ISSN print: 1224-5984
ISSN online: 2248-3942

Rom Biotechnol Lett. 2019; 24(4): 571-579
doi: 10.25083/rbl/24.4/571.579

Received for publication, December, 3, 2017
Accepted, June, 2, 2018

Review

The general biological relevance of the oxidative stress status in replicating some neuropsychiatric and digestive – related manifestations in zebrafish

MĂDĂLINA-ANDREEA ROBEA[1], ALEXANDRA SĂVUCĂ[1], ȘTEFAN-ADRIAN STRUNGARU[2], CHRISTIAN LENZI[3]*, CHIARA GRASSO[3], GABRIEL PLĂVAN[1], ALIN CIOBICĂ[2,4,5], MIRCEA NICOARĂ[1]

[1]"Alexandru Ioan Cuza" University of Iasi, Department of Biology, Faculty of Biology, Bd. Carol I, 11, 700505, Iasi, Romania
[2]"Alexandru Ioan Cuza" University of Iasi, Department of Research, Faculty of Biology, Bd. Carol I, 11, 700505, Iasi, Romania
[3]Faculty of Natural Sciences, University of Turin, via Accademia Albertina 13-10123 Torino, Italy
[4]Center of Biomedical Research, Romanian Academy, Iasi, Bdul Carol I, no 8, Romania
[5]Academy of Romanian Scientists, Splaiul Independentei nr. 54, sector 5, 050094 Bucuresti, Romania

Abstract Considering the awareness regarding the oxidative stress status implication in most of the actual neuropsychiatric disorders and also the ever increasing importance of zebrafish studies in this context, in the present paper we were interested in the general biological relevance of the oxidative stress status in replicating some neuropsychiatric manifestations in zebrafish, by focusing on the autism spectrum disorder, schizophrenia and Alzheimer's disease. Also considering the increased relevance of sleep-related studies in the neuropsychiatric pathology and the existence of some intriguing sleep studies in zebrafish, as well as the correlations between some digestive-related disorders such as irritable bowel syndrome and the neuropsychiatric aspects, some of these correlations and the possibility of studying them on zebrafish are also discussed and speculated during this mini-review.

Keywords Oxidative stress, zebrafish, disease, sleep, digestive.

To cite this article: ROBEA MA, SĂVUCĂ A, STRUNGARU ŞA, LENZI C, GRASSO C, PLĂVAN G, CIOBICĂ A, NICOARĂ M. The general biological relevance of the oxidative stress status in replicating some neuropsychiatric and digestive–related manifestations in zebrafish. *Rom Biotechnol Lett*. 2019; 24(4): 571-579. DOI: 10.25083/rbl/24.4/571.579

*Corresponding author: CHRISTIAN LENZI, Faculty of Natural Sciences, University of Turin, via Accademia Albertina 13-10123 Torino, Italy
E-mail: christian.lenzi@edu.unito.it

Introduction

Oxidative stress is a very important neuropathological aspect/component in several neuropsychiatric disorders, such as schizophrenia, Parkinson's disease, Alzheimer disease, anxiety or bipolar affective disorder, as our group has also previously demonstrated (M. PADURARIU & al [1], M. PADURARIU & al. [2], A. CIOBICA [3], A. CIOBICA & al [4], A. CIOBICA & al [5], C. STEFANESCU and A. CIOBICA [6]). On the other hand, human body and human brain have many strategies to defend themselves against oxidative stress, which under normal conditions is very effective. The antioxidant factors that form true protective systems of the body against the free radicals, are represented by antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase or aldehyde dehydrogenase (CAT) and nonenzymatic antioxidant factors (H. SIES [7]). The anti-oxidant enzymes catalyze the reaction of reduction of free radicals, which diminishes their power and hence oxidative cytotoxicity.

A variety of studies are proposing lately the zebrafish (*Danio rerio*) as a valid model organism in biomedical research and human brain diseases (A.V. KALUEFF & al [8]). The zebrafish is a small freshwater species native to the Indian subcontinent. This shoaling cyprinid (Cyprinidae) is currently used as model animal in various laboratories world-wide. Also, many transgenic subtypes of zebrafish are being developed in various scientific fields, since the gene manipulation and the husbandry are relatively easy to be performed in their case (A.R. WHITELEY & al [9]). There are also some genetic differences between wild populations and artificial selected danios which are used in laboratories (A.R. WHITELEY & al [9]). These can also occur for example at the phenotypic level as for body coloring (B.M. Vick & al [10]) or for temperature tolerance. In addition, according to Whiteley and colleagues, these differences should be taken into account when designing experimental studies, in order to improve the scientific knowledge about this species (A.R. WHITELEY & al [9]).

Moreover, a web network, The Zebrafish Information Network (ZFIN), was established as a community resource in order to implement the knowledge of zebrafish genetic and developmental data (J. SPRAGUE & al [10]). The availability of a huge number of studies in literature and the existence of many experimental advantages (e.g. small size, hundreds of offspring per week, embryos nearly transparent) make this species a very useful model organism, with several applications.

The biological consequences induced by oxidative stress is well-studied in zebrafish, both in adult (J.E. CHOI & al [11]; D. XIONG & al [12]) and in embryos (M. MUGONI & al [13]). Thus, Baulac and colleagues tried to deepen this subject in zebrafish models. In particular, they have studied mutations in the DJ-1 gene linked to autosomal recessive Parkinson's disease, observing the DJ-1 expression in both zebrafish and human brains (post mortem). They found that the DJ-1 gene was expressed early during zebrafish development and DJ-1 KD (Knock Down) embryos did not show any problem during the development but they were more susceptible to programmed cell death. Interestingly, DJ-1 expression was increased zebrafish brains under oxidative stress, indicating that DJ-1 is involved stress-responsive machinery. Since oxidative stress is linked to the development of Alzheimer's disease (AD), the researchers also examined DJ-1 expression in human AD brains. The results of this study strongly suggest that DJ-1, usually present in human AD brains, is not necessary during zebrafish development but can be induced in zebrafish exposed to oxidative stress (S. BAULAC & al [14]).

Considering the aforementioned aspects regarding the oxidative stress implications in most of the actual neuropsychiatric disorders and also the ever increasing importance of zebrafish studies in this context, in the present paper we were interested in the general biological relevance of the oxidative stress status in replicating some neuropsychiatric manifestations in zebrafish, by focusing on the autism spectrum disorder, schizophrenia and Alzheimer disease. Also considering the increased relevance of sleep-related studies in the neuropsychiatric pathology and the existence of some intriguing sleep studies in zebrafish, as well as the correlations between some digestive-related disorders such as irritable bowel syndrome and the neuropsychiatric aspects, some of these correlations and the possibility of studying them on zebrafish are also discussed and speculated during this mini-review.

Autism spectrum disorder

One of the most well-known disorders among children is autism, which is integrated in a group with Asperger's syndrome and nonspecific pervasive develop-mental disorder under the name autistic spectrum disorders, ASD (S.R. SHARMA & al [15]).

The causes of occurrence are not yet clarified, however, hereditary factors, premature births, family history of psychiatric illness, exposure or administration of substances during pregnancy as drugs or insecticides are some of the factors correlated with autism (K. LYALL & al [23]; S.R. SHARMA & al [15]).

Regarding the zebrafish models of autism, zebrafish models own structures capable to display symptoms of

ASD, being a useful tool for studying it (C. SHEN and Z. ZUO [18]; D.A.N. MESHALKINA & al [19]). Being social organisms, zebrafish models have been used to modulate behavioral symptoms of ASD through chemical substances intake as valproic acid (VPA) and several pesticides (D.A.N. MESHALKINA & al [19]). VPA is a fatty acid with an important role in seizures treatment (S.B.R. FAGUNDES [20]). Its secondary effects trigger ASD symptoms and because of that, it has a broad use in ASD medical research (D.A.N. MESHALKINA & al [19]). Zebrafish larvae exposed to 60 µM VPA present perturbation in brain cellularity according to a histo-pathological test made by A.B. VAN WOUDENBERG & al [21].

Thus, F.F. ZIMMERMANN and his group (2015) [21] studied the effect of VPA on 0-48 hours post-fertilization zebrafish embryos testing the locomotor activity, social interaction, aggression and anxiety. These parameters are well-known to be found in autistic individuals. 48 µM VPA was the dose administrated to zebrafish embryos. VPA has induced changes in locomotor activity as increase in the mean speed and distance travelled comparative with the control group (F.F. ZIMMERMANN & al [21]). No significant changes were observed in aggressive behaviour (F.F. ZIMMERMANN & al [21]). Furthermore, social behavior was affected by VPA intake. VPA zebrafish embryos became more solitary due to the time spent near the empty tank instead to stay longer next to its conspecifics (F.F. ZIMMERMANN & al [21]).

In a recent report which studied the effects of several concentrations of VPA as: 5, 50 and 500 µM on touch response, movement speed, light-dark explore, mirror attack, shoaling and social contact (D. CHEN & al [49]). The zebrafish embryos (8 hpf) were observed until 13 dpf. Hyperactivity, increase in cell proliferation and macrocephaly were the main results obtain after VPA administration on zebrafish embryos (D. CHEN & al [49]).

Further analyses are needed to prove a link between oxidative stress and onset of ASD through modelling specific symptoms in zebrafish which had demonstrated that it can be an alternative organism to investigate ASD.

Schizophrenia

Schizophrenia is also a chronic disabling neuro-developmental brain disease with heterogeneous genetic background. Symptoms of schizophrenia include positive symptoms consist of psychotic symptoms, such as hallucinations, delusions, and disorganized speech and behavior; negative symptoms such as poverty of speech, reduced emotions, and loss of interests and drive, while cognitive symptoms comprise of deficits in working memory, attention and in executive functions, such as the ability to organize, also the mood symptoms consist of depressive, cheerful or sad moods (R.S. KAHN & al [23]).

The zebrafish exhibits a powerful combination of attributes which makes this a good tool for the molecular genetic analysis of vertebrate in neurodevelopmental mechanisms. In addition, the rapid external development of the zebrafish embryo, together with its optical transparency, facilitate detailed in vivo visualization of molecular probes for analyzing cell type, cell behavior and subcellular structures in both living and fixed specimens.

A group investigated a role of oxidative damage in the central nervous system (CNS) effects of ethanol (EtOH) in zebrafish, which may be associated with changes in social behavior domain after REE (resting energy expenditure). For this experiment they used 64 adult (4-6 months–old) zebrafish. Animals were exposed for 8 consecutive days to water for control for 20 min per day and in 1% (v/v) EtOH for EtOH group for the same time, the EtOH was added directly to the tank water. In that way after these days, REE alters social behavior of zebrafish and changes oxidation mechanisms in the CNS by increasing lipid peroxidation promoting markedly changes in antioxidant mechanisms. They demonstrate that REE provide changes in the antioxidant enzyme mechanisms, and induces oxidative stress in brain samples because SOD and CAT activities, as well as NPSH levels decreased in brain tissue (E. TALISE & al [68]). Following comparisons made by WOOD and his colleagues in 2009 [25], according to requirements for Neuroregulin 1 (NRG1) in myelination and its status as a genetic risk factor for Schizophrenia, of oligodendrocyte specification in nrg1 morphant zebrafish embryos, they found very similar defects of oligodendrocyte development. In addition, they found that, both disc1 and nrg1 were required for specification of olig2-positive cerebellar neurones in zebrafish model (J.D. WOOD & al [25]).

In another genetic study, they use the zebrafish, like a model of schizophrenia, to investigate the neuro-anatomical and neurobehavioral function of miR-137 during development. This was realized by the modulation of miR-137 expression in the zebrafish embryos throughout early development by both direct administration of synthetic miR-137 mimic or morpholino (MO) miR-137 antagonist, and inducible transgenic expression of miR-137 or transgenic antimiR137 sponge-RNA expression. While upregulation produced no observable specific phenotype, both transgenic miR-sponge and MO-induced down-regulation of miR-137 inhibited touch-response behavior at embryonic and larval stages without modifying other swimming behaviors. They found that miR-137 in zebrafish is expressed and active in the sensory neurons responsible for touch-sensitivity of zebrafish embryos and larvae. However, no obvious anatomical abnormalities were detected, suggesting subtle defects or a possible change

in synaptic function or overall activity (J. GIACOMOTTO & al [26]).

Alzheimer's disease

Alzheimer's disease or AD, is a progressive and irreversible neurodegenerative disease. AD is characterized by progressive loss of memory and cognitive function (A.A. FAROOQUI [27]). The pathologic hallmarks of AD are extracellular amyloid-β (aβ) protein which is containing neuritic plaques and intracellular hyperphosphorylated tau-containing neurofibrillary tangles (Y. XI & al [28]).

Zebrafish could be a good alternative model in elucidating the molecular basis of human neurodegenerative diseases. In fact, zebrafish has been established as an excellent vertebrate model for the study of developmental biology and gene function and it is a promising model organism for studying molecular events in AD because of their faster development and shorter lifespan, compared to mice, that makes them a good choice for neurodegeneration modeling (C. SARACENO & al [30]). Also, several of the human genes encoding the enzymes required for the post-translational modifications of APP (amyloid precursor protein) gene have been found with a high percent of amino acid similarity in zebrafish (A. MUSA & al [31]). The zebrafish are easy to use in research because, embryos and larvae can be easily placed in to microtiter plates and can be treated with various chemicals in their aqueous support medium. This strategy can be used to reveal therapeutic compounds for various disease states. A published study in 2010 generated a transgenic Aβ toxicity model in zebrafish involved fusing the human Aβ-42 sequence to the promoter of the mitfa gene (M. NEWMAN & al [32]). This would drive expression of human Aβ-42 specifically in the melanocytes of the zebrafish. In that way it would produce an easily identifiable disrupted pigmentation pattern phenotype without being lethal to the zebrafish larvae. So, a disrupted pattern will be evident in the adult fish. Aβ toxicity canal was been analyzed in zebrafish simply by exposing embryos to amyloid-beta in their supportive aqueous environment. Treatment of embryos with 2.5 μM Aβ-40 caused defective development including that of the vasculature and also accelerated cell senescence (S. DONNINI & al [33]).

T.T. KAO & al studied in 2014 [34] the impact of the folate deficiency (FD) at zebrafish in AD. The aim for testing this deficiency is because folate or folic acid, vitamin B9, is vital for the development, regeneration and function of nervous system and have been linked to many diseases, especially neurological disorders. In that study, they established a zebrafish folate deficient model by over expressing a fusion of enhanced green fluorescent protein (EGFP) with γ-glutamylhydrolase (γGH) controlled by a heat-shock promoter (hsp). After the anatomical and pathological analyses of the fishes, their results related an increase of the reactive oxygen species (ROS) in FD embryos. Also, that FD-induced oxidative stress also impaired autophagic–lysosomal pathway and contributed to the AD-like pathology observed in aged FD fish that was caused by a nutrient deficiency.

We could mention here that some of the neuromediators implicated in AD are also exerting a role on the oxidative stress status and these were previously demonstrated to have significant effects in various zebrafish models. In this way, scopolamine (an acetylcholine muscarinic receptor antagonist), which was previously demonstrated to increase oxidative stress status (S. HAIDER & al [35], H.S. FOYET & al [36]) was demonstrated to induce also memory impairment in zebrafish, which were reversed by the administration of physostigmine, an acetylcholinesterase inhibitor (Y.H. KIM & al [37]).

Sleep disturbances in neuropsychiatric diseases

Sleep is a normal aspect which occurs in everybody lives and it is needed like food or water. Even if sleep retains a third of our lives, it is essential for maintain a healthy and a good function of body systems (G. MEDIC & al [38]). Approximately 20% of injuries which are a car accident result have been associated with drivers sleepiness (G. MEDIC & al [38]). Sleep disturbances are a common symptom in neuropsychiatric diseases such as ASD, schizophrenia and AD (T. PORKKA-HEISKANEN & al [39]).

Regarding the possible study of sleep in zebrafish, there are actually a considerable number of studies involving sleep processes and zebrafish, as this could have an increased relevance for the topic we presented in this mini report. In this way, the KARLSSON group in Iceland previously demonstrated for the first time from example the effects of the wakefulness-promoting drug modafinil on sleep and wakefulness in larval zebrafish in a manner resembling the mammalian processes (e.g. in connection to circadian rhythms, sleep homeostasis, and sleep pressure) (B. SIGURGEIRSSON & al [40]).

Of course, there is also a connection between oxidative stress and sleep, considering that oxidative stress could be an important pathophysiological mechanism in this context (R. VITALARU & al [41]). Also this can be related with our group's previous studies in oxidative stress status modifications in zebrafish (S.A. STRUNGARU & al [43], [44], as well as the aforementioned zebrafish sleep studies (B. SIGURGEIRSSON & al [40]).

Also, in this case there are some of the neuromediators implicated in sleep physiology that are exhibiting a role on the oxidative stress status, and were previously demonstrated

to have significant effects in zebrafish models. For example, it was previously reviewed from Chiu or Rihel groups that molecules such as noradrenaline, serotonin, dopamine, GABA, glutamate, histamine, adenosine and melatonin are exerting fundamental and intricate roles in regulating zebrafish sleep/wake behavior (C.N. CHIU & al [44], J. RIHEL & al [45]).

Digestive–related manifestations in zebrafish (focussing on the possible existence of irritable bowel syndrome in zebrafish)

Thus, in regards to the digestive –related dysfunctions and the animal modelling of these disorder by using the zebrafish, we can most certainly affirm that the study of gastrointestinal diseases in zebrafish advanced very significantly in the last 10 years, especially together with the focus on the wound healing-related processes, microbiome-host interactions, genetic diseases and drug screening, according to the review work of J.R. GOLDSMITH and C. JOBIN [46]. Interestingly enough, the aforementioned authors did not mention the neuropsychiatric and behavioural-related zebrafish studies in their description (J.R. GOLDSMITH and C. JOBIN [46]).

Thus, as described by the same authors mentioned above, the zebrafish gastrointestinal system is homologous to the mammalian one, although it is of course a little bit simpler in his general architecture (I. SHEPHERD and J. EISEN [47]), being composed by a liver, a pancreas, a gall bladder, and an intestinal area (J.R. GOLDSMITH and C. JOBIN [46]).

It (the zebrafish enteric nervous system) also express a variety of molecules that can be found in the mammals, such as adenylate cyclase-activating, polypeptide (PACAP), vasoactive intestinal polypeptide (VIP), calcitonin gene-related, polypeptide (CGRP), nitric oxide (NO) neurokinin-A (NKA), substance P, acetylcholine and serotonin (as reviewed by I. SHEPHERD and J. EISEN [47]).

Considering this, previous models of hepatocellular carcinoma (thioacetamide induced), pancreatic cancer (e.g. transgenic model), non-alcoholic fatty liver disease (induced by the administration of thioacetamide and/or by genetic manipulation) and inflammatory bowel disease-IBD (induced by the administration of oxazolone and 2,4,6-trinitrobenzene sulfonic acid-TNBS) were described in zebrafish (reviewed by J.R. GOLDSMITH and C. JOBIN [46]).

An exciting review on the latest frontiers in modelling and using zebrafish as an important animal model for the digestive disorders was also described by K.C. SADLER group in the Zebrafish specific journal (K.C. SADLER & al [48]).

In fact, the ultrastructural mapping of zebrafish digestive system, was also recently suggested as a very promising base for future experimental drug test studies in this area (D. CHENG & al [49]), which gained a lot of momentum in the last few years, although, as described by the previous mentioned authors there is still a "limit of confidence" regarding the passing from the classical rodent models to the zebrafish (the differences and resemblances from this two groups of animals being extensively compared on the liver, pancreas or gut levels by the CHENG group) (D. CHENG & al [49]).

In addition, considering that several gut functions and specific genes are kept from the zebrafish to mammals, the zebrafish was also lately proposed as an important candidate for the study of the mechanical aspects related to intestinal inflammation (S. BRUGMAN [50]). As mentioned above, there are quite a variety of larval and adult models of enterocolitis in zebrafish, mainly referring to oxazolone, TNBS or DSS administration (in larvae – e.g. larval immersion), as well as the intrarectal injection of oxazolone and TNBS in adults (S. BRUGMAN [50]). Furthermore, this is related to the genetic susceptibility of intestinal inflammation in zebrafish, especially considering that, as we said above, the zebrafish are exhibiting around 70% of homology to the human ones (D. CHENG & al [49]).

Actually, even in 2017 the HANYANG group also reviewed the possible applications of zebrafish models in the inflammatory bowel disease, considering its multi-factorial pathogenesis, and mainly insisting on the genetic susceptibility on this matter, as well as immunology behind these aspects, and the role of microbiota. The same group also reviews the main limitations of zebrafish models in this context, by mainly focusing on the duplicate genes from the zebrafish genome and differences in lymphocytes maturation [L. HANYANG & al [51]).

Even more, considering our latest interest in irritable bowel syndrome (IBS) animal models (R. LEFTER & al [52], [53]) and zebrafish behavioural studies (S.A. STRUNGARU & al [42], [43], we can speculate about IBS-related manifestations (e.g. IBS is basically a common disorder that affects the large intestine, with its main symptoms being cramping, abdominal pain, bloating, gas, and diarrhea or constipation.

However, IBS models in zebrafish are, according to our best of knowledge, not mentioned until now in the entire literature about the digestive disorder in zebrafish! The main reason related to this aspect can be related to the fact that IBS is a stress related and highly dependent on the environment – related disorder, which depends a lot on the nervous circuits and brain gut-axis (R. LEFTER & al [52]), with its aetiology being much more complex than in the case of the inflammatory bowel disease, where (as we

showed above in zebrafish) the intrarectal administration of some specific chemical agents can induce the pathological manifestations (and some goes in rats-as we discussed in R. LEFTER & al [52]. Thus, it is possible to generate some IBS models in rats, based on the initial administration of some intrarectal specific chemical, which are then combined and conditioned by some other complex factors implicated in this matter described in R. LEFTER & al [52]. However, the present subchapter could open perhaps some new ideas for further studies in this area of research, especially focussing on the IBS replication in zebrafish, as our group is currently working on preliminary studies in this area.

Thus, IBS is one of the most common functional (e.g. lack of a detectable organic cause) gastrointestinal disorders exhibiting complex and controversial pathological features, which were not yet replicated in the zebrafish, according to our best of knowledge. Still, some other functional digestive disorders were replicated in zebrafish level, such as the Hirschsprung's disease or the Goldberg-Shprintzen Syndrome, mainly based on some genetic manipulations and some clinic phenotype replication (as reviewed by I. SHEPHERD and J. EISEN [47]).

Also, perhaps if we want to focus even more about IBS modelling in zebrafish, we could concentrate our attention on the linearly segmented intestinal track of the zebrafish, which has of course the absorptive and the secretory functions (J.R. GOLDSMITH and C. JOBIN [46]).

In regards to the zebrafish enteric nervous system (I. SHEPHERD and J. EISEN [47]), one other aspect to be considered is the microbiota (which was previously demonstrated to be related in some extents to some IBS-like manifestations (E. DISTRUTTI & al [54], B.K. RODIÑO-JANEIRO & al [55]) that in zebrafish is actually related to the motility processes and possible even with the development of the enteric nervous system (I. SHEPHERD and J. EISEN [47]).

Another factor which can be discussed here is represented by the oxidative stress, with our group previously demonstrating the implication of the oxidative metabolism in the inflammatory bowel disease (I. BALMUS & al [56], D. ACHITEI & al [57]) and possibly IBS (R. LEFTER & al [58]).

Of course these aspects are also opening some therapeutical avenues for some antioxidants in this context, ranging from classical antioxidants (I. KHAN & al [59], F. TROFIN & al [60]) to plant extracts (S. MOZAFFARI & al [61], [F. IMBREA & al [62], [63], P. DOBRE & al. [64], I. TONCEA & al [65], S.M. PETRE & al [66], M. APOSTU & al [67], R. DOBRINOIU & al [68]).

Conclusions

The zebrafish still remains an underdeveloped and underutilized model in the study of the neuropsychiatric manifestations and it still has a lot of answers to offer regarding the specific mechanistic and the associated comorbidities of these disorders, as well as in a better understanding of specific treatments associated with them.

Acknowledgments

CA is supported by a research great for Young Teams offered by UEFISCDI Romania, no. PN-III-P1-1.1-TE-2016-1210, contract no. 58 din 02/05/2018, called "Complex study regarding the interactions between oxidative stress, inflammation and neurological manifestations in the pathophysiology of irritable bowel syndrome (animal models and human patients)".

References

1. M. PADURARIU, A. CIOBICA, I. DOBRIN, C. STEFANESCU, Evaluation of antioxidant enzymes activities and lipid peroxidation in schizophrenic patients treated with typical and atypical antipsychotics. *Neurosci Lett*, 479: 317-20 (2010).
2. M. PADURARIU, A. CIOBICA, L. HRITCU, B. STOICA, W. BILD, C. STEFANESCU, Changes of some oxidative stress markers in the serum of patients with mild cognitive impairment and Alzheimer's disease. *Neurosci Lett*, 469:6-10 (2010).
3. A. CIOBICA, L. HRITCU, M. PADURARIU, R. DOBRIN, V. BILD, Effects of serotonin depletion on behavior and neuronal oxidative stress status in rat: relevance for anxiety and affective disorders. *Adv Med Sci*, 55:289-296 (2010).
4. A. CIOBICA, M. PADURARIU, I. DOBRIN, C. STEFANESCU, R. DOBRIN, Oxidative stress in schizophrenia – focusing on the main markers. *Psychiatr Danub*, 23:237-45 (2011).
5. A. CIOBICA, Z. OLTEANU, M. PADURARIU, L. HRITCU, The effects of low-dose pergolide on memory and oxidative stress in a 6-OHDA induced rat model of Parkinson's disease. *J Physiol Biochem*, 68:59-69 (2012).
6. C. STEFANESCU, A. CIOBICA, The relevance of oxidative stress status in first episode and recurrent depression. *J Affect Disord*, 143:34-8 (2012).
7. A.I. ALEXA, A. CANTEMIR, A. CIOBICA, I. GARDIKIOTIS, I. ANTIOCH, C. HONCERIU,

C.L. ZAMFIR, V. POROCH, The Dynamics of the Main Oxidative Stress Chemical Markers in the Serum of Rats Stressed by Various Behavioural Tasks. *Revista de Chimie – Bucharest*, 68(2):350-353 (2017).

8. A.V. KALUEFF, A.M. STEWART, R. GERLAI, Zebrafish as an emerging model for studying complex brain disorders. *Trends in Pharmacological Sciences*, 35(2):63-75 (2014).
9. A.R. WHITELEY, A. BHAT, E.P. MARTINS, Population genomics of wild and laboratory zebrafish (Danio rerio). *Molecular Ecology*, 20:4259-4276 (2011).
10. J. SPRAGUE, L. BAYRAKTAROGLU, D. CLEMENTS, T. CONLIN, D. FASHENA, K. FRAZER, M. HAENDEL, D.G. HOWE, P. MANI, S. RAMACHANDRAN, K. SCHAPER, E. SEGERDELL, P. SONG, B. SPRUNGER, S. TAYLOR, C.E. VAN SLYKE, M. WESTERFIELD, The Zebrafish Information Network: the zebrafish model organism database. *Nucleic Acids Research*, 34(1):D581–D585 (2006).
11. J.E. CHOI, S. KIM, J.H. AHN, P. YOUN, J.S. KANG, K. PARK, J. YI, D.Y. RYU, Induction of oxidative stress and apoptosis by silver nanoparticles in the liver of adult zebrafish. *Aquatic Toxicology*, 100(2):151-159 (2010).
12. D. XIONG, T. FANG, L. YU, X. SIMA, W. ZHU, Effects of nano-scale TiO2, ZnO and their bulk counterparts on zebrafish: Acute toxicity, oxidative stress and oxidative damage. *Science of the Total Environment*, 15;409(8):1444-52 (2011).
13. M. MUGONI, A. CAMPOREALE, M.M. SANTORO, Analysis of Oxidative Stress in Zebrafish Embryos. *J Vis Exp*, 89: 513-28 (2014).
14. S. BAULAC, H. LU, J. STRAHLE, T. YANG, M.S. GOLDBERG, J. SHEN, M.G. SCHLOSSMACHER, C. LEMERE, Q. LU, W. XIA, Increased DJ-1 expression under oxidative stress and in Alzheimer's disease brains. *Molecular Neurodegeneration*, 4:12 (2009).
15. S.R. SHARMA, X. GONDA, F.I. TARAZI, Autism spectrum disorder: classification, diagnosis and therapy. *Pharmacology & Therapeutics* (2018).
16. K. LYALL, L. CROEN, J. DANIELS, M.D. FALLIN C. LADD-ACOSTA, B.K. LEE, B.O.Y. PARK, N.W. SNYDER, D. SCHENDEL, H. VOLK, G.C. WINDHAM, C. NEWSCHAFFER, The changing epidemiology of autism spectrum disorders. *The Annual Review of Public Health*, 38:81-102 (2017).
17. M.E. GONZÁLEZ-FRAGUELA, M.-L. DIAZ HUNG, H. VERA, C. MARAGOTO, E. NORIS, L. BLANCO, R. GALVIZU, M. ROBINSON, Oxidative stress markers in children with autism spectrum disorders. *British Journal of Medicine & Medical Research*, 3(2):307-317 (2013).
18. C. SHEN, Z. ZUO, Zebrafish as a model to study autism spectrum disorder caused by environmental chemicals exposure. *Journal of Alzheimers Disease & Parkinsonism*, 6:288 (2016).
19. D.A. MESHALKINA, M.N. KIZLYK, V.E. KYSIL, A.D. COLLIER, D. J. ECHEVARRIA, M. S. ABREU, L.J.G. BARCELLOS, C. SONG, J.E. WARNICK, E.J. KYZAR, A.V. KALUEFF, Zebrafish models of autism spectrum disorder. *Experimental Neurology*, 299:207-216 (2018).
20. S.B.R. FAGUNDES, Valproic acid: review. *Rev Neuroscienc*, 16(2):130-136 (2008).
21. A.B. VAN WOUDENBERG, C. SNEL, E. RIJKMANS D. DE GROOT, M. BOUMA, S. HERMSEN, A. PIERSMA, A. MENKE, A. WOLTERBEEK, Zebrafish embryotoxicity test for developmental (neuro) toxicity: Demo case of an integrated screening approach system using anti-epileptic drugs. *Reproductive Toxicology*, 49:101-116 (2014).
22. F.F. ZIMMERMANN, K.V. GASPARY, C.E. LEITE, D.P. COGNATO, C.D. BONAN, Embryological exposure to valproic acid induces social interaction deficits in zebrafish (Danio rerio): A developmental behavior analysis. *Neurotoxicology and Teratology*, 52:36-41 (2015).
23. R.S. KAHN, I.E. SOMMER, R.M. MURRAY, A. MEYER-LINDENBERG, D.R. WEINBERGER, T.D. CANNON, M. O'DONOVAN, C.U. CORRELL, J.M. KANE, J. VAN OS, Schizophrenia. *Nat. Rev. Dis. Prim*, 1:150-67 (2015).
24. T.E. MÜLLER, S. Z. NUNES, A. SILVEIRA, V.L. LORO, D.B. ROSEMBERG, Repeated ethanol exposure alters social behavior and oxidative stress parameters of zebrafish. *Pnp* (2017).
25. J.D. WOOD, F. BONATH, S. KUMAR, C.A. ROSS, V.T. CUNLIFFE, Disrupted-in-Schizophrenia 1 and neuregulin 1 are required for the specification of oligodendrocytes and neurones in the zebrafish brain *Human Molecular Genetics*, 18(3):391-404 (2009).
26. J. GIACOMOTTO, A.P. CARROLL, S. RINKWITZ, B. MOWRY, M.J. CAIRNS, T.S. BECKER, Developmental suppression of Schizophrenia-associated miR-137 alters sensorimotor function in zebrafish. *Transl Psychiatry*, 6:818 (2016).
27. A.A. FAROOQUI, Neurochemical aspects of neurotraumatic and neurodegenerative diseases. *Springer*, New York (2010).

28. Y. XI, S. NOBLE, M. EKKER, Modeling Neurodegeneration in Zebrafish. *Curr Neurol Neurosci Rep*, 11:274-282 (2011).
29. X. WANG, M. XU, A. F. JACQUELINE, Z.-JI KE, J. LUO, Thiamine deficiency induces endoplasmic reticulum stress and oxidative stress in human neurons derived from induced pluripotent stem cells. *Toxicology and Applied Pharmacology*, 320:26-31 (2017).
30. C. SARACENO, S. MUSARDO, E. MARCELLO, S. PELUCCHI, M.D. LUCA, Modeling Alzheimer's disease: from past to future. *Front Pharmacol*, 4:77 (2013).
31. A. MUSA, H. LEHRACH, V.A. RUSSO, Distinct expression patterns of two zebrafish homologues of the human APP gene during embryonic development. *Dev Genes Evol*, 211:563-7 (2001).
32. M. NEWMAN, L. WILSON, E. CAMP, G. VERDILE, R. MARTINS, M. LARDELLI, A zebrafish melanophore model of amyloidbeta toxicity. *Zebrafish*, 7:155-159 (2010).
33. S. DONNINI, R. SOLITO, E. CETTI, F. CORTI, A. GIACHETTI, S. CARRA,& al. A beta peptides accelerate the senescence of endothelial cells *in vitro* and *in vivo*, impairing angiogenesis. *FASEBJ*, 24:2385-2395 (2010).
34. T.-T. KAO & al. Folate deficiency-induced oxidative stress contributes to neuropathy in young and aged zebrafish—Implication in neural tube defects and Alzheimer's diseases. *Neurobiol. Dis*, (2014).
35. S. HAIDER, S. TABASSUM, T. PERVEEN, Scopolamine-induced greater alterations in neurochemical profile and increased oxidative stress demonstrated a better model of dementia: A comparative study. *Brain Res Bull*, 127:234-247 (2016).
36. H.S. FOYET, H.H. ABAÏSSOU, E. WADO, E.A. ACHA, C. ALIN C, Emilia coccinae (SIMS) G Extract improves memory impairment, cholinergic dysfunction, and oxidative stress damage in scopolamine-treated rats. *BMC Complement Altern Med*, 23(15):333 (2015).
37. Y.H. KIM, Y. LEE, D. KIM, M.W. JUNG, C.J. LEE. Scopolamine-induced learning impairment reversed by physostigmine in zebrafish. *Neurosci Res. Jun*, 67(2):156-61 (2010).
38. G. MEDIC, M. WILLE, M. HEMELS, Short- and long-term health consequences of sleep disruption. *Nature and Science of Sleep*, 9:151-161 (2017).
39. T. PORKKA-HEISKANEN, K.M. ZITTING, H.K. WIGREN, Sleep, its regulation and possible mechanisms of sleep disturbances. *Acta Physiologica*, 208(4):311-328 (2013).
40. B. SIGURGEIRSSON, H. ÞORSTEINSSON, H. ARNARDÓTTIR, I. JÓHANNESDÓTTIR, K.Æ. KARLSSON, Effects of modafinil on sleep-wake cycles in larval zebrafish. *Zebrafish* (2011).
41. R. VITALARU, M. PADURARIU, A. CANTEMIR, A. CIOBICA, C. STEFANESCU Implications of oxidative stress in patients with depressive disorder and insomnia, *European Neuropsychopharmacology* (2010).
42. S.A. STRUNGARU, M. A. ROBEA, G. PLAVAN, E. TODIRASCU-CIORNEA, A. CIOBICA, M. NICOARA, Acute exposure to methylmercury chloride induces fast changes in swimming performance, cognitive processes and oxidative stress of zebrafish (Danio rerio) as reference model for fish community, *Journal of Trace Elements in Medicine and Biology*, 47:115-123 (2018).
43. S.A. STRUNGARU, G. PLAVAN, A. CIOBICA, M. NICOARA, M.A. ROBEA, C. SOLCAN, E. TODIRASCU-CIORNEA, A. PETROVICI, Acute exposure to gold induces fast changes in social behavior and oxidative stress of zebrafish (Danio rerio), *Journal of Trace Elements in Medicine and Biology*, 50:249-256 (2018).
44. C.N. CHIU, D.A. PROBER. Regulation of zebrafish sleep and arousal states: current and prospective approaches. *Front Neural Circuits*, 9:7-58 (2013).
45. J. RIHEL, D. A. PROBER, A. ARVANITES, K. LAM, S. ZIMMERMAN, S. JANG, et al, Zebrafish behavioral profiling links drugs to biological targets and rest/wake regulation. *Science*, 327:348-351, (2010).
46. J. R. GOLDSMITH and C. JOBIN, Think Small: Zebrafish as a Model System of Human Pathology, *Journal of Biomedicine and Biotechnology* (2012).
47. I. SHEPHERD, J. EISEN, Development of the zebrafish enteric nervous system. *Methods Cell Biol*, 101:143-60 (2011).
48. K.C. SADLER, J.F. RAWLS, S.A. FARBER, Getting the inside tract: new frontiers in zebrafish digestive system biology. *Zebrafish*, 10(2):129-31 (2013).
49. D. CHENG, G.J. SHAMI, M. MORSCH, R.S. CHUNG, F. BRAET, Ultrastructural Mapping of the Zebrafish Gastrointestinal System as a Basis for Experimental Drug Studies. *BioMed Research International*, (2016).
50. S. BRUGMAN The zebrafish as a model to study intestinal inflammation. *Dev Comp Immunol*, 64:82-92 (2016).
51. L. HANYANG, L. XUANZHE, C. XUYANG & al Application of Zebrafish Models in Inflammatory Bowel Disease. *Front Immunol*, 8:501 (2017).

52. R. LEFTER, A. CIOBICA, S. GUENNE, M. COMPAORE, M. KIENDREBEOGO, C. STANCIU, A. TRIFAN, A Complex Behavioral Characterisation of a Rat Model of Irritable Bowel Syndrome generated by Chronic Stress Contention, *Neurophysiology*, 50: 266 (2018).
53. R. LEFTER, A. CIOBICA, D. TIMOFTE, D. ABABEI, R. DOBRIN, A. LUCA, A. TRIFAN, C. STANCIU, C. SFARTI, A new biological approach in generating an irritable bowel syndrome rat model-focusing on depression in sucrose splash test and body weight change, *Rom Biotech Lett*, 23 (2018).
54. E. DISTRUTTI, L. MONALDI, P. RICCI, S. FIORUCCI, Gut microbiota role in irritable bowel syndrome: New therapeutic strategies. *World J Gastroenterol*, 22(7):2219-41 (2016).
55. B.K. RODIÑO-JANEIRO, M. VICARIO, C. ALONSO-COTONER, R. PASCUA-GARCÍA, J.A. SANTOS, Review of Microbiota and Irritable Bowel Syndrome: Future in Therapies. *Adv Ther*, 35(3):289-310 (2018).
56. I.M. BALMUS, A. CIOBICA, A. TRIFAN, C. STANCIU, The implications of oxidative stress and antioxidant therapies in inflammatory bowel disease: clinical aspects and animal models, *Saudi Journal of Gastroenterology*, 22(1):3-17 (2016).
57. D. ACHITEI, A. CIOBICA, G. BALAN, E. GOLOGAN, C. STANCIU, G. STEFANESCU, Different Profile of Peripheral Antioxidant Enzymes and Lipid Peroxidation in Active and Non-active Inflammatory Bowel Disease Patients. *Digestive Diseases and Sciences*, 58:1244-1249 (2013).
58. R. LEFTER, M.I. BALMUS, A. TRIFAN, A. CIOBICA, C. STANCIU, Is irritable bowel disease a neurological disorder? The oxidative stress and inflammation connection, *International Journal of Clinical Neurosciences and Mental Health*, 3(1):116 (2016).
59. I. KHAN, S.E. SAMSON, A.K. GROVER, Antioxidant Supplements and Gastrointestinal Diseases: A Critical Appraisal. *Med Princ Pract*, 26(3):201-217 (2017).
60. F. TROFIN, A. CIOBICA, C. HONCERIU, S. COJOCARU, B. STOICA, D. COJOCARU, E. CIORNEA, D. TIMOFTE, Modulatory effects of vitamin C and smoking status in the relation between physical exercising and oxidative stress, *Rom Biotech Lett*, 22(2):12439-12447 (2017).
61. S. MOZAFFARI, H. ESMAILY, R. RAHIMI, M. BAEERI, Y. SANEI, A. ASADI-SHAHMIRZADI, M.H. SALEHI-SURMAGHI, M. ABDOLLAHI, Effects of Hypericum perforatum extract on rat irritable bowel syndrome. *Pharmacogn Mag. Jul*, 7(27):213-23 (2011).
62. F. IMBREA, S. JURCOANE, H.V. HALMAJAN, M. DUDA, L. BOTOS, Camelina sativa: A new source of vegetal oils. *Rom Biotech Lett*, 16 (3):6263-6270 (2011).
63. F. IMBREA, S. JURCOANE, G. POP, I. M. IMBREA, L. BOTOS, F. CRISTA, L. SMULEAC, In search of plant sources for serine protease inhibitors: I. Detection of serine protease inhibitors in callus cultures induced from somatic explants of flax (Linum usitatissimum L.). *Rom Biotech Lett*, 22(4):12762-12766 (2017).
64. P. DOBRE, S. JURCOANE, F. MATEI, C. STELICA, N. FARCAS, A.C. MORARU, Camelina sativa as a double crop using the minimal tillage system. *Rom Biotech Lett*, 19 (2):9190-9195 (2014).
65. I. TONCEA, D. NECSERIU, T. PRISECARU, L.N. BALINT, M.I. GHILVACS, M. POPA, The seed's and oil composition of Camelia – first romanian cultivar of camelina (Camelina sativa, L. Crantz). *Rom Biotech Lett*, 18(5):8594-8602 (2013).
66. S.M. PETRE, A. MORARU, P. DOBRE, S. JURCOANE, Research on Camelina sativa wintering, by genotype and fertilizer doses used, in the pedo-climatical conditions from the south of Romania. *Rom Biotech Lett*, 19(6):9964-9973 (2014).
67. M. APOSTU, G. TANTARU, M. VIERIU, A.D. PANAINTE, N. BIBIRE, L. AGOROAEI, Evaluation of in Vitro Reducing Effect of Several Vegetable Extracts on the Digestive Bioavailability of Heavy Metals. *Rev Chim*, 68(4):683-686 (2017).
68. R. DOBRINOIU, S. JURCOANE, S. DANAILA-GUIDEA, M. MORARU, M. DUMBRAVA, The impact of new technological approaches upon establishing production components and yield randament in Carthamus tinctorium L. culture. *Rom Biotech Lett*, 16(2):6125-6134 (2011).