Indian J. Applied & Pure Bio. Vol. 39(1), 135-144 (2024). **ISSN: 0970-2091**
A Web of Science Journal

Heavy metals as a risk factor for Parkinson's disease: An epidemiological and *insilico* study

[1]Bhargawi Mishra, [2]Sooraj Patil, [1]Neha Srivastava, [1]Abhishek Pathak, *[3]Geeta J. Gautam and *[1]V. N. Mishra

[1]Department of Neurology, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005 (India)
[2]Chinmaya Mission Hospital, Bengaluru-560038 (India)
[3]Department of Zoology, Mahila Mahavidyalaya, Banaras Hindu University, Varanasi-221005 (India)
Corresponding author: Prof. V.N. Mishra, Geeta J. Gautam

Abstract

The exact cause of Parkinson's disease is not known, but both genetic and environmental factors are thought to play a role in its development. Exposure to heavy metals such as lead, manganese, cadmium, copper, iron, and mercury has been linked to an increased risk of Parkinson's disease (PD). Our study aimed to find out the presence and concentration of lead (Pb), cadmium (Cd), and manganese (Mn) in the whole blood of PD patients and their age-match control group and find out the association of these metals with alpha-synuclein protein via bioinformatic approach.

48 Parkinson's disease patients and 47 healthy control people were screened for this study based on MRI report reading. The metal concentration was estimated in the blood sample after acid digestion and estimation was done via ICP MS technique. A patch dock tool was used for docking metals (Pb, Cd, and Mn) with targeted α-synuclein protein.

Our result suggested that all the three metals were significantly higher in the blood of PD patients than the control group. Docking analysis shows α-synuclein binding of metal-protein interaction in the order Cd>Mn>Pb. This study suggests that all three metals may contribute to the risk of PD. Further study is needed for mechanism of action of these heavy metals.

Key words : Parkinson's disease, Heavy metals, α-Synuclein, docking, ICP MS.

Revolution in the field of medical sciences resulted in an increase in life expectancy, thanks to advancements in healthcare and the medical sciences. But, due to this, we see an increase in the prevalence of various neurological disorders in the elderly. The World Health Organization (WHO) estimates 1-2% of adults over 65 years worldwide are suffering from Parkinson's disease (PD)[22], while in the Indian scenario, North India found a prevalence rate of 67.71/105 among the north Indian population, including Uttar Pradesh, Bihar, Jharkhand and Madhya Pradesh[20].

PD is a neurodegenerative disease with multifactorial etiology and may be an interplay between environmental and genetic factors. Exposure to heavy metals is linked with an increased risk of Parkinson's disease. A study showed exposure to Pb for more than 20 years has a stronger association with PD risk in a health system population-based case-control study (144 cases vs. 464 controls) from the metropolitan Detroit area[4].

Lead (Pb), cadmium (Cd), and manganese (Mn) are common environmental pollutants that may enter the human body via contaminated food and water through ingestion and by inhalation of heavy metals loaded dust particles. Inside the body, they either remain attached to RBC or plasma for some time and then get accumulated inside the bone or cross the blood-brain barrier (BBB) to deposit inside brain tissues before they get eliminated via the liver and excreted out of the body. These are among the neurotoxic metals that interrupt the redox balance and initiate free radical formation while decreasing the level of antioxidant enzymes[2]. This results in stimulating the pathway of oxidative reactions leading to the death of dopaminergic neurons in substantia nigra pars compacta (SNpc) of the basal ganglia of the brain. This results in stimulating the pathway of oxidative reactions leading to the death of dopaminergic neurons in substantia nigra pars compacta (SNpc) of the basal ganglia of the brain.

Mutations in genes like LRRK2, SNCA, ARK2, PARK7, PINK1, and VPS35 are among the major causes of PD pathogenesis. SNCA gene transcribes into α-Synuclein protein which is involved in the regulation of neurotransmitter release. When there is an accumulation of abnormal α-Synuclein protein in certain regions of the brain, it forms clumps called Lewy bodies, a hallmark of PD pathology, and it is believed that they contribute to the death of dopaminergic neurons in the brain, leading to the symptoms of PD.

It has also been hypothesized that metal dyshomeostasis is involved in neurodegeneration through modulation of protein aggregation (e.g α-Synuclein) and fibril synthesis, oxidative stress, neuroinflammation, and excitotoxicity. Exposure to lead can increase alpha-synuclein aggregation in cultured human neurons[19] and a low Pb exposure can increase alpha-synuclein levels in the brains of mice[17]. Similarly, Mn and Cd are also involved in increased alpha-synuclein expression and aggregation in the striatum, a brain region involved in motor control[6].

Some mechanisms are proposed for this accumulation. For example, cadmium exposure may promote alpha-synuclein

aggregation by disrupting the normal function of lysosomes, which is responsible for breaking down and removing unwanted proteins, including alpha-synuclein. When lysosome function is impaired, the clearance of alpha-synuclein may be reduced, leading to its accumulation[18]. Similarly to this, multiple pathways are involved which contribute to the development of Parkinson's disease**.** In the present study, we determined the blood concentration level of Pb, Cd, and Mn in Parkinson's disease patients and healthy control people and tried to investigate their potential mechanism of action in neurodegeneration via the interaction of these metals with alpha-synuclein protein of PD through the bioinformatics approaches.

Neurological examination :

The present study is a hospital-based cross-sectional study conducted on both out and in-patients presenting to the Department of Neurology from December 2017 to October 2020 with the PD. The study was conducted at SSL Hospital, Institute of Medical Sciences, a tertiary care centre affiliated with Banaras Hindu University, Varanasi in North India. Patient interviews and records were done for the collection of data by using questionnaires on their demographic details.

(a) Inclusion criteria :

Patients admitted to the Neurology and stroke ward of SSL Hospital, BHU with Parkinson's disease as per United Kingdom's Brain Bank criteria and atypical Parkinson's disease (Multiple system atrophy as per consensus criteria for the diagnosis of multiple system atrophy, adapted[3]. Progressive Supranuclear Palsy as per **Movement Disease Society criteria 2017) (APD)**[8] as cases and the relatives or helper of those patients were not having any such neurological disease as controls.

(b) Exclusion criteria :

Those denied written consent for the study.

Clinical diagnosis :

Pre-determined detailed case record forms were filled. All participants were subjected to full medical history and examination. Complete general physical examination and neurological examination were performed. Patients with PD were classified as per standard diagnostic criteria and detailed testing for Hoehn and Yahr scale (H and Y-scale), a commonly used system for describing how the symptoms of PD progress which includes stages from 1 through 5[7] and later modified with the addition of stages 1.5 and 2.5 help describe the intermediate course of the disease PD patients. Stages 4 and 5 are referred to as "severe impairment," Stage 3 as "moderate impairment," and others were labeled as having "mild impairment."

Ethical clearance for the present study was obtained from the Institutional Ethical Committee. Informed written consent was obtained from each participant using his or her native language.

Statistical analysis was performed with the help of GraphPad Prism 5.

Heavy metal estimation in blood :

2 ml blood from each individual was

drawn out from the superficial vein of the upper limb with a sterilized syringe in an EDTA vial, and kept at -20 °C for further analysis. Before performing the lab analysis, the blood sample was kept for 10 min. at 4°C and finally brought to room temperature.

Approximately 1 ml of blood was digested with 7 mL of HNO_3 (65 % v/v) in acid-prewashed PTFE vessels. Digestion was carried out using a closed-vessel microwave digestion system at 100 °C for 30 min. in a microwave digester system. After cooling down to room temperature, the digested samples were quantitatively transferred into pre-cleaned 25-mL volumetric flasks, dilution was done using deionized water, and stored at room temperature. Metal Analysis in whole blood was done by ICP MS technique. Perkin Elmer Optima 7000DV ICP MS was used for detection.

Retrieval of targeted proteins and selected heavy metal structures :

Tertiary structure of targeted proteins: SNCA was retrieved from PDB Database (https://www.rcsb.org/) and the structure of selected heavy/essential metals: Pb, Cd and Mn, were retrieved from Metal PDB database (https://metalpdb.cerm.unifi.it/). Further the analysis of these structures has been performed on Discovery Studio.

Molecular docking of SNCA protein with selected heavy metals :

Docking of ligands molecules with various selected targets was carried out by Patch Dock Server[16] (default parameter RMSD esteem 4.0 and complex type protein-small ligand) further visualization of the docked complexes through Discovery Studio 3.5**.** Docking investigation was based on the geometric shape complementarity score (GSC score), which was determined in PatchDock Server. The findings of results are based on the docking score and the interaction at the binding sites (catalytic pockets). The higher scores represent more binding affinity, and hence more steady is the complex. The docking assessment was done using Discovery Studio 3.0 to find out the contacting receptor residues involved in interaction with ligands.

Table-1. Characteristics and clinical data of PD patients and control

	PD patients	Control
Subjects (n)	62	52
Female (n)	15 (24.19%)	14 (26.92%)
Male (n)	47 (75.8%)	38 (73.07%)
Age (yrs)	54.60 ± 13.73	62.04±7.53
Duration of PD (years)	3.2±1.03	
Age of onset of PDYoung onset Late onset	 19 43	
Hoehn &Yahr score	2.10±1.03	

The summary of patients details and respective control is given in Table no.1, where we observed more percentage of male participants than female. The average PD patients were younger than the control group due to the young onset of PD in the patients.

In Fig. 1(A) the graph shows elevated whole blood Lead concentration in the control group as compared to the PD group. When an unpaired T-test is applied at a 95% confidence

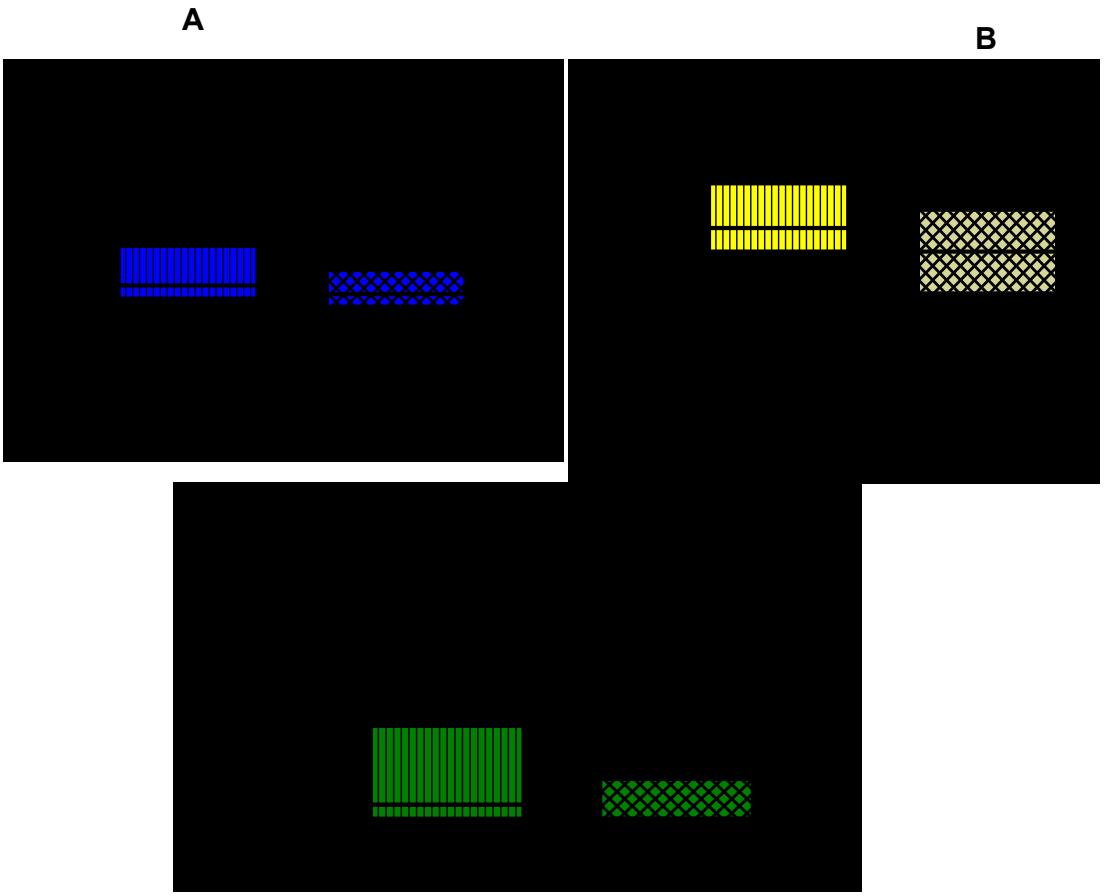

Fig. 1. Shows a comparison between whole blood concentrations of (A) lead, (B) cadmium, and (C) manganese in Parkinson's disease (PD) and the Control group.

interval, a significant difference with a p-value of 0.0356 was obtained between both groups. This indicates that Pb concentration in PD patients was significantly higher than in the control group. There are some other studies revealing contrasting results as less Pb concentration in the blood serum of PD patients than in the control group[5].

However, Pb is a neurotoxic metal, studies suggested a 2-3 times increase in PD risk as a consequence of Pb exposure as it is involved in dopaminergic neuron damage and in causing oxidative stress and neuroinflammation[14]. We also noticed an increase in Pb concentration in the blood of PD patients than the control group. Although, blood testing can provide results for relatively recent exposure of 4-6 weeks, PD patients have weaker bones over the time, so Pb stored in the bones comes out and increase their concentration in the blood, which is the reason for increased Pb concentration in the blood of PD patients. In Vitro studies demonstrate that Pb mimics

calcium via the calcium signaling channel and enters the brain[1].

Fig. 1. (B) gives a comparative graphical representation of Mn in PD patients and a healthy control group. At a 95% confidence interval, a significantly higher mean Mn concentration was obtained in the whole blood of PD patients than the control group with p value of 0.05. The result is significant in PD patients and shows that Mn concentration in blood is associated with PD. Although Mn plays an important role as a cofactor in various enzymes like glutamine synthetase, serine/ threonine-protein phosphatase-I, and pyruvate decarboxylase that are intended for neurotransmitters production and glial cell functioning its excess level is reported to be neurotoxic. In our result, Mn concentration ranges from 1.21 to 4.52 µg/l in PD patients whereas 1.4 µg/l to 3.7 µg/l in the control group. Excess Mn concentration in the blood of PD patients could also be due to a liver dsfunction which interferes with Mn excretion. Our result is supported by a study in where due to Mn toxicity, patients were detected with early psychiatric symptoms known as 'locura manganica' or 'manganese madness' and later succeeded with motor symptoms like rigidity, bradykinesia with little resting tremor, masked face, postural instability, very similar to Parkinson's disease[10]. In those patients Mn was found to be about >28 µg/l . In our study, we found much lower concentration indicating that Mn has not reached to that threshold to cause manganism but accumulation is taking place in the body higher than the control people.

In fig. 1(C), the graph shows higher whole blood mean cadmium concentration in PD patients as compared to a control group. When an unpaired T-test at a 95% confidence interval is applied, a significant difference with a p-value of 0.0117 was obtained between both groups. This indicates that whole blood Cd concentration in the control group was significantly higher in PD patients than in the control group. While a case study shows acute Cd poisoning leads to parkinsonian symptoms and higher cadmium concentration was observed in PD patients than in the control group[12,13]. Although chronic Cd is measured in urine, blood is useful for measuring recent intoxication[11,15]. Cd mainly disrupts the calcium homeostasis in neurons, which can result in abnormal intracellular calcium signaling and contribute to neuronal injury and cell death[21]. Studies also suggest that higher Cd concentration can be associated with the etiology of PD[10].

Table-2. Interaction of selected heavy/essential metals with targeted protein α-synuclein with their binding scores, pocket, volume and interacting residues

Protien-Metal	Binding Interaction	Interacting Score	Pocket Residues	Volume
α-synuclien-Pb	3283	Q270,Y273	P0	1015.66
α-synuclien-Cd	5451	E272,E279	P0, P1	1217.95
α-synuclien-Mn	4108	D288,E292	P0	1116.89

For bioinformatics study of the interaction between the target protein and selected metals, Patchdock server was used for docking calculation. Before performing docking analysis active site prediction was done using Discovery Studio 3.5. In PatchDock docking calculation, Pb, Cd, and Mn interacted with the prominent sites of α-synuclein protein, identified using discovery studio3.5 with docking scores: 3283, 5451, and 4108 respectively (Table-2; Fig. 2). The protein interacted residues were involved in interaction with selected metals, which belongs to the major active site of the α-synuclein protein. Further pocket identification analysis has shown that Pb, Cd, and Mn, were interacted with α-synuclein on catalytic pockets like P0 and P1 with the volume capacities 1015.66, 1217.95, and 1116.89 respectively (Table-2; Fig. 2).

Table-2 shows the binding score of Pb, Cd, and Mn with α-synuclein protein. The binding score is in the order Cd>Mn>Pb. This suggests that the least energy is involved in the interaction between Cd and α-synuclein protein followed by Mn and Pb.

A higher binding score of Cd with α-synuclein protein indicates that Cd can easily bind to alpha-synuclein protein and promote its aggregation, leading to the formation of toxic oligomers and fibrils[9]. Cd-induced aggregation of alpha-synuclein has been observed both in vitro and in vivo, and it has been suggested that this may be one of the mechanisms by which Cd contributes to the pathogenesis of PD**.** Higher Cd concentration in the blood of PD patients in our study also justifies the above finding.

The second-highest binding score of Mn (4108) with α-synuclein protein suggested strong interaction between both of them. Mn has been shown to be involved in the formation of aggregation in the alpha-synuclein protein, which may aid in the onset of Parkinson's disease[24]. Furthermore, research has revealed that mutation in the alpha-synuclein gene can make people more vulnerable to manganese toxicity. Thus from our study, the higher Mn concentration in the blood of PD patients than the control group supports the interaction between Mn and alpha-synuclein protein and thus aggregation of this protein in the brain of PD patients.

Pb accumulation in the body of PD patients and binding score 3283 with α-synuclein protein is supported by in vitro studies which showed that lead can bind to alpha-synuclein and promote the formation of twisted fibrils, which are thought to be a pathological feature of PD[25]. The study of in vivo and in vitro models has suggested that Pb exposure led to the aggregation of alpha-synuclein and increased the formation of Lewy bodies, a hallmark of Parkinson's. Although the binding score of Pb is least as compared to the other two metals, but Pb is somehow involved in alpha-synuclein aggregation in certain pathway. Synphilin-1 is a protein that has been shown to interact with alpha-synuclein. Pb can promote the inclusion formation of alpha-synuclein through its interaction with synphilin-1[23]. These studies suggest that lead exposure may contribute to the accumulation and aggregation of alpha-synuclein in the brain, which may play a role in the development of Parkinson's disease.

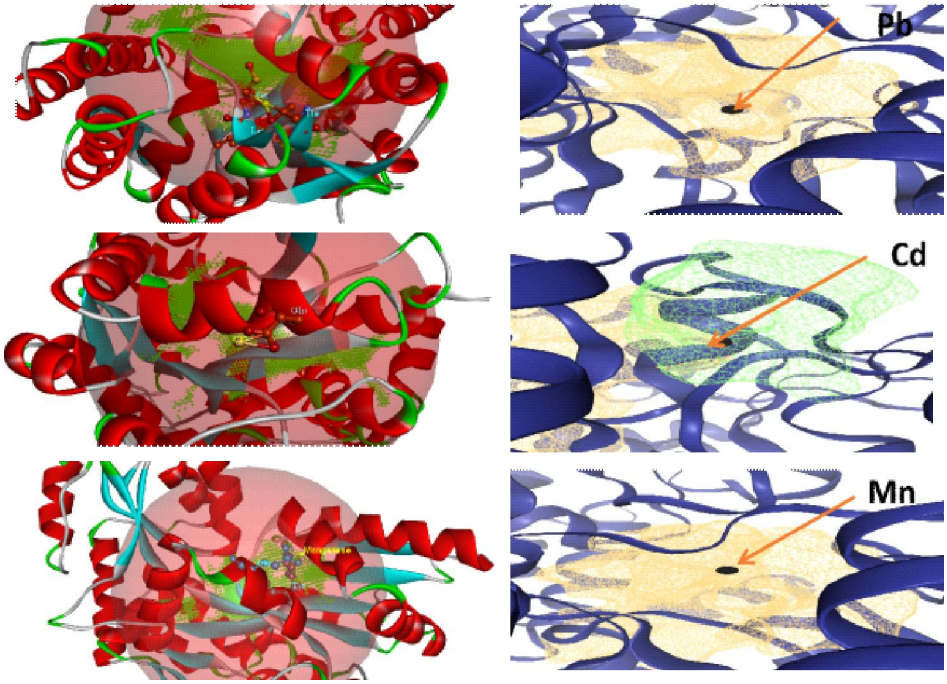

Fig. 2. Interaction of selected heavy metals (Pb, Cd, and Mn) with targeted protein SNCA.

It can be concluded from this study that Pb is vibrantly present in the environment and healthy individuals are vigorously exposed to this toxic metal. Significantly higher levels of Cd and Mn in the blood of PD patients as well as insilico interaction of the α-synuclein protein with Cd, Mn and Pb shows that all the metals are strongly associated with the risk of developing PD. However, it is important to note that humans are a complex system and numerous factors are involved for disease predisposition, heavy metal exposure may be one of the factorial causes of Parkinson's disease, along with other genetic predisposition and environmental toxins may also play a role.

We oblige our thanks to the Interdisciplinary School of Life Sciences-BHU (ISLS) for providing the instrumental facility. We thank all the patients and people who donated their blood samples for this study.

Conflict of interest None

Ethical statement The above clinical study was assessed and approved by the BHU ethical committee (ECR/Bhu/Inst/UP/2014/ Re-registration-2017 dt.31.01.2017)

References :

1. Bressler, J. P., and G. W. Goldstein (1991). *Biochem. Pharmacol., 41*(4): 479-484. https://doi.org/10.1016/0006-2952(91)90617-E
2. Chen P, MR Miah and M. Aschner (2016).

F1000Research, *5*.

3. Gilman, S., G. K. Wenning, P. A. Low, D. J. Brooks, C.J. Mathias, J.Q. Trojanowski, N. W. Wood, C. Colosimo, A. Dürr, C. J. Fowler, H. Kaufmann, T. Klockgether, A. Lees, W. Poewe, N. Quinn, T. Revesz, D. Robertson, P. Sandroni, K. Seppi, and M. Vidailhet, (2008). *Neurology. 71*(9): 670-676.
4. Gorell, J.M., E.L. Peterson, B. A. Rybicki, and C. C. Johnson, (2004). *J. Neurol. Sci., 217*: 169-174. https://doi.org/10.1016/j.jns.2003.09.014.
5. Gupta, V., M. K. Singh, R. K. Garg, K.K. Pant, S. Khattri, (2014) *Int. J. Neurosci., 124*(2): 88–92. https://doi.org/10.3109/00207454.2013.824438.
6. Harischandra, D.S., S. Ghaisas, G. Zenitsky, H. Jin, A. Kanthasamy, V. Anantharam, and A. G. Kanthasamy, (2019). *Front. Neurosci., 13:* 654. https://doi.org/10.3389/fnins.2019.00654
7. Hoehn, Margaret M., and Melvin D. Yahr. (1998). *Neurology, 50*(2): 318-318.
8. Hoglinger, GU., G. Respondek, M. Stamelou, C. Kurz, K. A. Josephs, A. E. Lang, B. Mollenhauer, U. Muller, C. Nilsson, J. L. Whitwell, T. Arzberger, E. Englund, E. Gelpi, A. Giese, D.J. Irwin, W.G. Meissner, A. Pantelyat, A. Rajput, J. C. Swieten, M. D., Troakes, C., Antonini, A., Bhatia, K. P., Bordelon, Y., Compta, Y., Corvol, J.-C., Colosimo, C., Dickson, D. W., Dodel, R., Ferguson, L., Grossman, M., Kassubek, J., Krismer, F., Levin, J., Lorenzl, S., Morris, H. R., Nestor, P., Oertel, W. H., Poewe, W., Rabinovici, G. M. D., Rowe, J. B., Schellenberg, G. D., Seppi, K., Eimeren, T., Wenning, G. K., Boxer, A.L., Golbe, L. I., and Litvan, I. (2017). *Movement Disorders, 32*(6): 853-864. https://doi.org/10.1002/mds.26987
9. Huerta, M. (2015). Investigating the Synergistic Effects of Chlorpyrifos and Cadmium Neurotoxicity in Alpha synuclein Overexpressing Dopaminergic Cell Model of Parkinson's Disease.
10. Mergler, D., M. Baldwin, S. Belanger, F. Larribe, A. Beuter, R. Bowler, M. Panisset, R. Edwards, A. Geoffroy, M. P. Sassine, and K. Hudnell, (1999). *Neurotoxicology, 20*(2-3): 327-342.
11. Nordberg, G.F., A. Bernard, G.L. Diamond, J.H. Duffus, P. Illing, M. Nordberg, I.A. Bergdahl, T. Jin, S. Skerfving, (2018). *Pure Appl. Chem., 90*(4): 755–808.
12. Okuda, B., I. Yasumichi, T. Hisao and S. Minoru (1997). *Clinical neurology and neurosurgery*, 263-265.
13. Parsian, H., P. Alipour, H. Gholinia, and P. Saadat (2021). *Archives of Neuroscience. 8*(3): 31.
14. Pyatha, S., H. Kim, D. Lee, and K. Kim, (2022). *Antioxidants*, *11*(12): 2467.
15. Satarug, S. (2018). *Toxics. 6*(1): 15. https://doi.org/10.3390/toxics6010015
16. Schneidman-Duhovny, D., Y. Inbar, R. Nussinov and H. J. Wolfson (2005). *Nucleic acids research*, *33* (Web Server issue), W363–W367. https://doi.org/10.1093/nar/gki481
17. Sulzer, D., C. Cassidy, G. Horga, U. J. Kang, S. Fahn, L. Casella, G. Pezzolim, J. Langley, X. P. Hu, F. A. Zucca, I. U. Isaias, and L. Zecca, (2018). *NPJ Parkinsons Dis., 10*(4): 11-23. http://

creativecommons.org/licenses/by/4.0/

18. Teixeira, M., R. Sheta, W. Idi, and A. Oueslati (2021). *Biomolecules, 11*(9): 1333. https://doi.org/10.3390/biom110913339.
19. Ullah, I., L. Zhao, Y. Hai, M. Fahim, D. Alwayli, X. Wang, and H. Li, (2021). *Toxicology Reports, 8:* 607-616.
20. Verma, A.K., J. Raj., V. Sharma, T.B. Singh, S. Srivastava, and R. Srivastava (2016). *Clinical epidemiology and global health, 5*(1): 8-13.
21. Wang, B., and Y. Du, (2013). *Review Oxid Med Cell Longev.* 898034. https://doi.org/10.1155/2013/898034
22. World Health Organisation (WHO). (2016). Neurological Disorders: Public Health Challenges.
23. Xie, Y.-Y., C.-J. Zhou, Z.-R. Zhou, J. Hong, M.-X. Che, Q.-S. Fu, A.-X. Song, D.-H. Lin, and H.-Y. Hu, (2010). *FASEB J., 24*(1): 954-966. https://doi.org/10.1096/fj.09-133082
24. Xu, B., S. Huang, Y. Liu, C. Wan, Y. Gu, D. Wang, and H. Yu, (2022). *JBC. 298*(1): 101469. https://doi.org/10.1016/j.jbc.2021.101469
25. Zuo, P.-J., A. Rabie and M. Bakr (2009). *Journal of Biomedical Science and Engineering, 2.2*: 86-89.