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Antioxidative and Anxiolytic Impact of Riboceine, a Dietary Supplement on Cytochrome Oxidase Inhibition in the Hippocampus

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ABSTRACT

Selectively reduced complex IV activity which causes inhibition of the mitochondrial respiratory chain produces free radicals, diminishes aerobic energy metabolism and causes excitotoxic damage to neurons, leading to neurodegenerative diseases. This study evaluated the putative therapeutic role of riboceine (*Ribcys*) against sodium azide induced Cytochrome oxidase inhibition in the hippocampus. A total number of thirty five rats were used. They were randomly distributed into 5 groups (A, B, C, D and E) with seven animals each (n = 7). Cytochrome oxidase inhibition was induced by treatment with 17mg/kg of NaN_3. Group A received only distilled water. Group B were treated with 17mg/kg of NaN_3 for 28 days. Group C were treated with 17mg/kg body weight of NaN_3 (28 days) followed by 100mg/kg body weight of Riboceine for 21 days. Group D were pre-treated with 100mg/kg of Riboceine for 21 days followed by 17mg/kg of NaN_3 for 28 days. Group E were treated with 100mg/kg of Riboceine for 21 days. Thereafter, elevated plus maze was used to determine the level of anxiety in the animals. The animals were sacrificed and histological demonstration of the hippocampus and colorimetric determination of oxidative stress were performed. NaN_3 induced cytochrome oxidase inhibition showed increased lipid peroxidation ($p<0.05$), elicited anxiety level ($p<0.05$) and increased chromatolysis in the hippocampus of the experimental animals. *Ribcys* treatment increased the concentrations of catalase (CAT), glutathione transferase (GSH) and superoxide dismutase (SOD) with $p<0.05$, reduced anxiety levels ($p<0.05$) and increased Nissl staining intensity of the pyramidal and granular layers of the hippocampus. The cytochrome oxidase inhibition induced by NaN_3 treatment imposed oxidative and chromatolytic damage to the hippocampus whereas *Ribcys* was able to modulate the activity of cytochrome oxidase in the hippocampus through its anxiolytic and antioxidant properties.

Keywords: Cytochrome oxidase inhibition, Riboceine, Hippocampus, anxiolytic, antioxidant.

INTRODUCTION

The use of dietary supplement is fast becoming common especially when nutritional needs are not being covered by daily dietary intake. It has been associated with providing essential nutrients for the overall health, reducing the risk of disease as well as managing some health related conditions[1]. Though dietary supplements may include vitamins, minerals, enzymes, herbs, amino acid, they are not drugs. Riboceine, which is one of the numerous dietary supplements also known as a glutathione supplement consist of D-ribose-L-cysteine. This supplement primarily helps to increase intracellular gluthathione production in the body. Riboceine has been reported to possess antioxidative property invitro and inhibit activities of some selected rat liver cytochrome enzymes[2], ameliorate amodaiquine-induced reproductive dysfunction in rats[3], regenerate intervertebral disc degeneration[4] as well as restore testicular damage[5,6]. Reduced level of intracellular glutathione has been implicated in most neurodegenerative diseases, hence the need to trigger its production by the use of dietary supplements such as Riboceine to prevent or retard the progression of memory loss[7].

Cytochrome oxidase inhibition as seem in Alzheimer's disease could contribute to neuronal dysfunction and cognitive impairment. The inhibition of this enzyme may be evoked by exposure to neurotoxins, such as sodium azide (NaN_3)[8,9]. Sodium azide inhibits cytochrome oxidase by binding to the heme cofactor irreversibly and selectively reduces the complex IV activity[10] which eventually activates free radicals production and decreased metabolic aerobic energy, resulting to excitotoxic damage, creating a deleterious spiral causing neurodegeneration[11]. The release of excitotoxins via mitochondria impairment may be induced by NaN_3 administration, and this in turn result in neuronal cell loss[9]. Increased oxidative stress levels, behavioural impairment as well as neuronal structural distortions as seen in several neurodegenerative diseases are also linked with NaN_3 induction[12-14]. As an acutely

neurotoxic substance[15,16], research has been carried out to elucidate sodium azide (NaN_3) as a neuronal mitochondrial toxin[17,18].

Bearing in mind that man is continually exposed to neurotoxins, and the need for therapeutic agents is critical to provide solution to problems associated with exposure to neurotoxins, this study is aimed at investigating the impact of Riboceine on cytochrome oxidase inhibition in the hippocampus of adult wistar rats.

MATERIALS AND METHODS

Animal procurement, handling and care: Animals were nurtured in the animal facility of Osun state University, Osogbo. The animals were caged in wired plastic cages and kept in a clean environment. Acclimatization for 14 days prior commencement of experiment was done under standard laboratory conditions. The rats were fed with rat chow with drinking water *ad libitum. A*ll the experimental procedures conformed with the guidelines of UNIOSUN Health Research Ethical Committee for studies involving experimental animals.

Animal grouping and treatment: A total number of thirty five rats were used. They were randomly distributed into 5 groups (A, B, C, D and E) with seven animals each (n = 7). The groups were treated as follows:

1. Group A received only distilled water.
2. Group B were treated with 17mg/kg of NaN_3 for 28 days.
3. Group C were treated with 17mg/kg body weight of NaN_3 (28 days) followed by 100mg/kg body weight of Riboceine for 21 days.
4. Group D were pre-treated with 100mg/kg of Riboceine for 21 days followed by NaN_3 for 28 days.
5. Group E were treated with Riboceine for 21 days.

Riboceine treatment was done orally using an oral gavage while sodium azide was administered via subcutaneous injection. Treatment was done daily at 0800 hours.

Elevated plus Maze Test: The anxiety responses of rodents were assessed by this protocol. Each animal was place at the centre of the apparatus and its exploratory activity for 5 mins was observed and recorded. The duration each animal spent in the close arm was determined.

Animal Sacrifice and Histology: After completion of treatments and neurobehavioral testing, blood was taken from the ocular sinus of the rats using capillary tube. Thereafter, the animals were euthanized with 20mg/kg ketamine administered intramuscularly and transfused with 50mL of 0.1M of PBS. Some of the brains were quickly dissected out, placed on pre-chilled metal for isolation of the hippocampus. The isolated hippocampus was post-fixed in 0.1 M of PBS and immediately processed for oxidative stress enzymes analysis whereas the remaining excised brain specimen were fixed in 4% paraformaldehyde and processed for histological analysis. The Nissl substance was demonstrated using Cresyl fast violet stain.

Colorimetrical analysis: The hippocampal region fixed in PBS were homogenized in 19 volumes of 0.1 M phosphate buffer (pH 7.4) using a homogenizer, centrifuged at 3000rpm for 15 min and the supernatant fractions were obtained for various biochemical parameters described below.

Estimation of catalase activity: Catalase activity was determined by the method of Sinha[19] (known as the dichromate method). 0.2ml of enzyme was added to the assay mixture containing 0.5 ml of H_2O_2 solution, l.0ml of phosphate buffer and 0.4ml water. Thereafter, 0.2 ml dichromate/acetic acid was added after 0,30,60,90 seconds of incubation. Absorbance was read at.610nm after heating for 10 mins.

Estimation of lipid peroxidation: The amount of malonyldialdehyde (MDA) was measured spectrophotometrically by the method described by Ohkawa *et al.,*[20]. 0.1 ml of sample was treated with 2ml of TBA-TCA-HCL i.e. 1:1:1 reagent (TBA 0.37%, 0.25M HCL and 15% TCA) and incubated in a water bath at 95^0C for 15 minutes. The tubes was then placed on ice and centrifuged at 3000 rpm for 10 minutes and the absorbance's of the clear supernatant was measured spectrophotometrically at 552 nm.

Glutathione (GSH) Estimation: The glutathione (GSH) was estimated following the procedure described by Beutler *et al.,*[21]. The sulfhydryl groups present in the glutathione forms a colour complex with DTNB {5, 5^1 dithiobis – (2- nitrobenzoic acid)}, which was measured colorimetrically at 412 nm.

Estimation of Superoxide Dismutase: The determination of SOD activities was according to the well-known spectrophotometrically assay introduced by McCord and Fredovich[22]. 75mM of tris-HCl buffer with the pH of 8.2, 30mM EDTA and 2mM of Pyrogallol was added to 50microliter of the supernatant. Absorbance was read spectrophotometrically at 420nm for 3min.

Statistical Analysis: Quantitative outcomes of neurobehavioral and biochemical examinations were analyzed using GraphPad Prism® (version 8) software. The outcomes were plotted in ANOVA followed with Turkey's multiple comparisons test. Significance was set at $p<0.05$*.

RESULTS

The effects of Riboceine before and after cytochrome oxidase inhibition was investigated on the hippocampus of adult male Wistar rats; by analysis of neurobehavioral

paradigms, hippocampi levels of CAT, SOD, MDA, GSH, cytochrome c oxidase; and structural modifications of the hippocampus using Cresyl fast violet stain.

Elevated Plus Maze: In figure 1, group B (110.4 ± 6.501) spent significant time in the closed arm when compared with group A (53.00 ± 2.449; $p<0.05$). *Ribcys* treatment significantly reduced the time spent in the closed arm of the ($p<0.05$) compared with the NaN_3 treated animals. However, *Ribcys* pretreatment resulted in performance responses that were almost indistinguishable from those of control rats.

Oxidative Stress Markers: From the result it was found that the NaN_3 (group B) treated rats showed significant decrease in activities of catalase, superoxide dismutase and glutathione in the hippocampus when compared with the control groups (group A) respectively.

In figure 2, animals pre-treated with *Ribcys* (group D) (8.730 ± 0.215) showed significant increase in hippocampi SOD activity when compared to group B (4.426 ± 0.2106); whereas, significant decrease in SOD activity was observed in group B (4.483 ± 0.4324) when compared to group C (7.35 ± 0.8606). However, in group D (8.142 ± 0.8465) and E (9.512 ± 0.6760), there was no significant difference in the level of SOD

Figure 3 showed significant decrease ($p<0.05$) in hippocampal catalase activity in the NaN_3 treated animals (group B) (4.483 ± 0.4324) when compared to group A. However, statistically significant increase at $p<0.05$ was observed in the Riboceine treated groups (C, D & E) when compared to group B.

Results obtained from differential expression of glutathione transferase (Fig. 4) showed significant increase ($p< 0.05$) in activities of glutathione (GSH) jn *Ribcys* treated animals (C,D and E) when compared with NaN^3 treated animals (group B) with a mean value of 18.60 ± 0.5099.

In figure 5, the MDA activity in group B (6.834 ± 0.1276) increased significantly ($p<0.05$) when compared to group A (2.186 ± 0.3815). However, significant reduction in MDA activity was observed in Riboceine treated groups (C; 3.992 ± 0.8697; D; 3.538 ± 0.1829 and E; 2.390 ± 0.3493) when compared to group B ($p<0.05$).

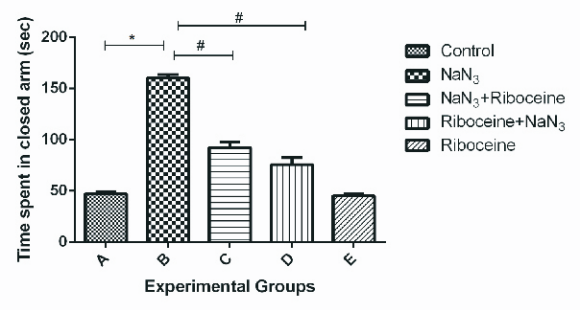

Figure 1: showing the effect of treatments on emotionality across the experimental animals using elevated plus maze test. The results are expressed as Mean ± SEM per treatment and respective control groups. Levels of significance values are *$p<0.05$, compared with control group; and #$p<0.05$, compared with group B (NaN_3 only). $p<0.05$ was considered to be statistically significant.

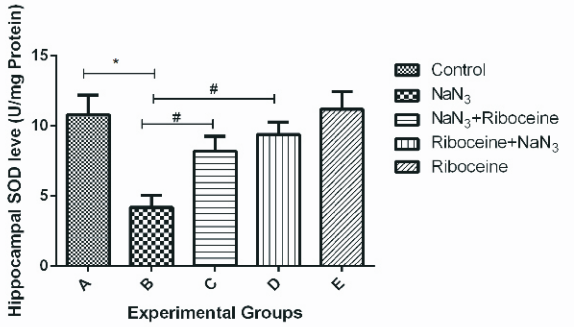

Figure 2: showing the estimation of Superoxide dismutase. The results are expressed as Mean ± SEM. Levels of significance values are *$p<0.05$, compared with control group; and #$p<0.05$, compared with group B (NaN_3 only). $p<0.05$ was considered to be statistically significant.

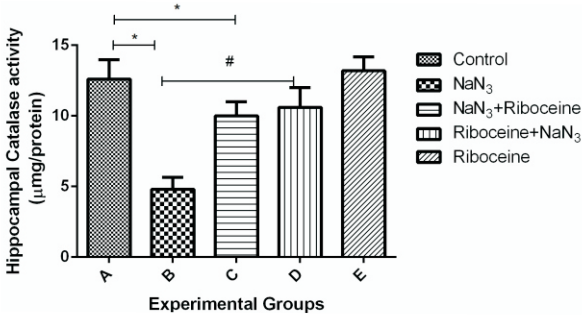

Figure 3: showing the estimation of Catalase. The results are expressed as Mean ± SEM. Levels of significance values are *$p<0.05$, compared with control group; and #$p<0.05$, compared with group B (NaN_3 only). $p<0.05$ was considered to be statistically significant.

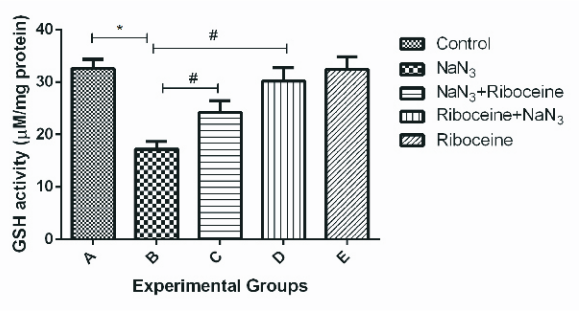

Figure 4: showing the estimation of Glutathione transferase. The results are expressed as Mean ± SEM. Levels of significance values are *$p<0.05$, compared with control group; and #$p<0.05$, compared with group B (NaN_3 only). $p<0.05$ was considered to be statistically significant.

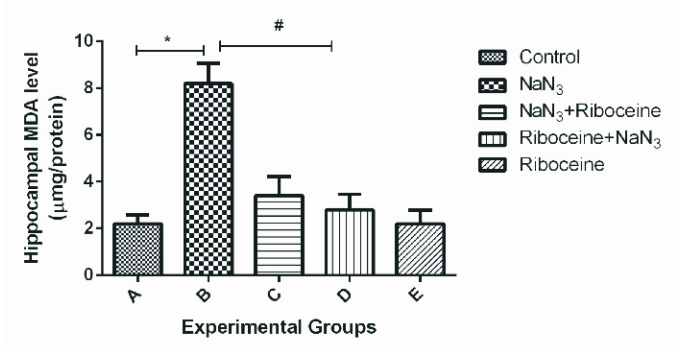

Figure 5: showing the estimation of MDA levels in the hippocampus. The results are expressed as Mean ± SEM. Levels of significance values are *p<0.05, compared with control group; and #p<0.05, compared with group B (NaN_3 only). p<0.05 was considered to be statistically significant.

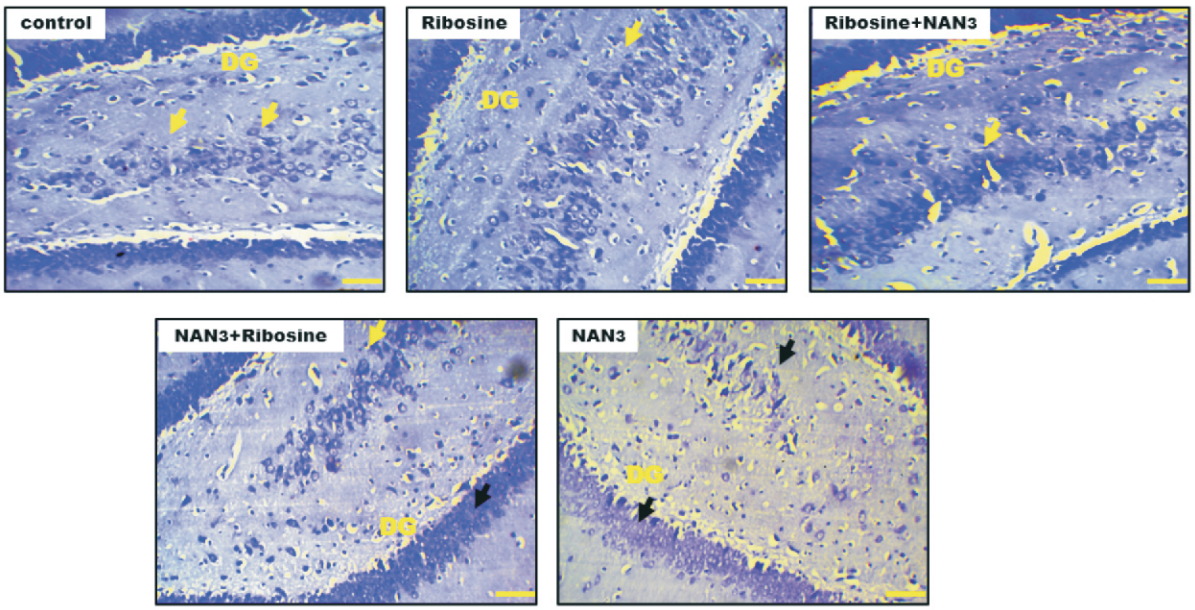

Figure 6: Photomicrographs showing panoramic views of hippocampus general morphological presentations in Wistar rats across the various study groups. Cresyl fast violet stain (*Scale bars:* 50µm). The Dentate gyrus (DG) composed of granule cells are well demonstrated and more conspicuous across the study groups.

Demonstration of Nissl intensity: Nissl profile demonstration by CFV stain across hippocampal sections within the study groups shows normal morphological presentations in control and Riboceine treated groups characterized with well stained and densely populated Nissl proteins in the pyramidal and granular layers. However, hippocampus of group B revealed reduced staining intensity of the Nissl substance in the pyramidal and granule cell layers.

DISCUSSION

The toxicity of sodium azide has been studied extensively and has been reviewed in details. Sodium azide produces toxicity by inhibiting cellular metabolism. It is known to inhibit cytochrome oxidase and may interfere with other enzymes. The consequence of this metabolic inhibition is cytotoxic anoxia; in which cellular damage occur despite the presence of adequate oxygen. From this study it was revealed that Riboceine exhibited not only an antioxidative but also an anxiolytic property against the impact of the inhibition of cytochrome oxidase enzyme induced by sodium azide in the hippocampus.

The ventral hippocampus has been implicated in anxiety-like behaviour which may possibly be due to the synchronisation between the ventral hippocampus and the medial prefrontal cortex in anxiety[23]. However, ventral hippocampus lesions have been shown to elicit anxiolytic effects which may not be directly linked with the amygdala[24]. Moreover, in our previous study[13] it was confirmed that sodium azide neurotoxicity produced an anxiogenic effect; with NaN_3 treated animals spending more time in the closed arm of the elevated plus maze as observed in this present investigation. Although the mechanism by which sodium azide induces anxiety is

not yet known, it is proposed that sodium azide produces hyper excitability of not only the amygdale but also the ventral hippocampus, hence increasing anxiety-related behavior. Supplementation with Riboceine was observed to reduce anxiety level in animals treated with NaN_3; this reveals that Riboceine have the ability to target some chemical messengers in the brain which eventually reduces brain excitability. Hence, the anxiogenic effect of Riboceine following cytochrome oxidase inhibition is hereby established.

Numerous studies have shown that NaN_3 neurotoxicity is implicated with increased production of reactive oxidative species as a result of significantly reduced levels of GSH, SOD and CAT and increased MDA[14,25] through the inhibition of the mitochondrial cytochrome oxidase[10,26]. In this study it was further confirmed that NaN_3 neurotoxicity triggered reduction in the levels of SOD, GSH and CAT as well as a reversed increased concentration of MDA which resulted to increased lipid peroxidation[27] and eventually led to elevated production of free radicals in the hippocampus. Damage to cells by these free radicals is evidenced in various pathological conditions including Alzheimer's disease, Parkinson's disease, cancer etc[28,29]. Therefore, exposure to sodium azide could possibly lead to pathological changes observed in neurodegenerative conditions such as Alzheimer's and Parkinson's disease[10]. However, Riboceine, a food supplement rich in antioxidant enzymes reduced the risk of increased free radical production in the body, specifically the hippocampus as observed in our study[3,30]. The antioxidative property of Riboceine provides the cells with a comprehensive defence mechanism against reactive oxygen species induced damage triggered by cytochrome oxidase inhibition. These mechanisms amongst others includes increased production of antioxidant enzymes (SOD, CAT and GSH) which eventually stabilize or deactivate free radicals before evoking cellular injury[31] as observed in pre/post-treatment with sodium azide in our study.

In this study, cytochrome oxidase inhibition was revealed to cause severe chromatolytic as well as some pyknotic changes in both the pyramidal and granule cell layers with a gross reduction in the cytoplasm Nissl proteins. Chromatolysis is caused by axonal injury and is associated with degranulation, degradation and dissagregation of ribosomes involved in protein synthesis[32]. However, reasonable regeneration of Nissl substance was evident in pyramidal and granular cell layers in animals post treated with riboceine while maintenance of the cytoarchitectural integrity of the hippocampus in animals pre-treated with Riboceine was observed. Hence, Riboceine was ameliorative and protective against Nissl substance damagen the hippocampus. 1

CONCLUSION

In this current study, NaN_3 induced cytochrome oxidase inhibition is anxiogenic and capable to exert neuronal injury on the morphology of the hippocampus as well as increase the oxidative stress level as a result of increase in free radical production. However, Riboceine served as both an anxiolytic and antioxidative agent, being able to protect and improve the integrity of the neurons in the brain against cytochrome oxidase inhibition induced damage and prevent cellular damage.

CONFLICT OF INTEREST

The authors declare no actual or potential conflict of interest

REFERENCES

1. Food and Drug Administration (FDA), Dietary Supplements: What You Need to Know, Rockville, MD, USA, Food and Drug Administration, 2017
2. N'guessan BB, Amponsah SK, Dugbartey GJ, Awuah KD, Dotse E, Aning A, Kukuia KK, Asiedu-Gyekye IJ, Appiah-Opong R. In Vitro Antioxidant Potential and Effect of a Glutathione-Enhancer Dietary Supplement on Selected Rat Liver Cytochrome P450 Enzyme Activity. Evidence-Based Complementary and Alternative Medicine. 2018.
3. Mobolaji-Jobi, S. O., Okoko, I. I. E., Medubi, L. J., & Okanlawon, A. O. Ameliorative Potential of Riboceine, Vitamin D and Calcium on Amodaiquine-Induced Reproductive Dysfunctions in Female Sprague-Dawley Rats. *The FASEB Journal*, 2020;*34*(S1), 1-1.
4. Ogunlade B, Osinubi AA, Duru FI, Adelakun SA, Ogunlade SA, Alao AA. histomorphological response of sulforaphane and riboceine on annular puncture-induced model of rabbits intervertebral disc degeneration. surgery. 2017;32:33.
5. Falana, B., Adeleke, O., Orenolu, M., Osinubi, A., & Oyewopo, A. Effect of D-ribose-L-cysteine on aluminum induced testicular damage in male Sprague-Dawley rats. *JBRA Assisted Reproduction*, 2017;*21*(2), 94.
6. Joseph, D. B., Samuel, O. O., Abiola, F. B., Ogueri, D. F. I., & AA, O. A. D-Riboce-L-Cystein Maintained Testicular Integrity in Rats Model (Rattus Novergicus) Exposed to X-Ray. *Cell Biology*, 2017;*5*(4), 38.
7. Emokpae O, Ben-Azu B, Ajayi AM, Umukoro S. D-ribose-L-cysteine enhances memory task, attenuates oxidative stress and acetyl-cholinesterase activity in scopolamine amnesic mice. Drug Development Research. 2020 Mar 27.
8. Szabados, T., Dul, C., Majtényi, K., Hargitai, J., Pénzes, Z., and Urbanics, R. A chronic Alzheimer's model evoked by mitochondrial poison sodium azide for pharmacological investigations. *Behav. Brain Res.* 2004;154, 31–40. doi: 10.1016/j.bbr.2004.01.016
9. Luques, L., Shoham, S., & Weinstock, M. Chronic brain cytochrome oxidase inhibition selectively alters hippocampal cholinergic innervation and impairs memory: prevention by ladostigil.

Experimental neurology, 2007;*206*(2), 209-219.
10. Gao, C., Chang, P., Yang, L., Wang, Y., Zhu, S., Shan, H., & Tao, L. Neuroprotective effects of hydrogen sulfide on sodium azide-induced oxidative stress in PC12 cells. *International journal of molecular medicine*, 2018;*41*(1), 242-250.
11. Liu B, Che W, Zheng C, Liu W, Wen J, Fu H, Tang K, Zhang J, Xu Y. SIR T5: A safeguard against oxidative stress- induced apoptosis in cardiomyocytes. Cell Physiol Biochem, 2013;32(4): 1050-1059
12. Marino S, Marani L, Nazzaro C, Beani L, Siniscalchi A. Mechanisms of sodium azide induced changes in intracellular calcium concentration in rat primary cortical neurons. Neurotoxicology, 2007;28(3): 622-629.
13. Abayomi TA, Tokunbo OS, Adebisi BT, Fadare MU, Gbadamosi IT, Akinwale JO, Sawyer EA and Arogundade T T. Neurobehavioral assessment of the impact of vitamins C and E following acute exposure to sodium azide-induced neurotoxicity. Journal of Environmental Toxicology and Public Health 2019a;4: 15-20. http://doi.org/10.5281/zenodo.2577031
14. Abayomi, T. A., Tokunbo, O. S., David, O., Obembe, O. O., & Dare, B. J. Neuroprotective effects of Buchholzia coriacea seed extract on sodium azide-induced neurotoxicity of the prefrontal cortex of Wistar rats. *Anatomy Journal of Africa*, 2019b;*8*(2), 1635-1646.
15. Smith RP, Louis CA, Kruszyna R, Kruszyna H: Acute neurotoxicity of sodium azide and nitric oxide. Toxicol Sci, 1991;17(1): 120-127.
16. Su AG., Zheng C, Liu W, Wen J, Fu H, Tang K, Zhang J, Xu Y: Acute neurotoxicity of sodium azide and nitric oxide. Toxicol Sci, 2010;17(1): 120-127.
17. Olajide OJ, Akinola BO, Ajao SM, Enaibe BU. Sodium azide-induced degenerative changes in the dorsolateral prefrontal cortex of rats: attenuating mechanisms of kolaviron. Eur J Anat. 2016;20(1):47-64.
18. Delgado-Cortés MJ, Espinosa-Oliva AM, Sarmiento M, Argüelles S, Herrera AJ, Maurino R, Villaran RF, Venero JL, Machado A, de Pablos RM. Synergistic deleterious effect of chronic stress and sodium azide in the mouse hippocampus. Chemical Research in Toxicology. 2015;28(4):651-61.
19. Sinha, A. K. Colorimetric assay of catalase. *Analytical biochemistry*, 1972;*47*(2), 389-394.
20. Ohkawa, H., Ohishi, N., & Yagi, K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical biochemistry*, 1979;*95*(2), 351-358.
21. Beutler E. Improved method for the determination of blood glutathione. J. lab. clin. Med.. 1963;61:882-8.
22. McCord JM, Fridovich I. The utility of superoxide dismutase in studying free radical reactions I. radicals generated by the interaction of sulfite, dimethyl sulfoxide, and oxygen. Journal of Biological Chemistry. 1969;244(22):6056-63.
23. Adhikari, A., Topiwala, M. A., & Gordon, J. A. Synchronized activity between the ventral hippocampus and the medial prefrontal cortex during anxiety. *Neuron*, 2010;*65*(2), 257-269.
24. McHugh, S. B., Deacon, R. M. J., Rawlins, J. N. P., & Bannerman, D. M. Amygdala and Ventral Hippocampus Contribute Differentially to Mechanisms of Fear and Anxiety. *Behavioral Neuroscience, 2004;118*(1), 63–78. https://doi.org/10.1037/0735-7044.118.1.63
25. Hamza, R. Z., Al-Harbi, M. S., & El-Shenawy, N. S. Ameliorative effect of vitamin E and selenium against oxidative stress induced by sodium azide in liver, kidney, testis and heart of male mice. *Biomedicine & Pharmacotherapy*, 2017;*91*, 602-610.
26. Srinivasan, S., & Avadhani, N. G. Cytochrome c oxidase dysfunction in oxidative stress. *Free Radical Biology and Medicine*, 2012;*53*(6), 1252-1263.
27. El-Demerdash FM, Yousef MI, Radwan FM. Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs. Food and Chemical Toxicology. 2009;47(1):249-54.
28. Zabel, M., Nackenoff, A., Kirsch, W. M., Harrison, F. E., Perry, G., & Schrag, M. Markers of oxidative damage to lipids, nucleic acids and proteins and antioxidant enzymes activities in Alzheimer's disease brain: a meta-analysis in human pathological specimens. *Free Radical Biology and Medicine*, 2018;*115*, 351-360.
29. Liu, Z., Zhou, T., Ziegler, A. C., Dimitrion, P., & Zuo, L. Oxidative stress in neurodegenerative diseases: from molecular mechanisms to clinical applications. *Oxidative medicine and cellular longevity*, *2017*.
30. Worthington K. Worthington Enzyme Manual. Worthington Biochemical Corporation. Lakewood, NJ. 2011.
31. Krishnamurthy P, Wadhwani A. Antioxidant enzymes and human health. Antioxidant enzyme. 2012 Oct 3;1:3-18. Available from: https://www.intechopen.com/books/enzyme-inhibitors-and-activators/inhibitors-and-activators-of-sod-gsh-px-and-cat
32. Moon LD. Chromatolysis: Do injured axons regenerate poorly when ribonucleases attack rough endoplasmic reticulum, ribosomes and RNA?. Developmental neurobiology. 2018;78(10):1011-24.