Ç. Çiçek / Hacettepe J. Biol. & Chem., 2023, (3), 309-315

Research Article

Hacettepe Journal of Biology and Chemistry

journal homepage: www.hjbc.hacettepe.edu.tr

Thiol/Disulfide Balance in Induced Phenylketonuria Model

İndüklenmiş Fenilketonüri Modelinde Tiyol/Disülfit Dengesi

Cigdem Cicek

Yuksek Ihtisas University, Faculty of Medicine, Department of Medical Biochemistry, Ankara, Turkey.

ABSTRACT

Objective The rare metabolic disorder phenylketonuria (PKU) is caused by a deficiency in the phenylalanine hydroxylase enzyme. Deficiency of this enzyme causes phenylalanine accumulation in the brain and irreversible neurological damage by increasing the blood phenylalanine level. The purpose of this study was to determine how the phenylketonuria model affected the balance of thiols and disulfides in the brain.

Method: Brain total thiol and native thiol levels were measured by the modified elman method in rat pups (n:7) in which PKU model was generated and control group (n:7). The disulfide level was calculated according to the total thiol and native thiol levels.

Results: The brain total thiol level of the PKU group was statistically decreased compared to the control group (*p=0.0369). Brain native thiol level of the PKU group was statistically decreased compared to the control group (****p<0.001). The brain disulfide level of the PKU group did not differ statistically compared to the control group (p=0.1107).

Conclusion: It was concluded that oxidative stress in PKU may affect thiol/disulfide levels. The study, which was reported for the first time in the literature, showed a change in the thiol/disulfide balance in the PKU model.

Key Words

Phenylketonuria model, thiol/disulfide balance, oxidative stress, brain.

ÖZ

Amaç: Nadir görülen bir metabolik bozukluk olan fenilketonüri (PKU), fenilalanin hidroksilaz enzimindeki bir eksiklikten kaynaklanır. Bu enzimin eksikliği beyinde fenilalanin birikimine ve kan fenilalanin düzeyini yükselterek geri dönüşü olmayan nörolojik hasara neden olur. Bu çalışmanın amacı fenilketonüri modelinin beyindeki tiyol ve disülfit dengesini nasıl etkilediğini belirlemektir.

Yöntem: PKU modeli oluşturulmuş sıçan yavrularında (n:7) ve kontrol grubunda beyin total tiyol ve serbest tiyol seviyesi modifiye elman yöntemi ile ölçüldü. Total tiyol ve native tiyol seviyelerine göre disülfit seviyesi hesaplandı.

Bulgular: PKU grubun beyin total tiyol seviyesi kontrol grubuna göre istatistiksel olarak azdır (*p=0.0369). PKU grubun beyin serbest tiyol seviyesi kontrol grubuna göre istatistiksel olarak azdır (****p<0.001). PKU grubun beyin disülfit seviyesi kontrol grubuna göre istatistiksel olarak değişiklik göstermemiştir (p=0.1107).

Sonuç: PKU'daki oksidatif stresin tiyol/disülfit seviyelerini etkileyebileceği sonucuna varıldı. Literatürde ilk kez bildirilen çalışma, PKU modelinde tiyol/disülfit dengesinde değişiklik olduğunu gösterdi.

Anahtar Kelimeler

Fenilketonüri modeli, tiyol/disülfit dengesi, oksidatif stres, beyin.

Article History: Feb 22, 2023; Revised: Apr 8, 2023; Accepted: Apr 8, 2023; Available Online: Jul 15, 2023.

DOI: https://doi.org/10.15671/hjbc.1254604

Correspondence to: C. Cicek, Department of Medical Biochemistry, Yuksek Ihtisas University, Ankara, Turkey.

E-Mail: cigdemcicek@yiu.edu.tr

INTRODUCTION

Phenylketonuria (PKU) is a rare genetic disease caused by a deficiency of the enzyme phenylalanine hydroxylase (PAH), which metabolizes the amino acid phenylalanine (Phe). Phenylalanine tyrosine conversion cannot occur due to the deficiency of the phenylalanine hydroxylase enzyme, and the phenylalanine level increases in the plasma, while the tyrosine level decrease [1]. Phenylalanine accumulates in the blood and other tissues, crosses the blood-brain barrier and creates a toxic effect on the brain. Accumulated phenylalanine causes irreversible progressive brain damage, mental retardation, and neuroinflammation [2].

In order to assess the oxidant/antioxidant balance, various markers are used. The thiol/disulfide balance created by Erel et al. is one of the most modern of these [3]. Thiol: It balances oxidative stress by accelerating inactivation or reducing the production of reactive oxygen species. Thiol groups in the environment are oxidized by reactive oxygen species and transform into reversible disulfide bonds. The oxidant molecules in the disulfide environment oxidize them, converting them into reversible bond structures. The thiol/disulfide balance can be preserved by reducing the disulfide bond structures created back to thiol groups. In antioxidant defense, detoxification, apoptosis, regulation of enzyme activities, mechanisms of transcription, and cellular signal transduction, thiol/disulfide balance plays crucial roles [4, 5]. Serum native thiol, total thiol and disulfide levels are measured with the method developed by Erel et al. With the same method, disulfide/thiol, disulfide/total thiol and native thiol/total thiol ratios and indices are calculated. Native thiol and total thiol values are predicted to fall under oxidative stress, but disulfide/thiol, disulfide/total thiol, and native thiol/total thiol indices are predicted to rise [3]. Disruption of this balance can lead to various diseases [6, 7]. When oxidative stress is reduced, these disulfide bonds are reduced to thiol groups, which can be a proponent of oxidative stress [8].

There has been no prior study on the thiol/disulfide balance reported, but the relationship of PKU with many original oxidative stress parameters has been evaluated in the literatüre [9-14]. We examined how it affects the thiol/disulfide balance in PKU in our study.

MATERIALS and METHODS

Animals

The experiments used 14 2-day-old Sprague-Dawley rat pups weighing an average of 9 grams (Kobay Dhl A.Ş. Local Ethics Committee, Protocol Number: 560). Pregnant rats were placed in cages prior to the experiment, and their offspring were housed in cages for 2 days from birth for 12 hours at night and 12 hours during the day in a circadian rhythm at 24-26 °C room temperature and 50-60% humidity. While the offspring were fed with the breast milk, the mother was subjected to standard feeding conditions. During the experimental injection, the pups were not separated from their mothers and continued to be breastfed.

Experimental design: PKU model

To establish a PKU model [15-17], daily injection of phenylalanine (Phe) (5.2 mmol/g body weight) was used, while the phenylalanine hydroxylase inhibitor, p-chlorophenylalanine (p-Cl-Phe) (0.9 mmol /g body weight) was administered every other day (PKU group). Saline solution (saline) was administered to the control group. All injections were started on the 2nd postpartum day and completed on the 10th postpartum day. Sacrification was performed on the 10th postpartum day and brain tissues were taken.

Solution preparation;

*p-Cl-Phenylalanine (Sigma, c8655) and Phe (Sigma, P1150000) stock solutions were prepared in 0.9% pH 7.2 saline, with concentrations of 26 µmol/ml and 152 µmol/ml, respectively [18, 19].

Brain tissue homogenization

Each brain size was measured by weight. Tissues were homogenized in 10% (w/v) 50 mM Tris pH 7.4 buffer containing 2 mM EDTA, 0.5% Triton X-100, ultimately containing protease inhibitor cocktail, for 3 x 10 seconds by OMNI Tissue Master 125 (F12520377). All procedures were performed on ice to prevent protein degradation. All solutions used were also kept on ice. Then, homogenates were centrifuged for 10 minutes at 5000 and 10000g at +4°C. After centrifugation, the supernatant was removed and stored at -80°C until used in protein analysis.

Before measurement of total and native thiol, the brain protein level of all samples was determined using the Protein Assay Kit (Thermo Fisher Coomassie (Bradford) catalog no: 23200). (Fig 2D.).

Total and native thiol measurement principle: For total thiol measurement, 10 µl of R1 (10 µl of R1' is used for native thiol measurement) and 10 µl of sample were mixed. Afterwards, R2 (110 µl) and R3 (10 µl) were added and the first absorbance (A1) was measured spectrophotometrically at 415 nm wavelength. The second absorbance (A2) was performed at the same wavelength at the 10th minute when the reaction plateaued. The measurement was completed by obtaining the A2-A1 absorbance difference [3]. The molar extinction coefficient of 14.100 mol/L-1 cm-1 of 5-thio-2-nitrobenzoic acid (TNB) was used to calculate total and native thiol levels. The disulfide level was calculated from the formula "(total disulfide-native disulfide)/2". All results were reported as micromoles per liter (µmol/L) [20].

Statistical analysis

It was examined whether the data in the experimental groups showed normal distribution with the Kolmogorov Smirnov test. If it had a normal distribution, comparisons between groups were made with One-Way Anova, one of the parametric tests. If the p value was below 0.05, pairwise comparisons were made with the Tukey HSD test. Independent T-test was used for two group comparisons. Statistical analyzes were performed in Graphpad 9.0 program.

RESULTS and DISCUSSION

As the total thiol level of the PKU group was 222.6 ± 30.76 µmol/L, the total thiol level of the control group was 314.1 ± 40.27 µmol/L. The total thiol level of the PKU group was statistically decreased compared to the control group (*p=0.0369) (Fig. 2A).

The native thiol level of the PKU group was 150.5 ± 11.42 µmol/L, while the native thiol level of the control group was 294.3.1 ± 22.06 µmol/L. The native thiol level of the PKU group was statistically decreased compared to the control group (****p<0.001) (Fig. 2B).

As disulfite levels are compared, the disulfide level of the control group is 36.04 ± 5.92 µmol/L, while the disulfide level of the PKU group is 9.89 ± 1.30 µmol/L. The disulfide level of the PKU group was statistically increased compared to the control group (ns; p=0.1107) (Fig. 2C).

The lack of PAH results in PKU, a genetic disorder. Due to the fact that PAH is necessary for the conversion of Phe to Tyr, a PAH deficiency leads to an accumulation of Phe and the breakdown products of its alternative metabolic pathway. Patients with classic PKU without dietary restrictions risk developing intellectual disability if untreated. Unfortunately, although recent research has demonstrated that oxidative stress plays a role in PKU the exact biochemical mechanisms underlying brain dysfunction remain unknown [21-23]. Because brain tissue lacks a robust antioxidant defense system, it is

Table 1. Reagents used

R1 reagent	R1' reagent	R2 reagent	R3 reagent
$NaBH_4$ (10 mM) (Carlo Erba, CE.478953)	NaCl (10 mM) (Sigma, S9888)	Formaldehyde (6.715mM) (Carlo Erba, CE.415666)	DTNB (10 mM) (AFG, AFG.179457
		EDTA (10mM) (Carlo Erba, CE.405494)	
In 1000 ml 1/1 water-methanol	In 1000 ml 1/1 water-methanol	In 1000 ml Tris buffer (100 mM, pH 8.2)	In 1000 ml of methanol

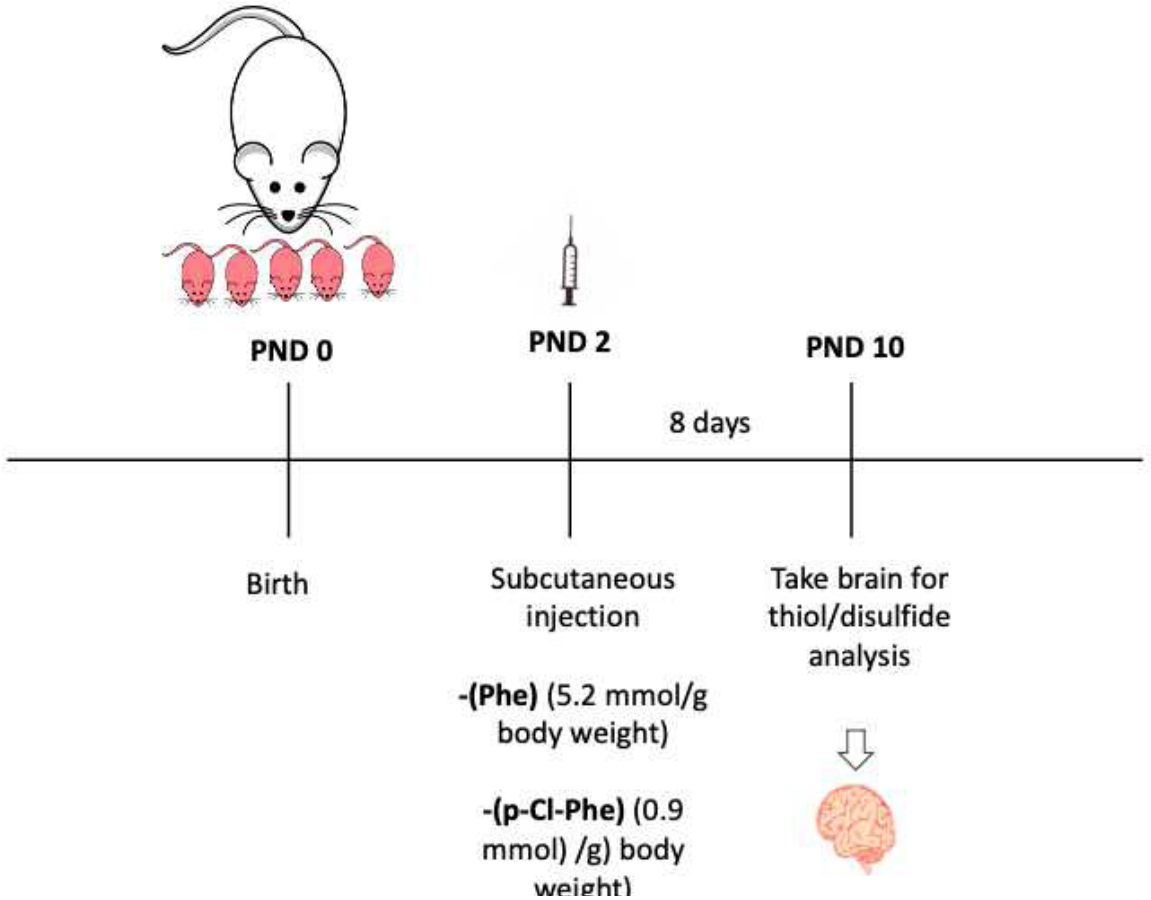

Figure 1. Experimental design.

well known that it is extremely susceptible to oxidative damage. Phenyl-ketone metabolites have previously been thought to be toxic to a developing brain. [24]. Oxidative stress may also increase their toxicity, but there is no evidence to suggest an association between mental retardation and oxidative stress. Oxidative stress is known to be associated with the pathogenesis of various PKU disease errors [25]

Even patients with early diagnoses who were receiving treatment had elevated serum levels of 8-hydroxy-2-deoxyguanosine, a marker of DNA oxidation, which was negatively correlated with these patients' antioxidant status. [26].. Additionally, PKU patients exhibited increased DNA damage in peripheral blood leukocytes, particularly in patients who were mismatched, indicating a connection between Phe levels and DNA damage in peripheral blood [27, 28]. Additionally, it has been noted in various studies that high Phe concentrations can damage proteins and lipids in plasma and erythrocytes, as shown by an increase in malondialdehyde concentration, carbonyl formation, and sulfhydryl oxidation [26, 27, 29-32].

It was possible to examine whether the oxidative damage to macromolecules seen in patients' peripheral fluids could affect the brain using animal models of PKU. Acute and chronic administration of Phe plus a PAH inhibitor, -methyl-L-phenylalanine (aMePhe), in the brain of rats after 6 days of age stimulated lipid peroxidation, according to research by Kienzle Hagen et al. in an in vitro model [19]. Another in vitro study found that Phe and its metabolites phenylpyruvate, phenylactate, and phenylacetate also induced lipid peroxidation in the cerebral cortex, leading to oxidative damage to proteins and lipids in the cerebral cortex and hippocampus of yo-

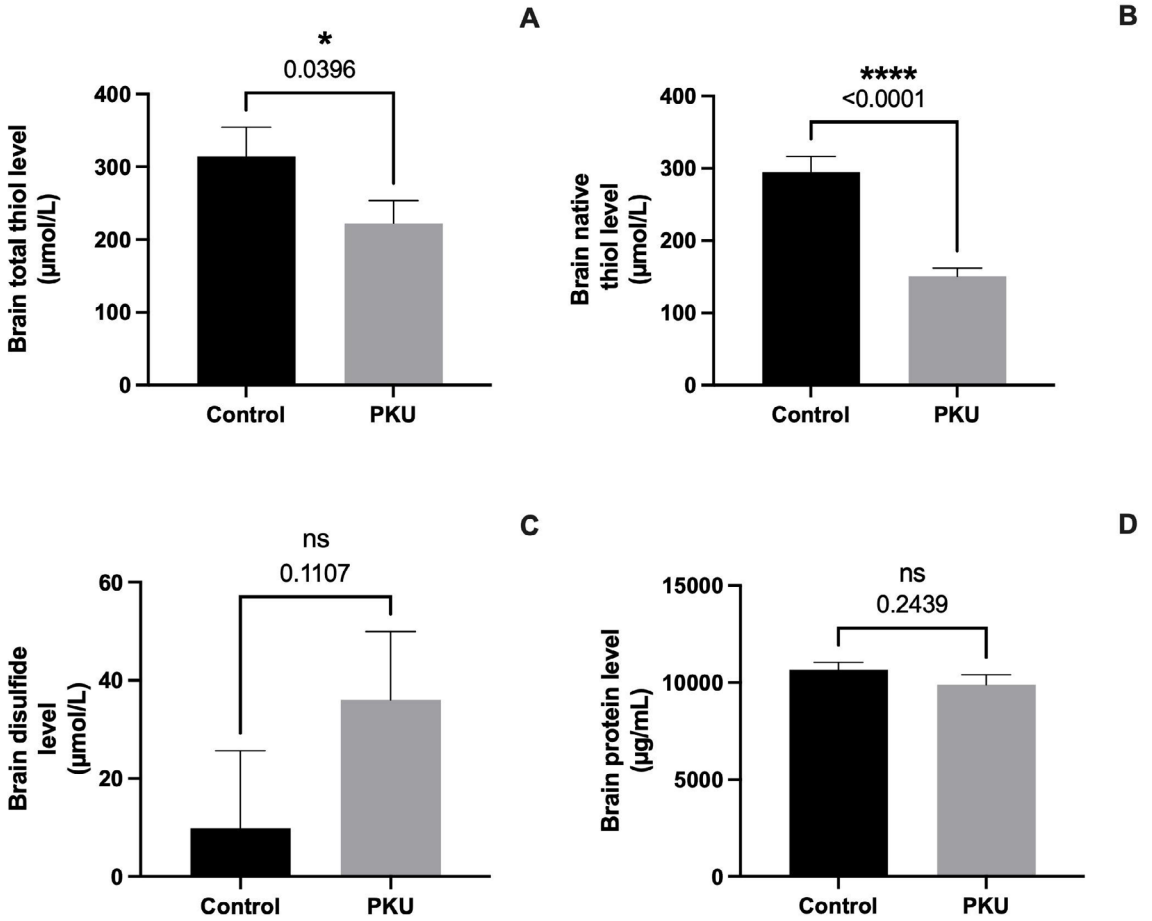

Figure 2. Total thiol, native thiol, disulfide and protein levels in the brains of control and induced-PKU rats: A) Total Thiol, B) Native thiol C) Disulfide D) Protein levels. Results showed that total thiol (*p=0.0396) and native thiol (****p<0.0001) levels were decreased in PKU compared to control rats. The disulfide level and protein level were not statistically different in the PKU group compared to the control group.

ung rats. Alpha-tocopherol and melatonin are effective at preventing lipid peroxidation, so the authors inferred that Phe causes the production of peroxyl and hydroxyl radicals [33].. One of our study, which is in the printing phase, is; enzymes associated with oxidative stress by gender Superoxide Dismutase (SOD), Glutathione Peroxidase (GPx), Glutathione reductase (GR); cytokines IL-1 β, IL-6, TNF-a; We analyzed monoamine oxidases [34] and Acetylcholinesterase (AChE) activity. SOD and GR activities were decreased in PKU rats compared to controls.

The difference in thiol/disulfide parameters from the control group was assessed in this study. In line with the results we obtained, the total thiol and native thiol levels of the PKU group decreased, while the disulfide level, which is an oxidative stress marker, was not changed compared to the control group. There aren't many studies in the literature that compare oxidative stress in metabolic diseases to thiol/disulfide balance. The impact of antioxidant therapy on thiol/disulfide parameters in patients with L-2-hydroxyglutaric aciduria was examined in the study reported by Cansever et al. There was no significant different in the thiol/disulfide parameters between the 14 participants who received antioxidant treatment and the control group [35]. The authors suggested that antioxidant therapy prevents oxidative damage. According to Zubarioglu et al. investigated the thiol/disulfide balance in metabolically controlled MSUD patients and showed no significant difference compared to the control group [36]. In the study by Cam et al, Thiol-Disulfide Ratio was analyzed in in-

herited metabolic disease. In inherited metabolic disease, it was shown that oxidative stress status increases during metabolic attacks [37]. In the study of McGuire et al., a relationship was found between the clinical status of patients with metabolic disorders and oxidative stress parameters [38]. In our study, it was concluded that oxidative stress in PKU affects the changes in thiol/disulfide levels. In the study, which was reported for the first time in the literature, it was shown that the thiol/disulfide balance may affect the pathophysiology of PKU.

CONCLUSION

In phenylketonuria, due to the deficiency of the phenylalanine hydroxylase enzyme, phenylalanine tyrosine conversion cannot occur and the level of phenylalanine increases in the plasma. Phenylalanine accumulates in the blood and other tissues and crosses the blood-brain barrier, creating a toxic effect on the brain. Accumulated phenylalanine causes irreversible progressive brain damage, mental retardation and neuroinflammation, while oxidative stress parameters increase. In this study, we reported that the thiol/disulfide balance was changed as an oxidative stress biomarker in PKU. The decreased thiol level in PKU may be due to the toxic effect of Phe.

Acknowledgments

The animal part of this study was supported by the Scientific Research Projects Unit of the Yüksek Ihtisas University (Project number: 2021/01.003).

References

1. C. Scriber, Hyperphenylalaninemia: phenylalanine hydroxylase deficiency, The metabolic and molecular bases of inherited disease., (2001) 1667-1724.
2. N. Blau, Genetics of phenylketonuria: then and now, Human mutation. 37 (2016) 508-515.
3. O. Erel and S. Neselioglu, A novel and automated assay for thiol/disulphide homeostasis, Clinical biochemistry., 47 (2014) 326-332.
4. P. V. Oliveira and F. R. Laurindo, Implications of plasma thiol redox in disease, Clinical Science. ,132 (2018) 1257-1280.
5. A. K. Erenler and T. Yardan, Clinical Utility of Thiol/Disulfide Homeostasis, Clinical laboratory., 63 (2017) 867-870.
6. S. Emre, D. D. Demirseren, M. Alisik, A. Aktas, S. Neselioglu, and O. Erel, Dynamic thiol/disulfide homeostasis and effects of smoking on homeostasis parameters in patients with psoriasis, Cutaneous and ocular toxicology., 36 (2017) 393-396.
7. B. Elmas, M. Karacan, P. Dervişoğlu, M. Kösecik, Ş. P. İşgüven, and C. Bal, Dynamic thiol/disulphide homeostasis as a novel indicator of oxidative stress in obese children and its relationship with inflammatory-cardiovascular markers, Anatolian journal of cardiology., 18 (2017) 361.
8. Z. K. Tufan, I. Hasanoglu, S. Kolgelier, M. Alisik, M. Ergin, G. R. Yilmaz, M. A. Tasyaran, O. Erel, and R. Guner, A retrospective controlled study of thiol disulfide homeostasis as a novel marker in Crimean Congo hemorrhagic fever, Redox Report., 22 (2017) 241-245.
9. Z. Wang, W. Zhao, X. Shen, H. Wan, and J. M. Yu, The role of P2Y6 receptors in the maintenance of neuropathic pain and its improvement of oxidative stress in rats, Journal of cellular biochemistry., 120 (2019) 17123-17130.
10. A. Horst, J. de Souza, M. Santos, A. Riffel, C. Kolberg, and W. Partata, Effects of N-acetylcysteine on spinal cord oxidative stress biomarkers in rats with neuropathic pain, Brazilian Journal of Medical and Biological Research., 50 (2017)
11. A. Gorlova, D. Pavlov, E. Zubkov, Y. Zorkina, A. Inozemtsev, A. Morozova, and V. Chekhonin, Alteration of oxidative stress markers and behavior of rats in a novel model of depression, Acta Neurobiol. Exp., 79 (2019) 232-238.
12. R. Rebai, L. Jasmin and A. Boudah, Agomelatine effects on fat-enriched diet induced neuroinflammation and depression-like behavior in rats, Biomedicine & Pharmacotherapy., 135 (2021) 111246.
13. O. Yakovleva, K. Bogatova, R. Mukhtarova, A. Yakovlev, V. Shakhmatova, E. Gerasimova, G. Ziyatdinova, A. Hermann, and G. Sitdikova, Hydrogen sulfide alleviates anxiety, motor, and cognitive dysfunctions in rats with maternal hyperhomocysteinemia via mitigation of oxidative stress, Biomolecules., 10 (2020) 995.
14. F. Beheshti, M. Hashemzehi, M. Hosseini, N. Marefati, and S. Memarpour, Inducible nitric oxide synthase plays a role in depression-and anxiety-like behaviors chronically induced by lipopolysaccharide in rats: Evidence from inflammation and oxidative stress, Behavioural brain research., 392 (2020) 112720.
15. N. W. Dimer, B. K. Ferreira, J. F. Agostini, M. L. Gomes, L. W. Kist, F. Malgarin, M. Carvalho-Silva, L. M. Gomes, J. Rebelo, and M. J. S. Frederico, Brain bioenergetics in rats with acute hyperphenylalaninemia, Neurochemistry international., 117 (2018) 188-203.
16. G. A. Dienel and N. F. Cruz, Biochemical, metabolic, and behavioral characteristics of immature chronic hyperphenylalanemic rats, Neurochemical research., 41 (2016) 16-32.
17. K. R. Simon, R. M. dos Santos, G. Scaini, D. D. Leffa, A. P. Damiani, C. B. Furlanetto, J. L. Machado, J. H. Cararo, T. P. Macan, and E. L. Streck, DNA damage induced by phenylalanine and its analogue p-chlorophenylalanine in blood and brain of rats subjected to a model of hyperphenylalaninemia, Biochemistry and Cell Biology., 91 (2013) 319-324.
18. L. R. Feksa, A. R. Cornelio, V. C. Rech, C. S. Dutra-Filho, A. T. S. Wyse, M. Wajner, and C. M. D. Wannmacher, Alanine prevents the reduction of pyruvate kinase activity in brain cortex of rats subjected to chemically induced hyperphenylalaninemia, Neurochemical research., 27 (2002) 947-952.
19. M. E. K. Hagen, C. D. Pederzolli, A. M. Sgaravatti, R. Bridi, M. Wajner, C. M. Wannmacher, A. T. Wyse, and C. S. Dutra-Filho, Experimental hyperphenylalaninemia provokes oxidative stress in rat brain, Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 1586 (2002) 344-352.

20. G. Ellman and H. Lysko, A precise method for the determination of whole blood and plasma sulfhydryl groups, Analytical biochemistry., 93 (1979) 98-102.
21. C. Sierra, M. A. Vilaseca, D. Moyano, N. Brandi, J. Campistol, N. Lambruschini, F. J. Cambra, R. Deulofeu, and A. Mira, Antioxidant status in hyperphenylalaninemia, Clinica chimica acta., 276 (1998) 1-9.
22. R. Artuch, C. Colomé, M. Vilaseca, C. Sierra, F. Cambra, N. Lambruschini, and J. Campistol, Plasma phenylalanine is asociated with decreased serum ubiquine-10 concentrations in phenylketonuria, Journal of inherited metabolic disease., 24 (2001) 359-366.
23. V. T. Bortoluzzi, C. S. Dutra Filho and C. M. D. Wannmacher, Oxidative stress in phenylketonuria—evidence from human studies and animal models, and possible implications for redox signaling, Metabolic Brain Disease., (2021) 1-21.
24. S. Kaufman, An evaluation of the possible neurotoxicity of metabolites of phenylalanine, The Journal of pediatrics., 114 (1989) 895-900.
25. N. Ercal, N. Aykin-Burns, H. Gurer-Orhan and J. D. McDonald, Oxidative stress in a phenylketonuria animal model, Free Radic Biol Med., 32 (2002) 906-11.
26. K. H. Schulpis, S. Tsakiris, J. Traeger-Synodinos and I. Papassotiriou, Low total antioxidant status is implicated with high 8-hydroxy-2-deoxyguanosine serum concentrations in phenylketonuria, Clinical biochemistry., 38 (2005) 239-242.
27. M. Deon, S. S. Landgraf, J. F. Lamberty, D. J. Moura, J. Saffi, M. Wajner, and C. R. Vargas, Protective effect of L-carnitine on Phenylalanine-induced DNA damage, Metabolic Brain Disease. , 30 (2015) 925-933.
28. A. Sitta, V. Manfredini, L. Biasi, R. Treméa, I. V. Schwartz, M. Wajner, and C. R. Vargas, Evidence that DNA damage is associated to phenylalanine blood levels in leukocytes from phenylketonuric patients, Mutation Research/Genetic Toxicology and Environmental Mutagenesis., 679 (2009) 13-16.
29. C. Colomé, R. Artuch, M.-A. Vilaseca, C. Sierra, N. Brandi, N. Lambruschini, F. J. Cambra, and J. Campistol, Lipophilic antioxidants in patients with phenylketonuria, The American Journal of Clinical Nutrition., 77 (2003) 185-188.
30. L. R. Sirtori, C. S. Dutra-Filho, D. Fitarelli, A. Sitta, A. Haeser, A. G. Barschak, M. Wajner, D. M. Coelho, S. Llesuy, A. Belló-Klein, R. Giugliani, M. Deon, and C. R. Vargas, Oxidative stress in patients with phenylketonuria, Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease., 1740 (2005) 68-73.
31. A. Sitta, A. G. Barschak, M. Deon, J. F. de Mari, A. T. Barden, C. S. Vanzin, G. B. Biancini, I. V. Schwartz, M. Wajner, and C. R. Vargas, L-carnitine blood levels and oxidative stress in treated phenylketonuric patients, Cellular and molecular neurobiology., 29 (2009) 211-218.
32. A. Sitta, A. G. Barschak, M. Deon, A. T. Barden, G. B. Biancini, P. R. Vargas, C. F. de Souza, C. Netto, M. Wajner, and C. R. Vargas, Effect of short-and long-term exposition to high phenylalanine blood levels on oxidative damage in phenylketonuric patients, International Journal of Developmental Neuroscience., 27 (2009) 243-247.
33. C. G. Fernandes, G. Leipnitz, B. Seminotti, A. U. Amaral, Â. Zanatta, C. R. Vargas, C. S. Dutra Filho, and M. Wajner, Experimental evidence that phenylalanine provokes oxidative stress in hippocampus and cerebral cortex of developing rats, Cellular and molecular neurobiology., 30 (2010) 317-326.
34. X. Qiu, Q. Mao, Y. Tang, L. Wang, R. Chawla, H. A. Pliner, and C. Trapnell, Reversed graph embedding resolves complex single-cell trajectories, Nature methods., 14 (2017) 979-982.
35. M. S. Cansever, T. Zubarioglu, C. Oruc, E. Kiykim, A. Gezdirici, S. Neselioglu, O. Erel, C. Yalcinkaya, and C. Aktuglu-Zeybek, Oxidative stress among L-2-hydroxyglutaric aciduria disease patients: evaluation of dynamic thiol/disulfide homeostasis, Metabolic brain disease., 34 (2019) 283-288.
36. T. Zubarioglu, E. Kiykim, M. S. Cansever, S. Neselioglu, C. Aktuglu-Zeybek, and O. Erel, Evaluation of dynamic thiol/disulphide homeostasis as a novel indicator of oxidative stress in maple syrup urine disease patients under treatment, Metabolic brain disease., 32 (2017) 179-184.
37. V. Cam, A. Olgac, M. Kilic, O. Erel, S. Neselioglu, and C. S. Kasapkara, Oxidative Stress in Intoxication Type Inborn Errors of Metabolism using Thiol-Disulfide Ratio, J Coll Physicians Surg Pak., 31 (2021) 663-7.
38. P. J. Mc Guire, A. Parikh and G. A. Diaz, Profiling of oxidative stress in patients with inborn errors of metabolism, Molecular genetics and metabolism., 98 (2009) 173-180.