HOWARD UNIVERSITY
APPLICATION SUMMARY

Charles and Mary Latham Fund Board Meeting

Request Date:	October 13, 2024
Project Title:	Novel Therapeutics for Trace Metal-Induced Neurotoxicity: Implications for Parkinson's Disease (PD)
Request Amount:	$20,000.00
Program Area:	Medical Research

Organization Information	**Contact Person for Application**
Howard University 2400 6th St NW, Washington, DC 20059 Washington, DC 20059 Tel: (202) 806-5790 Fax: 202-806-4465	Dr. Bruk Getachew Research Associate (202) 806-6311 bruk.getachew@howard.edu

Organization's annual operating budget: $311,000,000.00

Tax Status Notes
n/a

Organization Background
Howard University College of Medicine (HUCOM) dates from 1868 and serves a broad constituency, with about 70% of the students being U.S. underrepresented minorities and a substantial number being from foreign countries, making up a large percentage of the black physicians in this country. It provides students of high academic potential with a medical education of exceptional quality and prepares physicians to serve the underserved. Particular focus is on the education of disadvantaged students for careers in medicine. The Department of Physiology and Biophysics conducts programs of teaching and research in physiology and neuroscience that contribute to the training of medical practitioners and graduate (M.A., M.S. Ph.D., M.D./Ph.D.) level research scientists. In addition, the Department provides appropriate instruction of physiology to dental, allied health pharmacy and graduate students. Emphasis is placed on developing skills and habits of life-long learning and producing world leaders in medicine. Special attention is directed to teaching and research activities that address health care disparities. Our mission also includes the discovery of new knowledge through research. The goal of the HUCOM is to enhance our global recognition as a medical school of the first rank, known for the excellence of our teaching, research and service. In addition, the College envisions that it will be an exemplar in eliminating health disparities and in finding

solutions through research and public health programs for medical problems disproportionately found in disadvantaged communities, both in this nation and abroad.

Project/Program Budget (if applicable):

Project/Program Title: Novel Therapeutics for Trace Metal-Induced Neurotoxicity: Implications for Parkinson's Disease (PD)

Project Summary/Abstract

Summary: Manganese (Mn) and iron (Fe) are essential metals required for many physiological functions. These trace metals, albeit indispensable for human life, have much lower dietary requirements and need to be maintained within strict limits in each cell and tissue of the body. High accumulation of such metals in different tissues can be toxic. In neuronal tissues, it can cause neurodegeneration, resulting in the loss of dopamine containing neurons in the Substantia Nigra typified by movement disorders known as Parkinson's Disease (PD)-like syndromes. Current therapies aim to control the symptoms and minimize side effects. However, emerging therapeutic strategies include slowing pathology, reducing neuronal loss, and attenuating disease course. Pertaining to neuronal loss reduction, nicotine and butyrate have been shown to have neuroprotective effects against various endogenous or exogenous dopaminergic toxins. A widely used cellular dopaminergic cell model is neuroblastoma-derived SH-SY5Y cells. Butyrate is a short chain fatty acid (SCFA) fermentation product of dietary fiber by gut microbiota. Our group recently investigated the effects of nicotine on toxicity induced by manganese or iron in these cells where exposure of SH-SY5Y cells for 24 h to manganese (20 μM) or iron (20 μM) resulted in approximately 30% and 35% toxicities respectively. Pretreatment with nicotine (1 μM) completely blocked the toxicities of Mn and Fe. In turn, the neuroprotective effects of nicotine were blocked by selective and non-selective nicotinic receptor antagonists. Thus, dihydro-beta erythroidine (DHBE), a selective alpha4-beta2 subtype antagonist and methyllycaconitine (MLA), a selective alpha7 antagonist, as well as mecamylamine, a non-selective nicotinic antagonist all dose-dependently blocked the neuroprotective effects of nicotine against both Mn and Fe. Similar neuroprotective effects were observed with butyrate treatment which can be blocked free fatty acid receptors (FA3Rs) antagonists. Therefore, our findings lend a support for the potential utility of nicotine or nicotinic agonists as well as butyrate in PD-like neurodegenerative disorders, including those that might be precipitated by heavy metals, such as Fe and Mn. In fact, prebiotics such as dietary fiber which can be used as fuel source for probiotics which produce beneficial substances like butyrate. While alpha4-beta2 and alpha7 nicotinic receptors appear to mediate the neuroprotective effects of nicotine against Fe and Mn toxicities, FA3R appears to mediate the effect of butyrate. Nonetheless, the exact mechanisms for neither the neurotoxicity of the trace metals nor the neuroprotection of nicotine and butyrate are clear. This information is critical to fully understand pathological mechanisms that lead to PD-like syndrome as well as the development of novel pharmacotherapy for this devastating disease. There are mounting evidence to suggest the role of oxidative stress in neurodegenerative diseases. Therefore, our focus on this project is understand the role oxidative stress in metal-induced

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Specific Aims
The specific aims of this project are: 1. Determine the role of oxidative stress in the toxicities of Mn and Fe in dopaminergic cells 2. Determine the role of oxidative stress in the neuroprotective effects of nicotine and butyrate in dopaminergic cells 3. Determine effects of Mn, Fe, nicotine and butyrate on epigenetic parameters (e.g., DNA methylation) in dopaminergic cells.

Research Strategy: Significance
Significance
Although PD-like syndromes have shared clinical features, they could have different pathologies. Identifying the underlying causes and the understanding the mechanisms of disease pathology are imperative steps towards development of novel therapeutic strategies. Currently, the available treatments for PD-like syndrome are limited because they are based on restoration of dopaminergic tone in the striatum. Yet, this strategy neither change disease course nor treat some features of the disorder such as non-motor symptoms that often have the greatest impact on quality of life. Thus, the results of this pilot project will not only provide essential answers to some of these critical questions regarding not only the pathological mechanisms of metals-induced toxicities, but also, about the mechanism of neuroprotection against such toxicities by selective agents. It would also provide the framework for a larger study whereby potential novel pharmacotherapies in devastating neurodegenerative disease including PD could be suggested.

Research Strategy: Innovation
INNOVATION
This is a very novel project. First, a human dopaminergic cell line and an in vitro model of PD will be used in this project which offers construct validation. Second, experimental protocols will use state-of-the-art measurements to quantify oxidative and epigenetic parameters during Mn/Fe toxicities and nicotine/butyrate neuroprotection. Finally, quantifying DNA methylation is a unique and appropriate approach to elucidate the epigenetic changes following Mn, Fe, nicotine and butyrate treatment because, DNA methylation regulates gene expression. Although as an HDAC inhibitor, butyrate's effect as on histone modification is well known, little is known about its impact on other epigenetic mechanisms such as DNA methylation. Therefore, our approach is unique and befitting.
Approach
Design
SH-SY5Y cells incubation under the following experimental conditions (i.e., expose of undifferentiated neuroblastoma-derived SH-SY5Y cells to toxic concentration of Mn (60 μM) or Fe (60 μM) with pre (1h) exposure to nicotine (1.0 μM) or butyrate (10 μM)) followed by measurement of cell viability, oxidative stress, and epigenetic alterations.

Research Strategy: Design and Methods

Method
Cell culture and Cell viability Analysis: Following incubation of cells under experimental conditions, cell viability will be determined by MTT colorimetric assay according to the manufacturer's protocol as described previously (Getachew et al. 2019, 2021, 2022, Tizabi et al. 2023). Briefly, the yellow MTT tetrazolium salt (0.5 mg/ml) will be dissolved in phosphate-buffered saline (PBS). Thirty microliters of MTT will be added to each well and incubated for 3 h at 37 °C. The live cells will cause a reduction of the yellow salt to insoluble purple formazan crystals. The wells will be carefully aspirated, and 50 µl of dimethyl sulfoxide (DMSO) will be added to the wells to solubilize the crystals; the plates will be placed in a shaker for an hour and read spectrophotometrically at 570 nm with a background of 630 nm in a plate reader. Cell viability will be determined by subtracting the test results from the background and is presented as a percentage of the control.
ROS/Superoxide Detection: Following SH-SY5Y cells treatment under the following experimental conditions, the contents of ROS will be measured by flow cytometry using the Cell-based ROS/Superoxide Detection Assay kit (cat. no. ab139476; Abcam) according to the manufacturer's instructions. Briefly, SH-SH5Y cells after specified treatments will be collected into 5 ml round-bottom polystyrene tubes, washed with 1X Wash Buffer, resuspended in 500 µl ROS/Superoxide Detection Solution and incubated for 30 min at 37˚C in the dark. The ROS contents were determined using a flow cytometer (BD-C6, CFLOW plus 1.0, Becton, Dickinson and Company) equipped with a blue laser (488 nm).

Epigenetic Change Analysis: DNA methylation for candidate genes such as PRKN and PINK1, LRRK2 and SYN as well as those involved in mitochondrial function (e.g., mitochondrial genes associated with oxidative phosphorylation will be carried out as detailed previously (Kanherkar et al. 2018: Heinbockel and Csoka, 2018). Following SH-SY5Y cells treatment under the following experimental conditions whole-genome DNA methylation analysis using the NimbleGen Human DNA Methylation 3x720K Promoter Plus CpG Island Array will be performed to query gene promoters and genes on the array.

Research Strategy: Challenges
Challenges
Although it is ideal to conduct all studies in sufficient replications (at least 4 for each experiment) to assure reproducible and reliable results, it is likely that completion of the entire treatment protocol as well as the relication thereof might not be feasible for the duration and the budget specifications. However, it is possible to generate preliminary data for future grant application.

Future Plans
Future Plan
Preliminary data generated from this grant proposal will be used for future external grant application to study neurotoxic, inflammatory, and epigenetic effects of trace metals using animal models. Animal model is the next logical step up to probe aspects of metal toxicity that are more relevant to human condition such as homeostasis of essential

metals and neuroprotection including modulation of signal transduction pathways of inflammation, attenuation of oxidative stress. Information from such studies is crucial for development of novel therapeutics for metal-induced toxicities and PD-like syndromes.

Detailed Budget Breakdown and Justification
Budget and Justification for Latham Medical Research Grant Application

Personnel: $0
Equipment: Funds are requested for the following equipment:
$6,500 for a Fluorescence Absorbance Microplate Reader with Software for the purpose of oxidative stress analysis'
Expendable supplies: are budgeted at $10,000. The money will be spent on various drugs, reagents, assay materials and supplies. [Due to the fundamental research nature of the project, materials costs are estimated based on the PI's knowledge of prior projects of similar scope, and no further breakdown of materials is available.]

Travel: Funds are requested for travel:
$1,500 for the PI to attend one domestic conference to present research results.

Publication: Funds are requested for publication.
$2,000 for one publication associated with this project.

Other: 0

Total requested: $20,000.

Recommendation/Notes

Novel Therapeutics for Trace Metal-Induced Neurotoxicity: Implications for Parkinson's Disease (PD)

Summary: Manganese (Mn) and iron (Fe) are essential metals required for many physiological functions. These trace metals, albeit indispensable for human life, have much lower dietary requirements and need to be maintained within strict limits in each cell and tissue of the body. High accumulation of such metals in different tissues can be toxic. In neuronal tissues, it can cause neurodegeneration, resulting in the loss of dopamine containing neurons in the Substantia Nigra typified by movement disorders known as Parkinson's Disease (PD)-like syndromes. Current therapies aim to control the symptoms and minimize side effects. However, emerging therapeutic strategies include slowing pathology, reducing neuronal loss, and attenuating disease course. Pertaining to neuronal loss reduction, nicotine and butyrate have been shown to have neuroprotective effects against various endogenous or exogenous dopaminergic toxins. A widely used cellular dopaminergic cell model is neuroblastoma-derived SH-SY5Y cells. Butyrate is a short chain fatty acid (SCFA) fermentation product of dietary fiber by gut microbiota. Our group recently investigated the effects of nicotine on toxicity induced by manganese or iron in these cells where exposure of SH-SY5Y cells for 24 h to manganese (20 μM) or iron (20 μM) resulted in approximately 30% and 35% toxicities respectively. Pretreatment with nicotine (1 μM) completely blocked the toxicities of Mn and Fe. In turn, the neuroprotective effects of nicotine were blocked by selective and non-selective nicotinic receptor antagonists. Thus, dihydro-beta erythroidine (DHBE), a selective alpha4-beta2 subtype antagonist and methyllycaconitine (MLA), a selective alpha7 antagonist, as well as mecamylamine, a non-selective nicotinic antagonist all dose-dependently blocked the neuroprotective effects of nicotine against both Mn and Fe. Similar neuroprotective effects were observed with butyrate treatment which can be blocked free fatty acid receptors (FA3Rs) antagonists. Therefore, our findings lend a support for the potential utility of nicotine or nicotinic agonists as well as butyrate in PD-like neurodegenerative disorders, including those that might be precipitated by heavy metals, such as Fe and Mn. In fact, prebiotics such as dietary fiber which can be used as fuel source for probiotics which produce beneficial substances like butyrate. While alpha4-beta2 and alpha7 nicotinic receptors appear to mediate the neuroprotective effects of nicotine against Fe and Mn toxicities, FA3R appears to mediate the effect of butyrate. Nonetheless, the exact mechanisms for neither the neurotoxicity of the trace metals nor the neuroprotection of nicotine and butyrate are clear. This information is critical to fully understand pathological mechanisms that lead to PD-like syndrome as well as the development of novel pharmacotherapy for this devastating disease. There are mounting evidence to suggest the role of oxidative stress in neurodegenerative diseases. Therefore, our focus on this project is understand the role oxidative stress in metal-induced neurotoxicity. Therefore, our focus on this project is understand the role oxidative stress in metal-induced neurotoxicity. The specific aims of this project are: 1. Determine the role of oxidative stress in the toxicities of Mn and Fe in dopaminergic cells 2. Determine the role of oxidative stress in the neuroprotective effects of nicotine and butyrate in dopaminergic cells 3. Determine effects of Mn, Fe, nicotine and butyrate on epigenetic parameters (e.g., DNA methylation) in dopaminergic cells.

Preliminary Data

To better understand the potential utility of nicotine or butyrate in PD-like symptoms that might be brought about by accumulation or exposure to excess mounts of trace elements such as Mn and Fe, we evaluated the effects of these two drugs against toxicities induced by the two metals in dopaminergic SH-SY5Y cells. Moreover, we aimed to determine the involvement of specific nicotinic and fatty acid 3 receptors (FA3Rs), respectively, in potential protective effects of nicotine and butyrate.

In the first series of experiments, we observed that exposure of SH-SY5Y cells for 24 h to manganese (60 μM) or iron (60 μM) resulted in approximately 35% toxicity. Pretreatment with nicotine (1 μM) completely blocked the toxicities of Mn and Fe. The effects of nicotine, in turn, were blocked by selective nicotinic receptor antagonists. Thus, dihydro-beta erythroidine (DHBE), a selective alpha4-beta2 subtype antagonist and methyllycaconitine (MLA), a selective alpha7 antagonist, as well as mecamylamine, a non-selective nicotinic antagonist all at 1.0 μM blocked the protective effects of nicotine against both Mn and Fe (please see Fig 1). Thus, our findings provided further support for the potential utility of nicotine or nicotinic agonists in PD-like neurodegenerative disorders, including those that might be precipitated by high levels of Fe and Mn. Moreover, both alpha4-beta2 and alpha7 nicotinic receptor subtypes appear to mediate the neuroprotective effects of nicotine against toxicity induced by these two trace metals.

In the second series of experiments, we observed that pretreatment with butyrate (10 μM) completely blocked the toxicities of both Mn and Fe. The neuroprotective effects of butyrate on Fe, in turn, were completely blocked by beta-hydroxy butyrate (BHB), a selective FA3R antagonist, whereas the same antagonist only partially blocked the protective effects of butyrate against Mn toxicity, indicating involvement of other mechanisms in addition to FA3Rs in butyrate's protection against Mn. One of such mechanisms of butyrate is epigenetic regulation of DNA expression by inhibiting histone deacetylase (HDAC), an enzyme that allows the histones to wrap the DNA more tightly, (Liu et al 2018; Cantu-Jungles et al. 20190. The involvement of epigenetic mechanisms in neurodegenerative disorders including PD is well documented (Rroji et al. 2021; Mohd Murshid et al. 2022). In addition to HDAC inhibition, DNA methylation is also an important epigenetic mechanism (Rroji et al. 2021; Mohd Murshid et al. 2022). However, the epigenetic effects of Fe, Mn, nicotine or butyrate are far from clear. Hence, this study will provide some basic knowledge on these interactions, which can be expanded and eventually exploited pharmacologically.

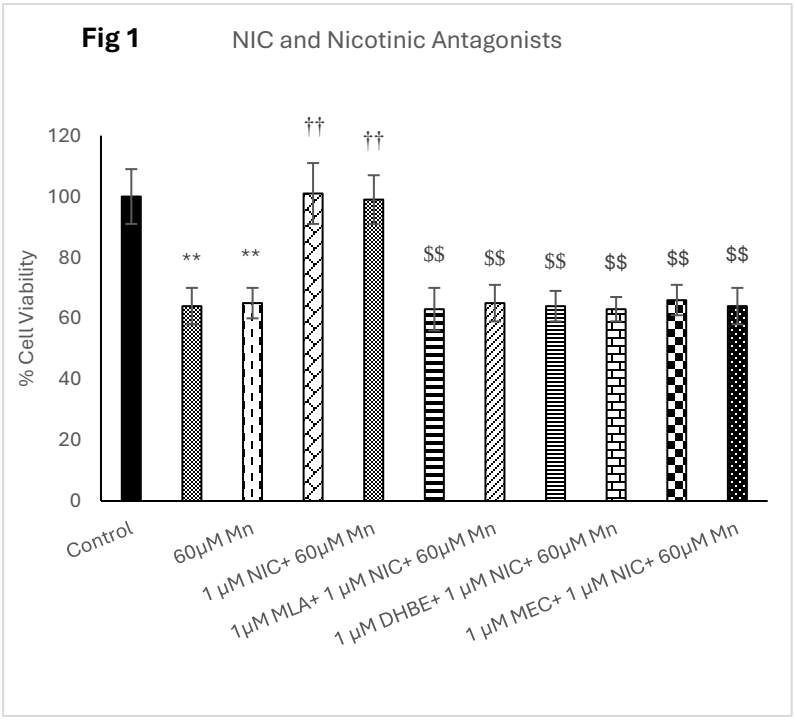

Fig 1. Effects of nicotinic receptor antagonists: MLA, DHBE and MEC against neuroprotective effects of NIC on Fe- and Mn-induced toxicities in SH-SY5Y cells. Cells were treated with the antagonists 1 h prior to NIC (1 uM), which was applied 1 h prior to Fe (60 μM) or Mn (60 μM) and cell viability was determined by MTT assay 24 h later. Values are mean ± SEM. **p<0.01 compared to control. ††p<0.01 compared to Fe or Mn alone. $p<0.05,$$p<0.01 compared to NIC + Fe or Nic + Mn. N=5 per treatment

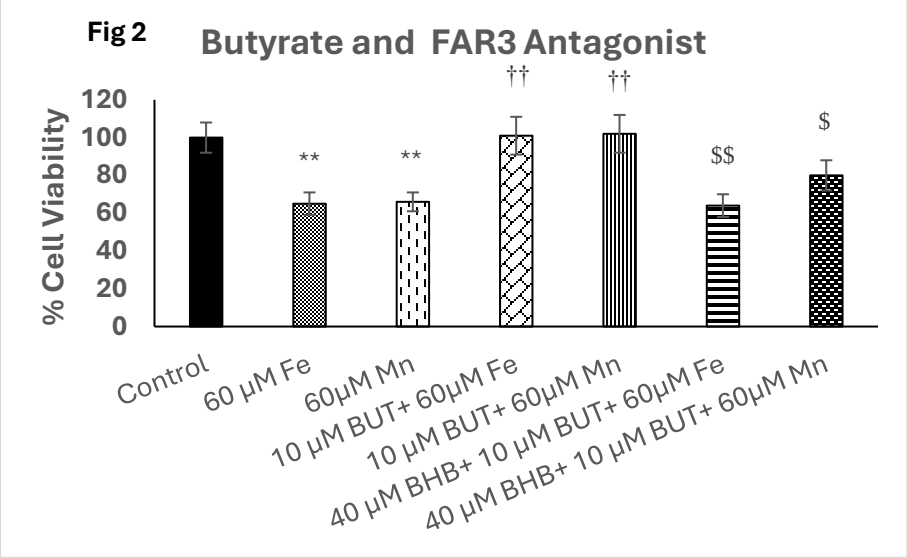

Fig 2. Effects of BHB, a FA3R antagonist on neuroprotective effects of butyrate against Fe- and Mn-induced toxicities in SH-SY5Y cells. Cells were treated with the antagonist 1 h prior to butyrate (BUT), which was applied 1 h prior to Fe or Mn. Cell viability was determined by MTT assay, 24 h later. Values are mean ± SEM. **p<0.01 compared to control. ††p<0.01 compared to Fe or Mn alone. $^{\$}$p<0.05,$^{\$\$}$p<0.01 compared to BUT + Fe or BUT + Mn. N=5 per treatment

Significance

Although PD-like syndromes have shared clinical features, they could have different pathologies. Identifying the underlying causes and the understanding the mechanisms of disease pathology are imperative steps towards development of novel therapeutic strategies. Currently, the available treatments for PD-like syndrome are limited because they are based on restoration of dopaminergic tone in the striatum. Yet, this strategy neither change disease course nor treat some features of the disorder such as non-motor symptoms that often have the greatest impact on quality of life. Thus, the results of this pilot project will not only provide essential answers to some of these critical questions regarding not only the pathological mechanisms of metals-induced toxicities, but also, about the mechanism of neuroprotection against such toxicities by selective agents. It would also provide the framework for a larger study whereby potential novel pharmacotherapies in devastating neurodegenerative disease including PD could be suggested.

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Challenges

Although it is ideal to conduct all studies in sufficient replications (at least 4 for each experiment) to assure reproducible and reliable results, it is likely that completion of the entire treatment protocol as well as the replication thereof might not be feasible for the duration and the budget specifications. However, it is possible to generate preliminary data for future grant application.

Future Plan

Preliminary data generated from this grant proposal will be used for future external grant application to study neurotoxic, inflammatory, and epigenetic effects of trace metals using animal models. Animal model is the next logical step up to probe aspects of metal toxicity that are more relevant to human condition such as homeostasis of essential metals and neuroprotection including modulation of signal transduction pathways of inflammation, attenuation of oxidative stress. Information from such studies is crucial for development of novel therapeutics for metal-induced toxicities and PD-like syndromes.

References and Citations

Cantu-Jungles TM, Rasmussen HE, Hamaker BR. (2019) Potential of prebiotic butyrogenic fibers in Parkinson's disease. *Front Neurol*. 10:663.

Getachew B, Csoka AB, Aschner M, Tizabi Y. (2019) Nicotine protects against manganese and iron-induced toxicity in SH-SY5Y cells: Implication for Parkinson's disease. *Neurochem Int*.124:19-24.

Getachew B, Csoka AB, Garden AR, Copeland RL, Tizabi Y. Sodium Butyrate Protects Against Ethanol-Induced Toxicity in SH-SY5Y Cell Line. Neurotox Res. 2021 Dec;39(6):2186-2193.

Getachew B, Csoka AB, Tizabi Y. Dihydromyricetin Protects Against Ethanol-Induced Toxicity in SH-SY5Y Cell Line: Role of $GABA_A$ Receptor. Neurotox Res. 2022 Jun;40(3):892-899.

Heinbockel T, Csoka AB. (2018) Epigenetic Effects of Drugs of Abuse. *Int J Environ Res Public Health*. 15(10):2098.

Kanherkar RR, Getachew B, Ben-Sheetrit J, et al. (2018) The Effect of Citalopram on Genome-Wide DNA Methylation of Human Cells. *Int J Genomics*. 8929057.

Liu H, Wang J, He T. et al. (2018) Butyrate: A Double-Edged Sword for Health? *Adv Nutr*. 9(1):21-29.

Mohd Murshid N, Aminullah Lubis F, Makpol S. (2022) Epigenetic Changes and Its Intervention in Age-Related Neurodegenerative Diseases. *Cell Mol Neurobiol*. 42(3):577-595.

Newell ME, Babbrah A, Aravindan A, Rathnam R, Kiernan R, Driver EM, Bowes DA, Halden RU. Prevalence rates of neurodegenerative diseases versus human exposures to heavy metals across the United States. Sci Total Environ. 2024 Jun 10;928:172260.

Rroji O, Kumar A, Karuppagounder SS, Ratan RR. (2021) Epigenetic regulators of neuronal ferroptosis identify novel therapeutics for neurological diseases: HDACs, transglutaminases, and HIF prolyl hydroxylases. *Neurobiol Dis*.147:105145.

Tizabi Y, Getachew B, Aschner M. Butyrate Protects and Synergizes with Nicotine against Iron- and Manganese-induced Toxicities in Cell Culture. Neurotox Res. 2023 Dec 14;42(1):3.

Wei R, Wei P, Yuan H, Yi X, Aschner M, Jiang YM, Li SJ. Inflammation in Metal-Induced Neurological Disorders and Neurodegenerative Diseases. Biol Trace Elem Res. 2024 Oct;202(10):4459-4481.

Budget and Justification for Latham Medical Research Grant Application

Personnel: $0

Equipment: Funds are requested for the following equipment:
$6,500 for a Fluorescence Absorbance Microplate Reader with Software for the purpose of oxidative stress analysis'

Expendable supplies: are budgeted at $10,000. The money will be spent on various drugs, reagents, assay materials and supplies. [*Due to the fundamental research nature of the project, materials costs are estimated based on the PI's knowledge of prior projects of similar scope, and no further breakdown of materials is available*.]

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$1500 for the PI to attend one domestic conference to present research results.

Publication: Funds are requested for publication.
$2,000 for one publication associated with this project.

Other: 0

Total requested: $20,000.

Biographical Sketch

Provide the following information for each individual included in the Research & Related Senior/Key Person Profile (Expanded) Form.			
NAME BRUK GETACHEW		POSITION TITLE RESEARCH ASSOCIATE	
EDUCATION/TRAINING (Begin with baccalaureate or other initial professional education, such as nursing, and include postdoctoral training).			
INSTITUTION AND LOCATION	DEGREE (IF APPLICABLE)	YEAR(S)	FIELD OF STUDY
Peru State College, Peru, NE	BSc	1995	Biological Science
Howard University, Washington, DC	M.S.	2002	Genetics
Howard University, Washington, DC	Ph.D.	2007	Pharmacology

RESEARCH AND PROFESSIONAL EXPERIENCE: Concluding with present position, list in chronological order, previous employment, experience, and honors. Include present membership on any Federal Government public advisory committee. List in chronological order the titles, all authors, and complete references to all publications during the past 3 years and to representative earlier publications pertinent to this application. If the list of publications in the last 3 years exceeds 2 pages, select the most pertinent publications. PAGE LIMITATIONS APPLY. DO NOT EXCEED 5 PAGES FOR THE ENTIRE BIOGRAPHICAL SKETCH PER INDIVIDUAL.

Professional Appointments

Research Associate, Department of Pharmacology 2016-Present

Speed Laboratory INC., Norcross, GA Intern, 2013- 2014

Post-doctoral fellow, Indiana University School of Medicine, Indianapolis, IN 2007-2010

Honors and Awards:

Toffler Scholar, 2024

Dr. Eliot Gardner Travel Award, 6th International Drug Abuse Research Society meeting, Dubrovnik, Croatia, 2017.

Institutional National Research Service Award, Postdoctoral Fellowship (T32 AA07462-21), Indiana University school of Medicine, 2007-2010

Trustee Scholarship, Howard University, 2002-2007

Guze 6th, 7th, 8th, and 10th Annual Symposium on Alcoholism travel award, Washington University, St Louis, MO, 2006, 2007, 2008 and 2010

Membership

American Society for Pharmacology and Experimental Therapeutics; Society for Neuroscience; Research Society on Alcoholism; International Drug Abuse Research Society.

A. Personal Statement

I have a rich research experience in the areas of behavioral neuroscience, neuropharmacology, and neurotoxicity. During my graduate studies at Howard University College of Medicine, I have developed a keen interest in understanding of the biological substrates of alcohol abuse disorders (AUDs) and co-morbid mental disorders. Particularly, I was interested in understanding the causal factors in AUD-associated depressive behaviors. To such end, I employed state-of-the-art inhalation chambers and examined the effects of alcohol withdrawal on central biogenic amines using animal model of depression (Wistar Kyoto) and its counterparts (Wistar). We demonstrated that alcohol withdrawal can cause deficiencies in monoamine levels in the specific regions of brain that can engender depressive-like behaviors. These fascinating findings were not only published in prestigious peer-reviewed journals, but also landed me caveated fellowship--Institutional National Research Service Award, Postdoctoral Fellowship (T32 AA07462-21), at Indiana University school of Medicine in the Institute of psychiatric Research. There, I spent three years studying the genetic aspects of AUDs. For the past 8 years, I have been involved in neurotoxicological and pharmacological research related to development of novel therapeutics for neurodegenerative diseases. I employ a mechanistic approach focused for testing, natural products (such as flavonoid of Asian medicinal plant extract-dihydromyricetin (DHM), gut-microbiota derived-short chain fatty acid (SCFA, butyrate), and repurposed drugs such as anthelmintic moxidectin. I am particularly interested in discovery of novel therapeutics for neurodegenerative diseases by understanding their neurobiological substrates including biochemical, cellular, genetic, and epigenetics. Our current pilot project is focus on investigating the effectiveness of butyrate and nicotine in metal-induced toxicity in Parkinson's disease (PD) in vitro model. The preliminary results are promising and show neuroprotective effects of these drugs. Currently, efforts are underway to elucidate the mechanism of their neuroprotective actions, particularly in relationship to epigenetic changes as well as alteration in the inflammatory pathways. This proposal, in response to Latham Medical Grant application dealing with finding a cure for devastating diseases such as PD fits perfectly with our current interest as we have shown that metals can have neurotoxic effects on cellular model of PD. Thus, identifying the molecular cites for these toxic effects can lead to discovery of druggable targets for potential development of novel therapeutics for treatment of neurodegenerative disorders.

PUBLICATIONS (PAST 4 YEARS, SINCE 2020), FROM 56 TOTAL

Getachew B, Hauser SR, Bennani S, El Kouhen N, Sari Y, Tizabi Y. Adolescent alcohol drinking interaction with the gut microbiome: implications for adult alcohol use disorder. Adv Drug Alcohol Res. 2024;4:11881. doi: 10.3389/adar.2024.11881. Epub 2024 Jan 15. PMID: 38322648; PMCID: PMC10846679.

Carvalho FV, Landis HE, **Getachew B**, Silva VDA, Ribeiro PR, Aschner M, Tizabi Y. Iron toxicity**,** ferroptosis and microbiota in Parkinson's disease: Implications for novel targets. Adv Neurotoxicol. 2024;11:105-132. doi: 10.1016/bs.ant.2024.02.001. Epub 2024 Feb 15. PMID: 38770370; PMCID: PMC11105119.

Tizabi Y, Bennani S, El Kouhen N, **Getachew B**, Aschner M. Heavy Metal Interactions with Neuroglia and Gut Microbiota: Implications for Huntington's Disease. Cells. 2024 Jul 3;13(13):1144. doi: 10.3390/cells13131144. PMID: 38994995; PMCID: PMC11240758.

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Active Support:

Toffler Scholar Grant Getachew (PI)

5/1/24 – present

Title: Novel Therapeutics for Drug-Induced Neurotoxicity: Implications for Parkinson's Disease

The main objective of this small grant is to determine the effectiveness of butyrate and nicotine in alleviation of drug-induced neurotoxicity in human neuroblastoma cell line and a Parkinson's disease model.

There is some overlap between this project and the current submission.

Pending Support:

Pending

N/A

Completed Project:

N/A

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