10.005.
Savas HA, Gergerlioglu HS, Armutcu F, Herken H, Yilmaz HR, Kocoglu E, et al. Elevated serum nitric oxide and superoxide dismutase in euthymic bipolar patients: impact of past episodes. World Journal of Biological Psychiatry 2006;7(1):51–5. doi:10.1080/15622970510029993.
Selek S, Herken H, Bulut M, Ceylan MF, Celik H, Savas HA, et al. Oxidative imbalance in obsessive compulsive disorder patients: a total evaluation of oxidant-antioxidant status. Progress in Neuropsychopharmacology and Biological Psychiatry 2008a;32(2):487–91. doi:10.1016/j.pnpbp.2007.10.002.
Selek S, Savas HA, Gergerlioglu HS, Bulbul F, Uz E, Yumru M. The course of nitric oxide and superoxide dismutase during treatment of bipolar depressive episode. Journal of Affective Disorders 2008b;107(1–3):89–94. doi:10.1016/j.jad.2007.08.006.
Selek S, Savas HA, Gergerlioglu HS, Bulut M, Yilmaz HR. Oxidative imbalance in adult attention deficit/hyperactivity disorder. Biological Psychology 2008c;79(2):256–9. doi:10.1016/j.biopsycho.2008.06.005.
Trebatická J, Kopasová S, Hradečná Z, Činovský K, Škodáček I, Šuba J, et al. Treatment of ADHD with French maritime pine bark extract, Pycnogenol®. European Child & Adolescent Psychiatry 2006;15(6):329–35.
Valko M, Rhodes C, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions 2006;160(1):1–40.
Viggiano D, Vallone D, Sadile A. Dysfunctions in dopamine systems and ADHD: evidence from animals and modeling. Neural Plasticity 2004;11:1–2.
Wender P. Attention deficit hyperactivity disorder in adults. New York: Oxford University Press; 1995.
Wilens TE, Biederman J, Spencer TJ. Attention deficit/hyperactivity disorder across the lifespan. Annu Rev Med 2002;53:113–31. doi:10.1146/annurev.med.53.082901.103945.
Wilens TE, Dodson W. A clinical perspective of attention-deficit/hyperactivity disorder into adulthood. The Journal of Clinical Psychiatry 2004;65(10):1301–13.
Wood DR, Reimherr FW, Wender PH, Johnson GE. Diagnosis and treatment of minimal brain dysfunction in adults: a preliminary report. Archives of General Psychiatry 1976;33(12):1453.