JOURNAL OF PHARMACOLOGICAL SCIENCES AND EXPERIMENTAL THERAPEUTICS
(Official Journal of the Nigerian Society for Pharmacological Sciences and Experimental Therapeutics)

ISSN 3115 - 6487

Neuroprotective Potentials of Pleurotus tuberregium Polysccharides and Ganoderma lucidum Polysaccharides against Lead-Induced Neurotoxicity in Wistar Rats

Sobowale Michael Tosin[1*], Olorundare Olufunke Esan[2], Ajayi Abayomi Mayowa[3], Ogunbodede Adedeji David[4], Emaleku Sunday Adeola[5]

[1]Department of Pharmacology, Faculty of Basic Medical Sciences, Igbinedion University Okada, Edo state, Nigeria
[2]Department of Pharmacology and Therapeutics, Faculty of Basic Clinical Sciences, University of Ilorin, Ilorin, Nigeria.
[3]Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Nigeria
[4]Department of Chemical Science, Faculty of Science, Augustine University, Ilara Epe Lagos State Nigeria.
[5]Department of Biochemistry, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State, Nigeria.

ARTICLE HISTORY

Received 16th July 2025
Revised 11th September 2025
Accepted 12th September 2025

Published 15th September 2025

Keywords:
Neurodegenerative disorder,
Lead acetate,
Bioactive compounds,
Wistar rats

***Corresponding author**
Dr. Sobowale Michael Tosin,
[1]Department of Pharmacology, Faculty of Basic Medical Sciences, Igbinedion University Okada, Edo state, Nigeria
Email:
sobowale.tosin@iuokada.edu.ng;
Tel: +2347062541722

ABSTRACT

Background: One of the neurotoxic chemical agents that is responsible for neurodegenerative disorder is lead acetate (Pb). *Pleurotus tuberregium* polysaccharide (PTP) and *Ganoderma lucidum* polysaccharide (GLP) are potent bioactive compounds that can be used in the management of neurological disorder. *Pleurotus tuberregium* polysaccharide and *Ganoderma lucidum* polysaccharide were investigated for their neuroprotective effects against lead-induced neuronal degeneration and neurobehavioral impairments in striatum, prefrontal cortex and hippocampus of the rats.

Materials and methods.: Forty-eight male Wistar rats were randomly divided into six groups (n=8). Group 1 served as control (Distilled water, *ad libtum*), group 2 received lead acetate (Pb, 25 mg/kg), group 3 were administered with PTP (100 mg/kg) + Pb (25 mg/kg), group 4 received GLP (100 mg/kg) +Pb (25 mg/kg), group 5 were administered with dimercaptosuccinic acid (50 mg/kg) +Pb (25 mg/kg) and group 6 received penicillamine (30 mg/kg) +Pb (25 mg/kg). Treatment was daily orally administered for 28 days. Neurobehavioral deficits were assessed using Y-Maze test (YMT), sucrose splash test (SST) and tail suspension test (TST). Determination of brain interleukin-6, tumor necrosis factor-α, anti-oxidants parameters, acetyl-cholinesterase activity, glutamic acid decarboxylase, monoamine oxidase, dopamine, serotonin,, caspase 3, caspase 9 and histological of the brain regions were equally carried out

Results: Results showed that extract of PTP and GLP ameliorated neurobehavioural alteration in SST and YMT, enhanced antioxidants parameters in catalase and superoxide dismutase, increased neurosignalling molecules. Expression of lipid peroxidation, Interleukin -6 (IL-6), caspase 3 and caspase 9 were significantly ($P < 0.05$) decreased in PTP and GLP treated group

Histology of the brain administered with lead acetate showed degenerated neuronal cell while the PTP and GLP treated group revealed normal neurofibrillary network

Conclusion: The study showed the neuroprotective effects of PTP and GLP and their ability to ameliorate mechanism related to the released of oxidative stress, proinflammatory cytokines, apoptotic marker enzymes and histoarchitectural alteration in the brain regions.

INTRODUCTION

Neurodegenerative disorder encompasses a wide range of conditions that results from progressive damage to cell and nervous system that are essential for mobility, coordination, strength and sensation. Neurodegenerative diseases affect millions of people worldwide, although, there is no complete cure for most of these complex neurological diseases[1]. With their progression, these disorders can affect every aspect of an individual's life such as memory and cognitive abilities, mood, speech and sleep. This explains why the impacts of neurotoxic chemicals in the body cause a sizable and rising percentage of illness and mortality in the developed world. One of these neurotoxic chemicals or metals that responsible for this neurodegenerative disorder is lead acetate $(Pb)^2$ .Lead acetate (Pb) is a prevalent environmental neurotoxin that has a detrimental effect on the host and causes neuronal deformity even in trace quantities of exposure. The developing central nervous system (CNS) is susceptible to Pb neurotoxicity and when the CNS is exposed to Pb during formative age, it can cause permanent negative effect on the CNS pathway of development, which may alter cognitive and behavioral features that can last long till the old age. Although, this Pb mediated changes that occurs in the CNS are not yet fully understood[3-4]. The CNS appears to be the most susceptible and main target for Pb-induced toxicity when compared to other organs of the body, as it can lead to permanent CNS damage at higher doses and eventually death[4]. Neural signaling disturbances which occurred in the brain is as a result of Pb induced toxicity which has been linked to a number of molecular processes, which include changes in the biochemistry of neurotransmitter, mitochondrial dysfunction and increased oxidative stress. Exposure to Lead acetate results in oxidative stress by generating reactive oxygen species (ROS) which seems to play an important role in the pathophysiology of lead neurotoxicity. The effect of the oxidative stress initiated by lead exposure destroys a number of intracellular and extracellular structures including the antioxidant defense system through mechanisms related to neuroimmune alterations. Ability to inhibit the effects of neuroinflammatory-mediated lead-induced neurotoxicity by chelating agents such as Calcium disodium ethylenediaminetetraacetic acid ($CaNa_2EDTA$), 2-3 dimercaptosucciic acid (DMSA), ascorbic acid and Pennicllliamine (PENN) enhanced with antioxidant activities would attenuate the detrimental effects caused by lead acetate [4]. However, this chelating agents are not without secondary toxic effects, hence the reason for natural product even though, the use of natural products as alternative agents in the treatment of heavy metal poisoning have not been fully utilized. Medicinal mushrooms work as chelating agents which helps in the cleaning of tissues and organs, thereby removing the heavy metals accumulated [5]. Mushrooms which occurs as a source of biological active compound contains both medicinal and therapeutic properties and are available in Nigeria, both during the early and late rainy season [6]. and they are usually found on the damp rotten log, grassland and in the forest. *Pleurotus. tuberregium* (*P. tuberregium*) is an edible mushroom that produces a sclerotium as well as fruiting body which can be used to produce delicacy. It is usually consumed because of its nutritional values, aroma, taste, and medicinal effects in the body[7]. Both the mushroom and sclerotium are edible [8]. About 85% of the countries in the world relied on traditional medicines to take care of their health, most of which involve the use of mushrooms and plants extract [9]. Compounds with important pharmacological properties have been isolated from mushroom, which include Polysaccharides with immuno-enhancing properties [10]. Polysaccharide extract obtained from *P. tuberregium* (PTP) is a novel edible mushroom which is potent in the management of neurological disorder and resulted in apoptosis of HL-60[11]. *Ganoderma lucidum* , one of the highly nutritious and significantly effective medicinal mushroom, has been used for clinical applications over the years[12]. Several researches have shown that it has a wide range of brain damage protection, such as amelioration of Alzheimer's disease, therapeutic effect on epilepsy, and the protective effect on neural cells in stroke injury [13]. Several bioactive chemical substances such as polysaccharides, tannis, alkaloids, triterpenoids, and proteins are shown to be found in the cultured mycelia, fruiting bodies, and spores of G. lucidum by many researchers.[14]. Research has also shown that bioactive compound obtained from *G. lucidum* such as polysaccharides have antioxidant, anti-tumour, anti-inflammatory, and immunomodulatory properties [15]. Although,[16] reported the antioxidant activities of GLP in hippocampus region of rat brain but expression of pro-inflammatory mediators and the alteration of neuronal structural integrity following exposure to lead acetate in striatum and prefrontal cortex were not studied. Hence, this present study evaluated the anti-inflammatory, anti-apoptotic, and neuroprotective effects of GLP and PTP against lead-induced neuronal degeneration and neurobehavioral impairments in striatum, prefrontal cortex and hippocampus of rats' brain.

MATERIAS AND METHODS.

Drugs and Reagents

Lead acetate was obtained from LOBA Chemie, Mumbai, India. Tumor necrosis factor-alpha (TNF-α)

and interleukin-6 (IL-6) ELISA kits were products of BioLegend (USA). 2, 3- dimercaptosuccinic acid (DMSA), pennicilamine (PENN), Caspase 3 and Caspase 9 were purchased from sigma- Aldrich (3050 Spruce St.., Saint Louis Missouri, 63103, United State). Ketamine hydrochloride was obtained from Rotex Medica GmBH Arzneimittelwerk 4, Trittau, Schleswig-Holstein, Germany.

Experimental Animals

The study was conducted in the Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Nigeria. Forty-eight male Wistar rats weighing between 184 - 225 g were procured from the Mctemmy concept animal husbandry services, Ogbomoso, Oyo state and were allowed to acclimatize for the period of two weeks before the commencement of the experiment. Throughout the experiment, the animals were housed under natural light and temperature condition as well as regular rat chow and drinking water *ad libitum*. Approval for the use of animals and the study protocol was granted by the University ethical committee (UERC/ASN/2023/2572) which followed strictly with National guideline for Laboratory Animal Care.

Preparation of the Mushroom and Treatment Protocol

G. lucidum and *P. tuberregium* were obtained through the help of botany technologist from the Department of Plant Science and Biotechnology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo State. The mushrooms were identified by Dr Ademola Olatokunbo of the Department of Plant Science and Biotechnology, Faculty of Science, Adekunle Ajasin University, Akungba Akoko, Ondo state. Adulterants were carefully picked out of the mushrooms and the mushrooms were thoroughly rinsed in tap water, cut into smaller pieces. *Ganoderma lucidum* were allowed to dry at room temperature (25ºC) for about 15 days. While *Plerotus tuberregium* were oven dried at 60^0C for seven days. After drying, the mushrooms were then pulverized into powdered forms with the aid of mechanical engine. The mushroom extract (GLP and PTP) were extracted according to the method described by [17]. GLP, PTP and lead acetate were dissolved in distilled water and administered orally with the aid of cannula for 28 days. The selected doses of GLP, PTP, DMSA, PENN and Lead acetate were based on the results obtained from the previous reports and preliminary investigation [18, 19, 20, 21,]. Forty-eight adult male Wistar rats were randomly divided into six groups (n=8); Control (Distilled water, *ad libtum*), Lead (Pb, 25 mg/kg), PTP (100 mg/kg) + Pb (25 mg/kg), GLP (100 mg/kg) +Pb (25 mg/kg), DMSA (50 mg/kg) +Pb (25 mg/kg) and pennicilamine (30mg/kg) +Pb (25mg/kg) **17-20.** Treatment was daily orally administered for 28 days. Neurobehavioral deficits were assessed using Y-Maze test (YMT), sucrose splash test (SST) and tail suspension test (TST). Brain were harvested, dissected into hippocampus, prefrontal cortex and striatum. Antioxidant parameters, neurotransmitter enzymes, inflammatory marker enzymes, neurosignalling enzymes, apoptotic marker enzymes and histological evaluation of striatum, prefrontal cortex and hippocampus were carried out

Extraction of Polysaccharides from *P. tuberregium* and *G. lucidum*

The dried powdered (500 g) each of *G. lucidum* and *P. tuberregium* fruiting bodies were added separately to 2 L of water and boiled for 1 h at 60ºC separately. The mixtures were then filtered using Whatman filter paper #1 to obtain the filtrate and residue. The filtrate was centrifuged at 4000 rpm for 10 min at room temperature to obtain the supernatants. The supernatants were concentrated in a water bath set at 55ºC. The concentrates were precipitated with four volumes of absolute ethanol (i.e., in ratio 1:4) and centrifuged to separate the precipitates. The precipitates were dissolved in small volumes of distilled water and concentrated in the water bath to obtain *Pleurotus tuberregium* polysaccharide (PTP) and *Ganoderma lucidum* polysaccharide (GLP) extracts. The extracts were stored in an opaque container in a refrigerator at 4°C and were reconstituted in distilled water before being administered to the rats, according to the experimental protocol

Y-maze Test (YMT)

The effect of PTP and GLP on cognitive impairment using the Y-maze test was assessed as an index of spatial memory dysfunction. The test was conducted by placing rats in an apparatus made of three identical arms (labeled A, B, C), ach arm measuring 33 × 11 × 12 cm and separated symmetrically at 120°. Rats exhibit alternation behavior when they make consecutive entries into all three arms (i.e., ABC, CAB, or BCA but not BAB). Ability of rats to visit a different arm option than the one previously visited while trying to remember the correct sequence of arms' visitation was considered spontaneous alternation performance. Percentage correct alternation performance (a measure of spatial working memory) was calculated as: (Total alternation number/Total number of entries – 2) X 100. After each test session, the maze was cleaned with 70% ethanol to remove residual odor from the previous animal [22].

Tail Suspension Test (TST)

Tail suspension test was carried out as previously reported[22]. The rats were assessed for anti-depressive –like behavior after treatment with GLP and PTP co-administered with lead acetate. After assessing the animals for anxiety on day 27, each rat was individually suspended 60cm above the floor with adhesive masking tape place appropriately 1cm from the tip of the tail. The test was carried out for 6 minutes, the first 2 minutes was ignored and the last 4 minutes was considered as the freezing/immobility time. The rats were recorded immobile when they were motionless.

Sucrose Splash Test (SST)

Splash test was carried out as previously reported[23] . The rat was placed in a standard empty rat cage under dim red light. The dorsal part of the rat was sprayed twice with 10% sucrose solution. Because of the viscosity of sucrose solution, the grooming behavior was initiated (i.e., the cleaning of the fur by licking or scratching), and the total grooming time was manually recorded using stop watch for five minutes by a blinded experimenter. A decrease in total grooming time was an indication of depression or anxiety -like behaviors.

Preparation of rat brain for biochemical and enzyme-linked immunosorbent assays

Following the assessment for neurobehavioral deficit, the rats were euthanized using ketamine, the brains were excised, the striatum, prefrontal cortex and hippocampus were isolated. Each samples were individually homogenized in a cold iced with 10% phosphate buffer (0.1 M, pH 7.4), centrifuged at 10,000 rpm at 4 °C for 10 min with the supernatants and homogenates immediately frozen and stored at (−4 °C) for biochemical assays and ELISA activity

Determination of brain IL-6 and TNF-α level.

The levels of IL-6 and TNF-α in the supernatants of the striatum, prefrontal cortex and hippocampus were determined according to the protocol given by the manufacturer. The striatum, prefrontal cortex and hippocampus of IL-6 and TNF-α levels was determined by specific rat TNF-α and IL-6 (BioLegend ELISA MAX™ Deluxe kit, USA) with sensitivity limit of 4 pg/mL. Measurement was taken at room temperature as described by Bio Legend protocol using microplate reader at a wavelength of 450 nm. The level of IL-6 and TNF-α in the striatum, prefrontal cortex and hippocampus were taken from the standard curve of the IL-6 and TNF-α kits and then expressed as pg/mg protein.

Determination of Anti-oxidants parameters.

The supernatant obtained from the brain region of the striatum, prefrontal cortex and hippocampus were used to assay for the antioxidant parameters , catalase (CAT) and superoxide dismutase (SOD) were measured colorimetrically using Hydrogen peroxide as substrate according to the method of [24,] reduced glutathione level was assayed using Ellman reagent according to the manufacturer guide of [25] and malondialdehyde (MDA) level was estimated using thiobarbituric reacting substance according to the methods of [25]. Nitric oxide measurement was determined using Griess reaction protocol. The colour changes were measured with a micro plate reader at a wavelength of 540 nm as previously described by McCord and Fridovich[25].

Determination of Acetyl-Cholinesterase Activity, Glutamic Acid Decarboxylase, Monoamine Oxidase, Dopamine and Serotonine

The procedure described by[26] was used to estimate AChE activity in the supernatant of the brain tissues. Glutamic acid decarboxylase was determined following the method described by[26]. Monoamine oxidase-A (MAO) activity was estimated using benzyl-amine as the substrate[24]. Dopamine and serotonin levels were determined in the brain regions (prefrontal cortex, striatum and hippocampus) using Elabscience ELISA kits according to manufacturer's instruction.

Determination of Apoptotic Enzymes in the Brain (Caspase 3 and Caspase 9)

Apoptotic markers, caspase-3 and caspase-9 were assessed in the brain supernatants using commercially available ELISA test kits (Elabscience Wuhan, Hubei, China). The apoptotic markers were assayed according to manufacturer's instruction and the representative standard curve was generated using the caspase-3 and caspase-9 standards, respectively.

Histological Examination of the Brain Regions

At n= 3, the striatum, prefrontal cortex and hippocampus were submerged in Bouin's fixative, then dehydrated in alcohol before embedding in paraffin. Sections (5 μm) thick were cut, deparaffinized and stained with haematoxylin and eosin. The stained slides were examined with a light microscope (Olympus BX63 model) at ×400 magnification, and the resulting photomicrographs were used to evaluate histoarchitecture of the brain regions.

Statistical analysis

The data were subjected to one-way analysis of variance (ANOVA) and post hoc Tukey test using GraphPad prism 5.0 (GraphPad Software, San Diego,

CA) and the resulting data were expressed as mean ± standard error of mean (SEM), level of significant was considered at p-value less than 5% ($p < 0.05$).

RESULTS

NEUROBEHAVIOURAL STUDIES

The effects of *Pleurotus tuberegium* polysaccharide and *Ganoderma lucidum* polysaccharide on neurobehavioral studies

Figures 1a and 1b shows the effects of Pb only when compared with the control group in Y-maze test in total arm entries. Pb significantly (#$P < 0.05$) increased the number of entries in Pb only group when compared with the control. However, extract of *Pleurotus tuberregium* polysaccharide (PTP) significantly (*$P < 0.05$) decreased the numbers of entries in Y-maze test. In the test for correct alternation, Pb significantly (#$P < 0.05$) decreased the number of correct alternation when compared with the control group. The extract of *Pleurotus tuberregium* polysaccharide (PTP) and *Ganoderma lucidum* polysaccharide (PTP) significantly (*$P < 0.05$) increased the number of correct alternation when compared with the Pb only group in Y-maze test. 2, 3- dimercaptosuccinic acid (DMSA) and pennicillamine (PENN) also significantly (*$P < 0.05$) increased the number of correct alternation when compared with the Pb only group in Y-maze test.

Rats exposed daily to lead acetate (Pb) for 28 days showed symptoms of depression with significant (#$p< 0.005$) increased in immobility period (freezing time spent) compared with the control group. As shown in figure 1c, PTP and GLP (100mg/kg) did not reversed the increase in the number of immobility time when compared with Pb only group.

Also, in sucrose splash test (SST) figure 1d, rats exposed to Pb only has a significant (#$p< 0.005$) decreased in grooming time when compared with control group. However, administration of the extract of GLP significantly (*$P < 0.05$) increased the grooming time when compared with Pb only group. Also, DMSA and PENN significantly (*$p< 0.005$) increased the grooming time when compared with the Pb only.

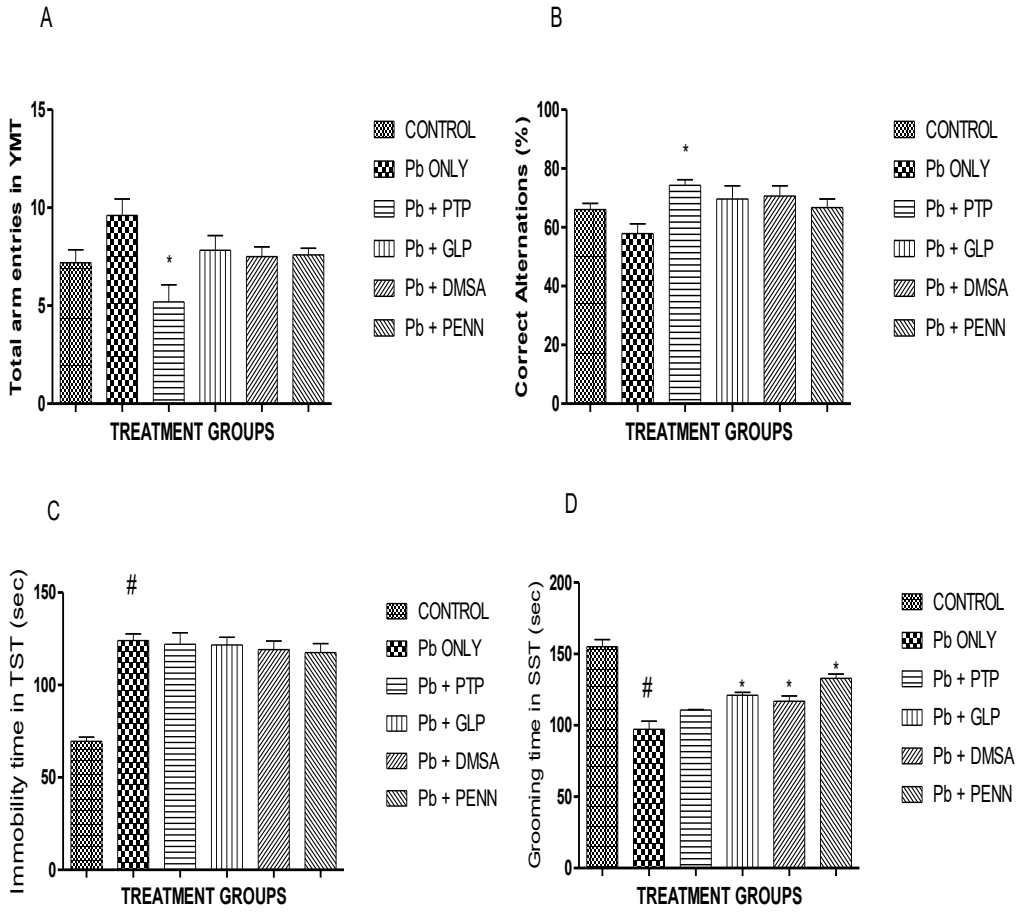

Figure 1: Effects of *Ganderma lucidum* polysaccharide (GLP) and *Pleurotus tuberregium* polysaccharide (PTP) on memory and depressive-like behaviours in Pb-treated rats. (A) Total arm entries in YMT, (B) Correct alternations in YMT, (C) Immobility time in TST, and (D) Grooming time in SST. Values are represented as Mean ± SEM (n=6). #$p<0.05$ compared with control and *$p<0.05$ compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine. PTP - *Plerotus tuberregium* polysaccharide and GLP - *Ganoderma lucidum* polysaccharide, Pb- Lead

Effects of *Pleurotus tuberegium* polysaccharide and *Ganoderma lucidum* polysaccharide on lipid peroxidation and antioxidant markers in the brain

As shown in figure 2, PTP and GLP reduced lipid peroxidation activity of malondialdehyde (MDA) in the rat brain after 28 days exposure to lead acetate (25mg/kg) increased the activity of MDA in the brain regions of striatum (STR), prefrontal cortex (PFC) and hippocampus (HPC) relatively to non-lead induced control group. However, the increase in lipid peroxidation activity of MDA in the brain regions were significantly (*P < 0.05) ameliorated by GLP (100mg/kg) in MDA HPC. Also, DMSA (50mg/kg) significantly (*P < 0.05) attenuated the effect of Lead-induced toxicity in the brain regions of hippocampus (HPC) of the wistar rats.

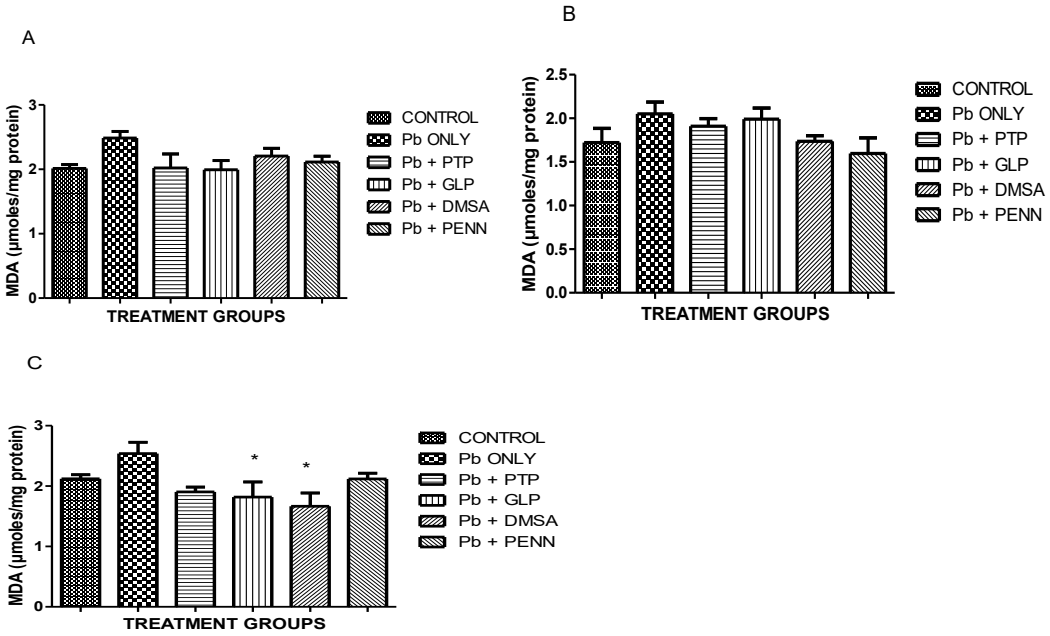

Figure 2: Effects of *Ganoderma lucidum* polysaccharide (GLP) and *Pleurotus tuberregium* polysaccharide (PTP) on antioxidant parameters in brain. (A) MDA STR (B) MDA PFC (C) MDA HPC (D). Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and *p<0.05 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine

Pleurotus tuberregium polysaccharide and Ganoderma lucidum polysaccharide abate lead-induced oxidative stress

Exposure to lead acetate significantly (#P < 0.05) decreased enzymatic reduced glutathione (GST) activity in the brain regions of striatum and prefrontal cortex of the group given Pb only (25 mg/kg) when compared with the control group.. However, treatment with PTP (100 mg/kg) and GLP (100 mg/kg) significantly (*P < 0.05) (***P < 0.001) increased the antioxidant activity of the enzymatic reduced glutathione. More so, administration of DMSA (50mg/kg) and PENN (30 mg/kg) significantly (***P < 0.05) increased the level of enzymatic reduced glutathione activity in the brain regions of striatum and prefrontal cortex.

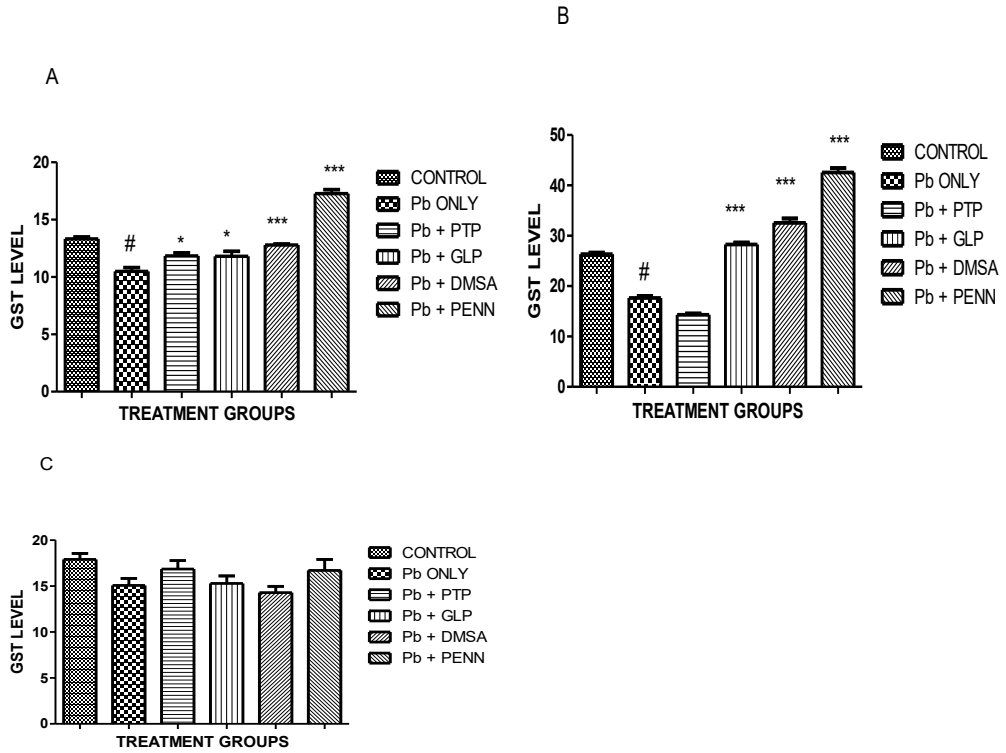

Figure 3: Effects of GLP and PTP on antioxidant parameters in brain. (A) GST STR (B) GST PFC (C) GST HPC (D). All values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and *p<0.05, ***p<0.001 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine

Pleurotus tuberregium polysaccharide and Ganoderma lucidum polysaccharide downregulated Nitric oxide activity

Exposure to lead acetate significantly (#P < 0.05) increased lipid peroxidation activity of NO in the group treated with lead acetate only in striatum, prefrontal cortex and hippocampus when compared with the control group. However, treatment with PTP (10 0mg/kg) downregulated the pro-oxidant activity of NO in STR but DMSA (50 mg/kg) and PENN (30 mg/kg) did not abrogate the effect of lead-induced toxicity in the brain regions of striatum, prefrontal cortex and hippocampus of the rat.

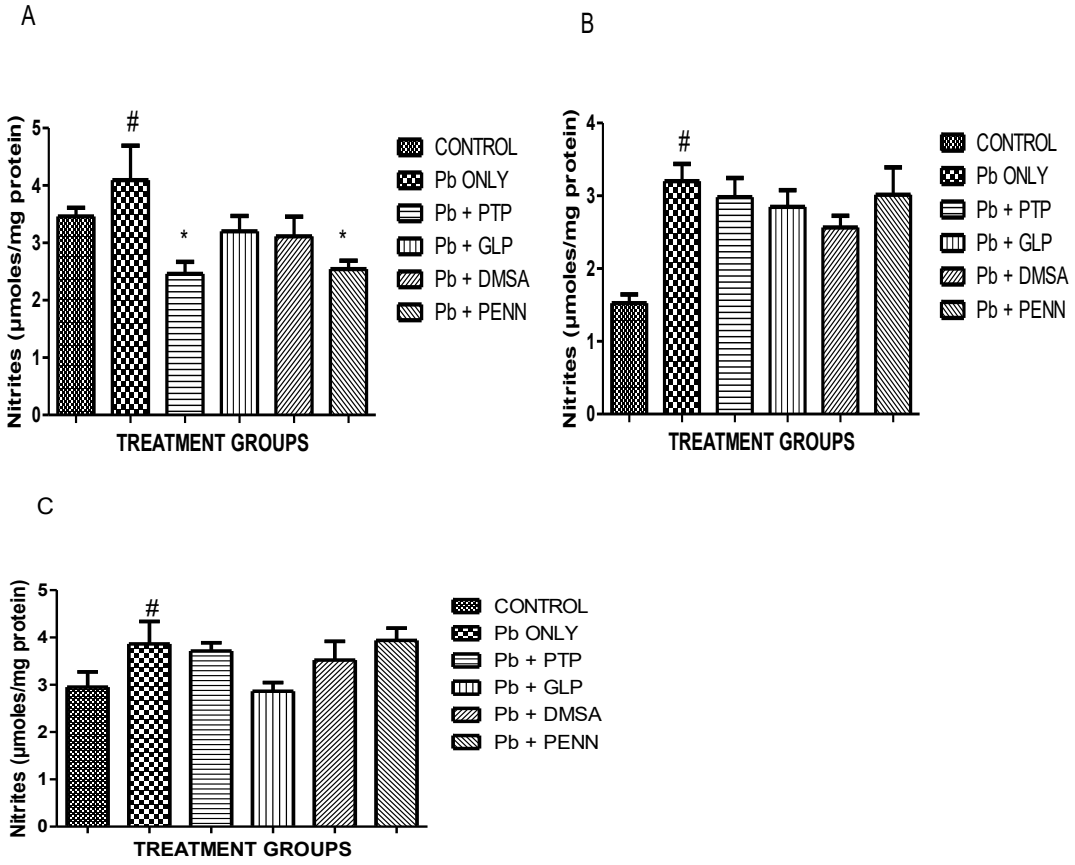

Figure 4: Effects of GLP and PTP on antioxidant parameters in brain. (A) NITRITE STR (B) NITRITE PFC (C) NITRITE HPC. Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and *p<0.05 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine.

***Pleurotus tuberregium* polysaccharide and *Ganoderma lucidum* polysaccharide upregulated catalase activity**

Exposure to lead acetate significantly (#P < 0.05) reduced catalase activity in the brain regions of striatum, prefrontal cortex and hippocampus in the group administered with lead acetate only (25 mg/kg) when compared with the control group. However, treatment with PTP (100 mg/kg) and GLP (100 mg/kg) significantly (***P < 0.001) upregulated the antioxidant activity of catalase. More so, administration of DMSA (50mg/kg) and PENN (30 mg/kg) significantly (***P < 0.001) increased the level of catalase activity in the brain regions of striatum, prefrontal cortex and hippocampus.

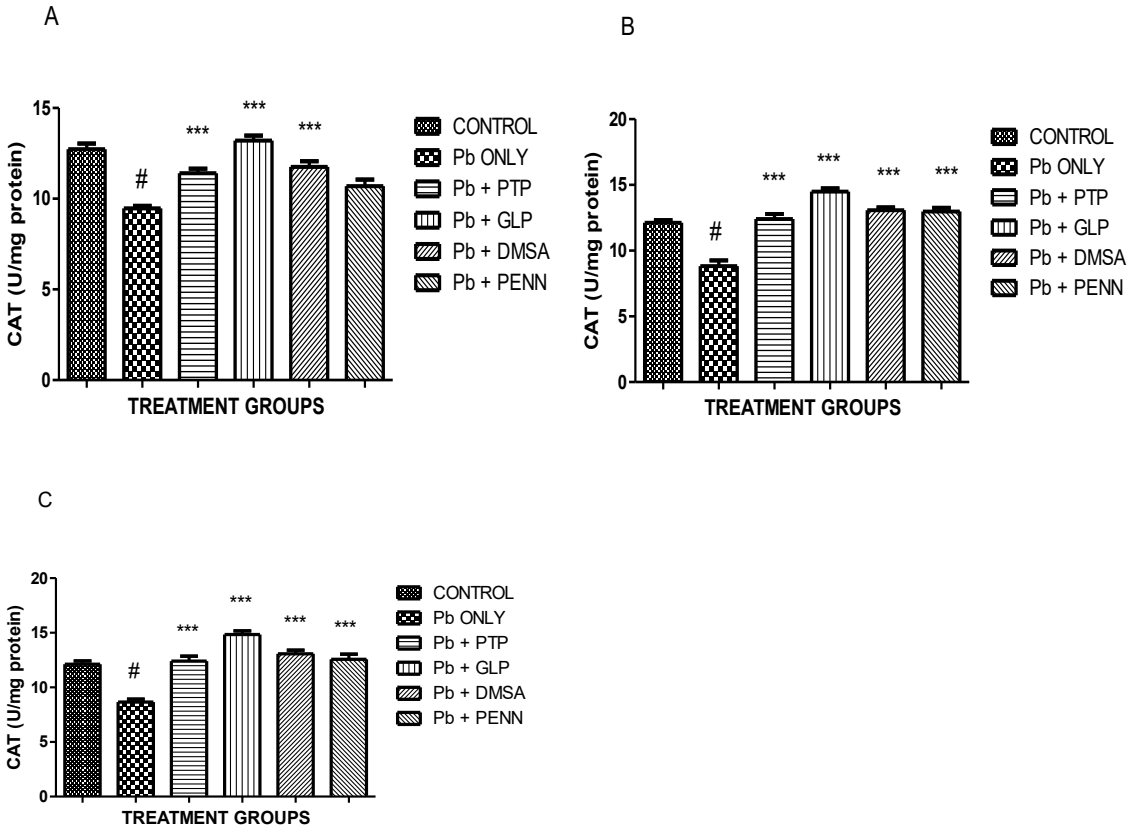

Figure 5: Effects of GLP and PTP on antioxidant parameters in brain. (A) CAT STR (B) CAT PFC (C) CAT HPC. All values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and (***p<0.001) compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine.

Pleurotus tuberreegium **polysaccharide and** ***Ganoderma lucidum*** **polysaccharide abated lead-induced free radicals of superoxide dismutase**

The groups that were given lead acetate only (25 mg/kg) has low level of superoxide dismutase in the brain regions of striatum, prefrontal cortex and hippocampus when compared with the control group. However, treatment with PTP (100 mg/kg) and GLP (100 mg/kg) significantly (***P < 0.001)u pregulated the antioxidant activity of superoxide dismutase. More so, administration of DMSA (50 mg/kg) and PENN (30 mg/kg) significantly (***P < 0.001) scavenged the free radical activity induced by lead toxicity in the brain regions of striatum, prefrontal cortex and hippocampus.

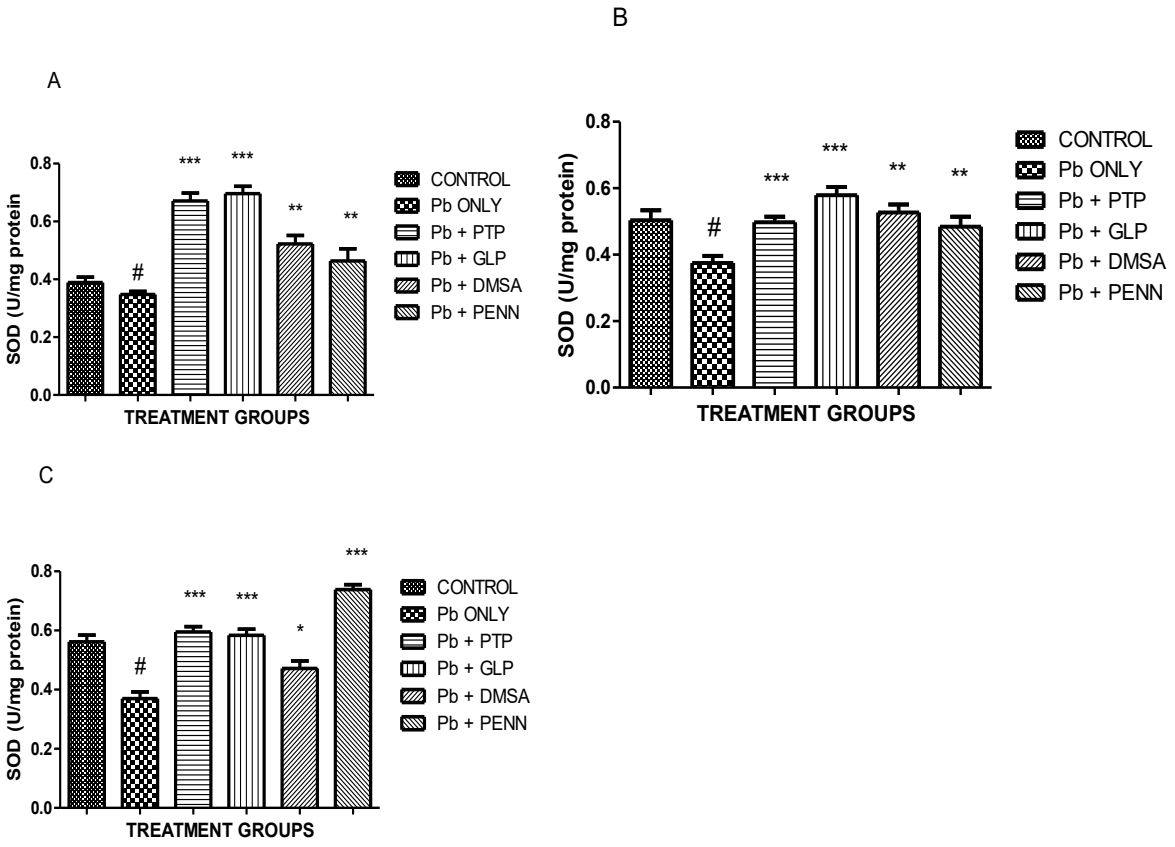

Figure 6: Effects of *G. lucidum* (GLP) and *P. tuberregium* (PTP) on antioxidant parameters in brain. (A) SOD STR (B) SOD PFC (C) SOD HPC (D). All values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and *p<0.05, **p<0.01, ***p<0.001 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine

NEUROTRANSMITTER ENZYMES

Effects of *Pleurotus tuberreegium* polysaccharide and *Ganoderma lucidum* polysaccharide on acetylcholinesterase level in rat brain.

The effect of PTP and GLP on acetylcholinesterase (ACHE) level in rat brain shows that lead acetate (Pb) produced significant (#p< 0.005) increased in brain level of acetylcholinesterase in the group treated with lead only (25 mg/kg) when compared with control group. However, treatment with GLP (100 mg/kg) significantly (***p< 0.001) reduced the effects of acetylcholinesterase in the brain regions of the rats. But, DMSA (50mg/kg) and PENN (30 mg/kg) did not significantly reduce the effects of acetylcholinesterase in brain regions of striatum, prefrontal cortex and hippocampus.

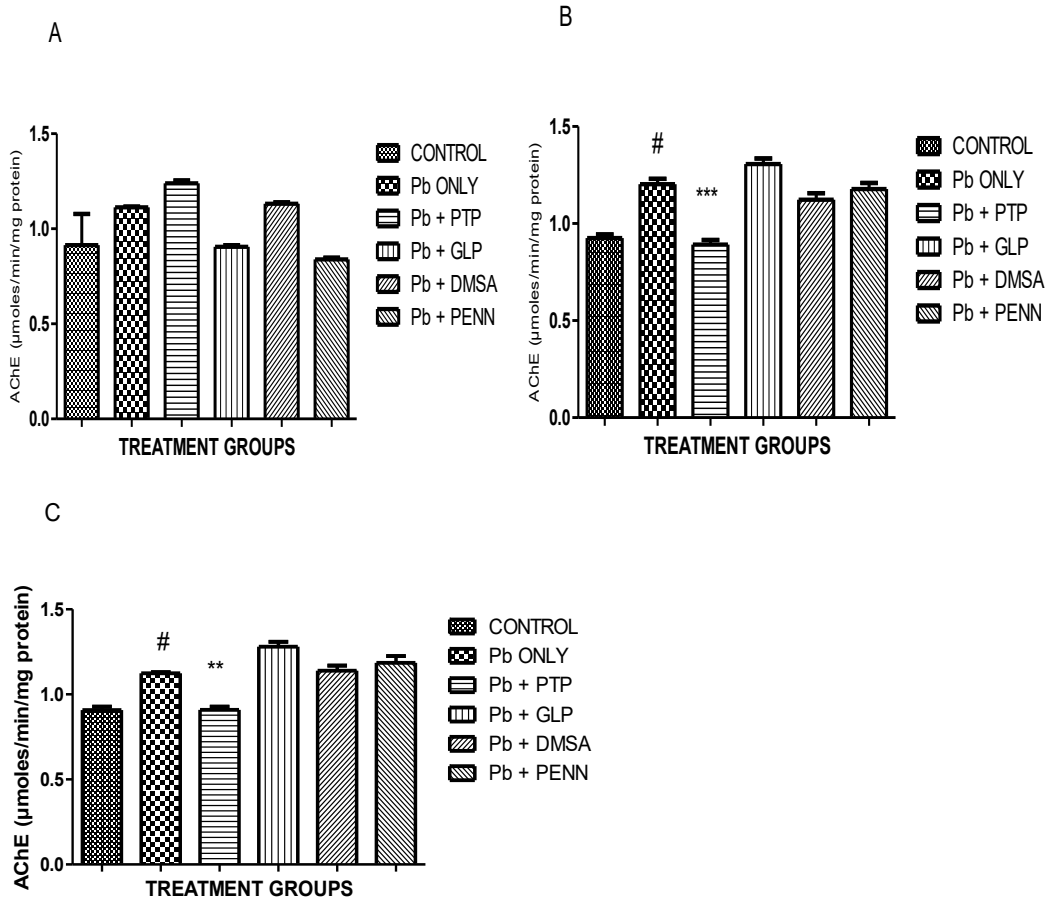

Figure 7: Effects of *G. lucidum* (GLP) and *P. tuberregium* (PTP) on Neurotransmitter enzymes in brain. (A) ACHE STR (B) ACH PFC (C) ACHE HPC. Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and **p<0.05, ***p<0.001 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine.

Effects of *Pleurotus tuberreegium* polysaccharide and *Ganoderma lucidum* polysaccharide on glutamic acid decarboxylase level in rat brain.

Unlike acetylcholinesterase which is highly expressed in the group treated with lead acetate only, the glutamic acid decarboxylase is significantly (#p< 0.05) reduced in the group treated with lead acetate only as shown in fig. 8. PTP (100mg/kg) and GLP (100 mg/kg) produced significant (*p< 0.05) (***p< 0.001) increase in the level of glutamic acid decarboxylase. Also, DMSA (50 mg/kg) and PENN (30 mg/kg) attenuated the effect of lead induced toxicity in the brain region of hippocampus of the rats.

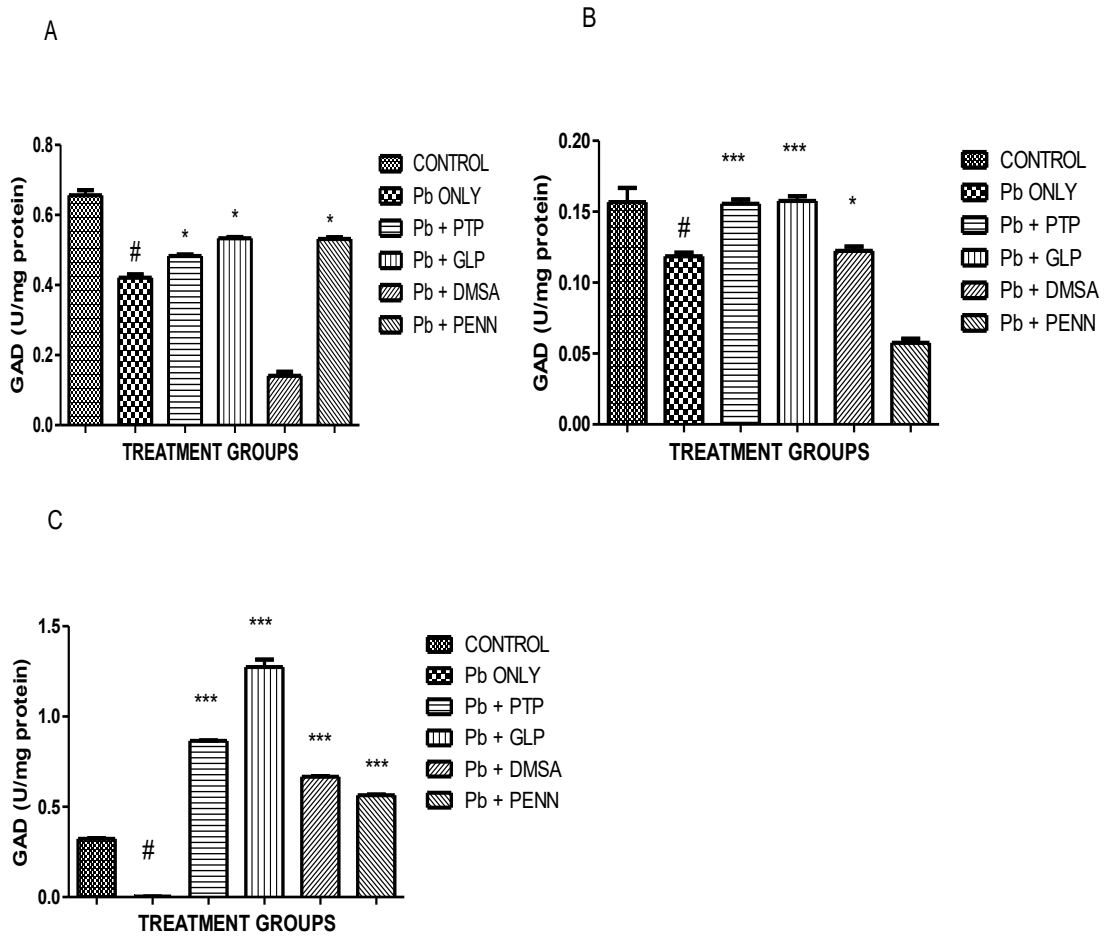

Figure 8: Effects of GLP) and PTP on Neurotransmitter enzymes in brain. (A) GAD STR (B) GAD PFC (C) GAD HPC. Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and *p<0.05, ***p<0.001 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine.

Effects of *Pleurotus tuberreegium* polysaccharide and *Ganoderma lucidum* polysaccharide on monoamine oxidase level in rat brain.

The effect of PTP and GLP on monoamine oxidase (MAO) level in rat brain is depicted in fig 9. The group given lead acetate only (25 mg/kg) has low level of MAO when compared with the control group. However, PTP (100mg/kg) and GLP (100 mg/kg) significantly (*p< 0.05) (***p< 0.001) increased the level of MAO when compared with the Lead only group (25 mg/kg). Unlike DMSA which was able to increase the level of MAO in PFC, PENN was only able to increase the level of MAO in striatum and hippocampus region of the brain.

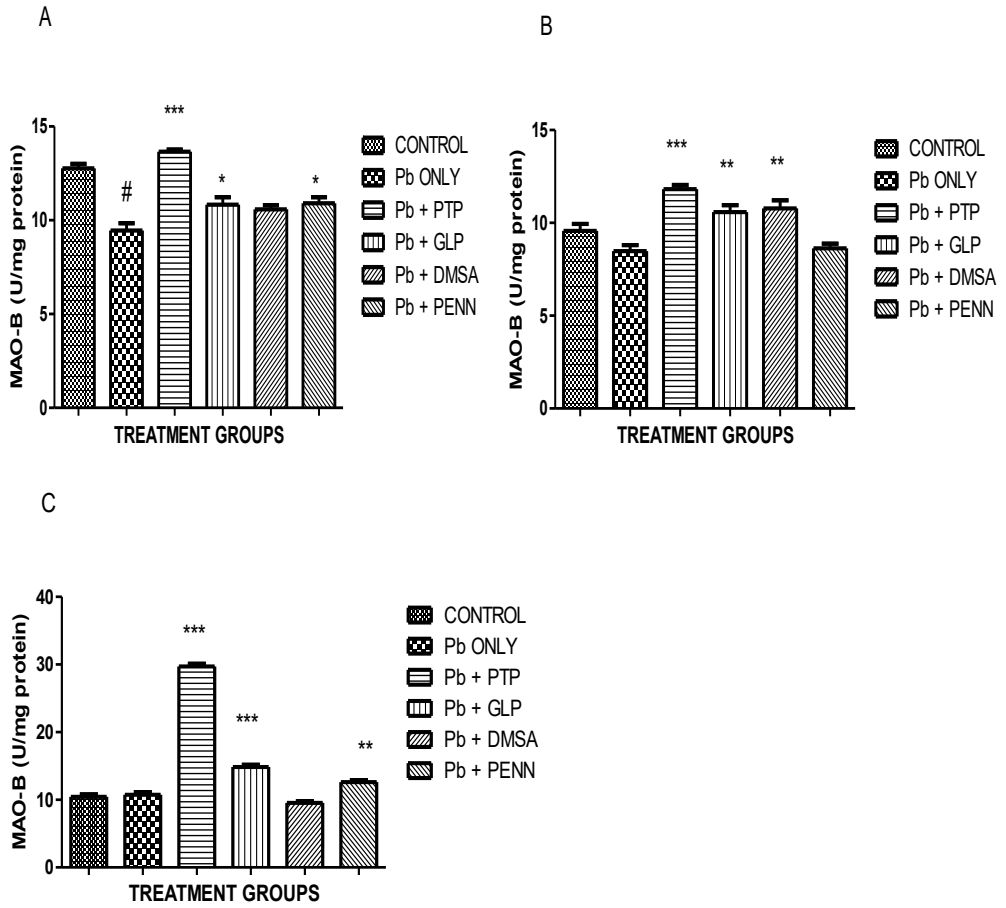

Figure 9: Effects of *G. lucidum* (GLP) and *P. tuberregium* (PTP) on Neurotransmitter enzymes in brain. (A) MAO-B STR (B) MAO-B PFC (C) MAO-B HPC. Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and *p<0.05, **p<0.01, ***p<0.001 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine.

INFLAMMATORY ENZYMES

***Pleurotus tuberregium* polysaccharides and *Ganoderma lucidum* polysaccharide abated lead – induced tumor necrosis factor**

Tumor necrosis factor was represented in figure 10 where lead acetate significantly (#p< 0.05) increased TNF-α level in the group given Pb only whereas, PTP (100 mg/kg) and GLP (100mg/kg) significantly (*p< 0.05) (***p< 0.001) downregulated the effects of TNF-α in the brain regions of striatum, prefrontal cortex and hippocampus. DMSA (50 mg/kg) and PENN (30mg/kg) also significantly (*p< 0.05) (***p< 0.001) reduced the effects of TNF-α

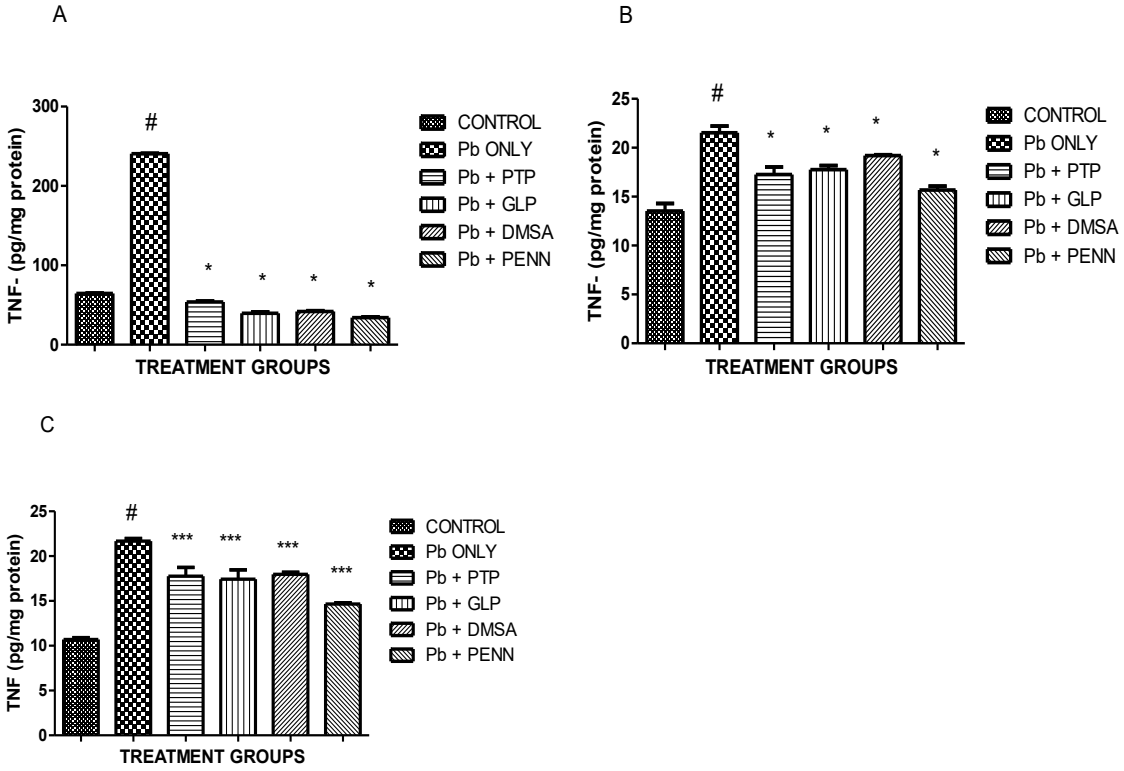

Figure 10: Effects of *G. lucidum* (GLP) and *P. tuberregium* (PTP) on Inflammatory enzymes. (A) TNF STR (B) TNF PFC (C) TNF HPC. Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and *p<0.05, **p<0.01, ***p<0.001 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine.

Effects of *Pleurotus tuberregium* polysaccharides and *Ganoderma lucidum* polysaccharide on lead-induced Interleukin-6

Administration of Pb significantly (#p<0.05) increased the level of Interleukin -6 (IL – 6) when compared with the control group. Whereas, PTP (100 mg/kg) and GLP (100 mg/kg) significantly (*p< 0.05) (***p< 0.001) decreased the effects of IL - 6 in the brain regions of striatum, prefrontal cortex and hippocampus. DMSA (50 mg/kg) and PENN (30 mg/kg) also significantly (*p< 0.05) (***p< 0.001) reduced the level of IL – 6.

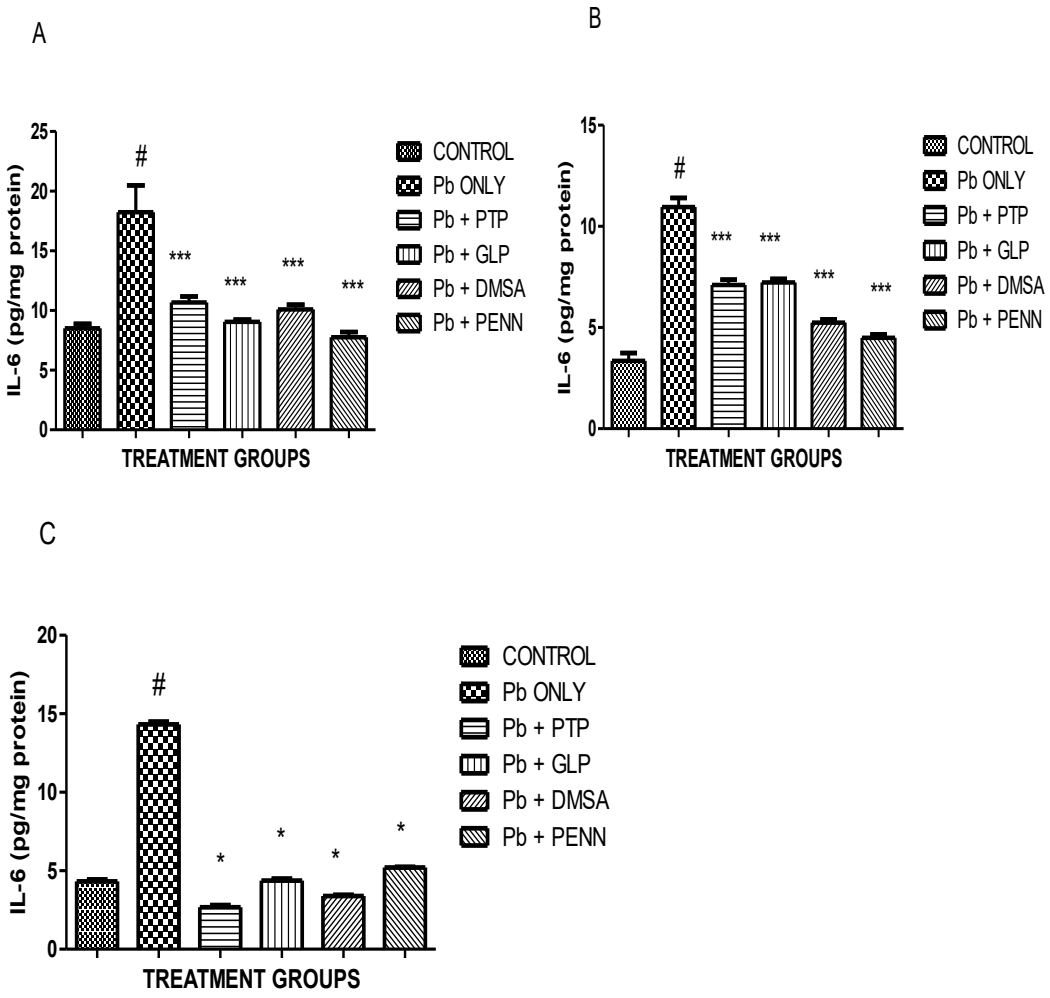

Figure 11: Effects of *G. lucidum* (GLP) and *P. tuberregium* (PTP) on Inflammatory enzymes. (A) IL - 6 STR (B) IL - 6 PFC (C) IL - 6 HPC. Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and *p<0.05, ***p<0.001 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine.

NUROSIGNALLING MOLECULES

***Pleurotus tuberregium* polysaccharides and *Ganoderma lucidum* polysaccharide increased the dopaminergic activity on lead exposed rats.**

One-way ANOVA showed that there was significant (#p< 0.05) decreased between the treatments groups in the brain regions of striatum, prefrontal cortex and hippocampus of dopamine in Pb only group when compared with control group '. However, there was significant (***p< 0.001) increased in the concentration of dopamine in the brain regions of striatum, prefrontal cortex and hippocampus after treatment with PTP (100mg/kg) and GLP (100 mg/kg). Also, DMSA (50 mg/kg) and PENN (30 mg/kg) significantly (*p< 0.05) (***p< 0.001) increased the level of dopamine in striatum and prefrontal cortex.

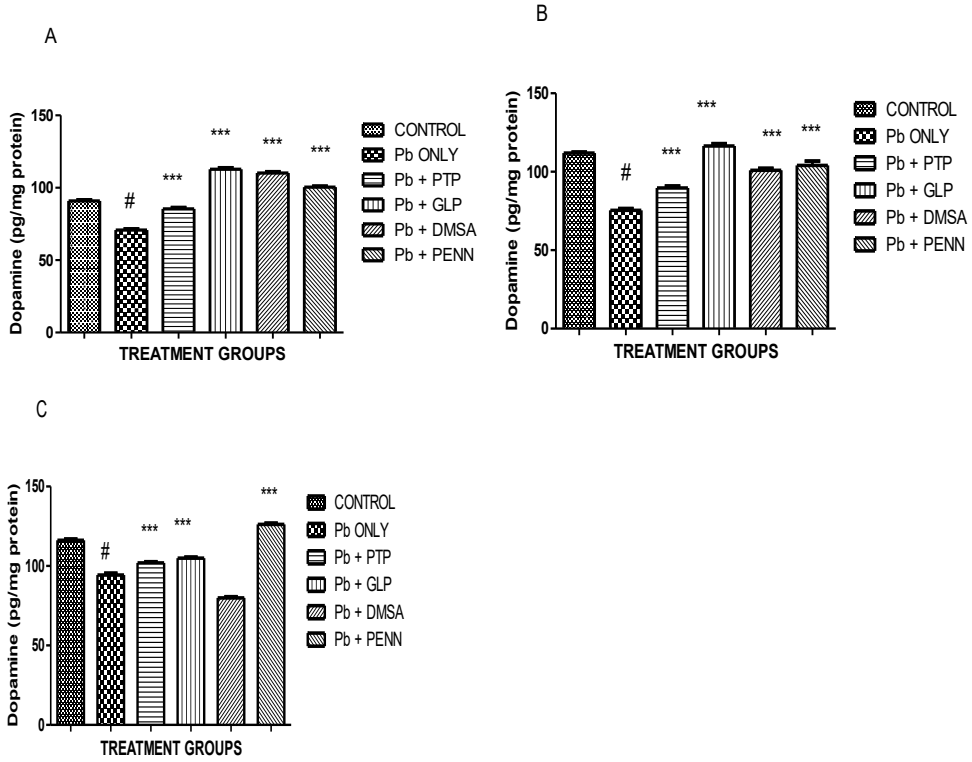

Figure 12: Effects of *G. lucidum* (GLP) and *P. tuberregium* (PTP) on Neurosignalling molecules. (A) Dopamine STR (B) Dopamine PFC (C) Dopamine HPC. Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and ***p<0.001 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine.

Pleurotus tuberregium **polysaccharides and** ***Ganoderma lucidum*** **polysaccharide increased the released of serotonin in lead exposed rats**

Figure 13 showed that administration of lead acetate produced significant (#p< 0.05) decreased in the level of serotonin in the rat brain regions with lead only group (25mg/kg) when compared with control group as revealed by one-way ANOVA followed by Post-hoc analysis. Meanwhile, PTP (100mg/kg) and GLP (100 mg/kg) increased significantly (**p< 0.01) (***p< 0.001) the concentration of serotonin. DMSA (50 mg/kg) and PENN (30 mg/kg) also significantly (***p< 0.001) increased the level of serotonin when compared with the lead acetate (25 mg/kg) only.

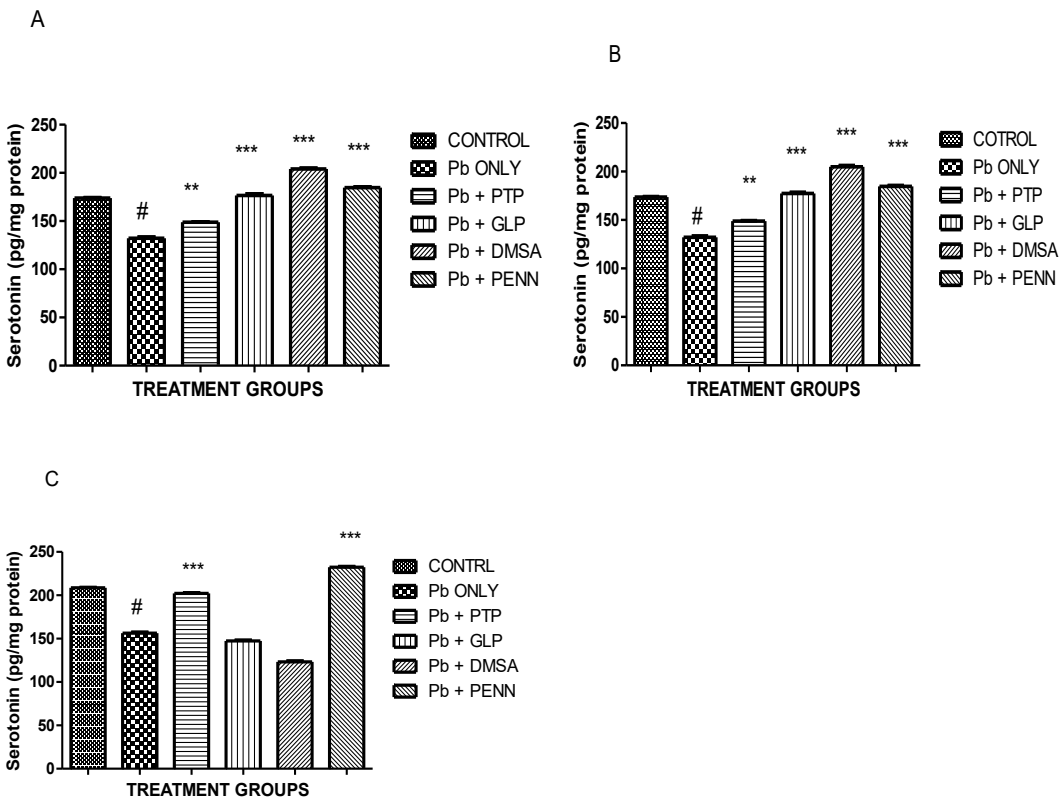

Figure 13: Effects of *G. lucidum* (GLP) and *P. tuberregium* (PTP) on Neurosignalling molecules. (A) Serotonin STR (B) Serotonin PFC (C) Serotonin HPC. Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and **p<0.05, ***p<0.05 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine

APOPTOTICS ENZYMES

Anti – apoptotic effects of *Pleurotus tuberregium* polysaccharides and *Ganoderma lucidum* polysaccharide on lead induced apoptosis in caspase 9

Represented in figure 14 is the result for the analysis of caspase 9. There was significant increased (#p< 0.05) in the expression of caspase 9 following exposure to lead acetate in Pb only group. However, treatment with PTP (100 mg/kg) and GLP (100 mg/kg) significantly (***p< 0.001) downregulated caspase 9 in HPC region of the brain when compared with lead only group.

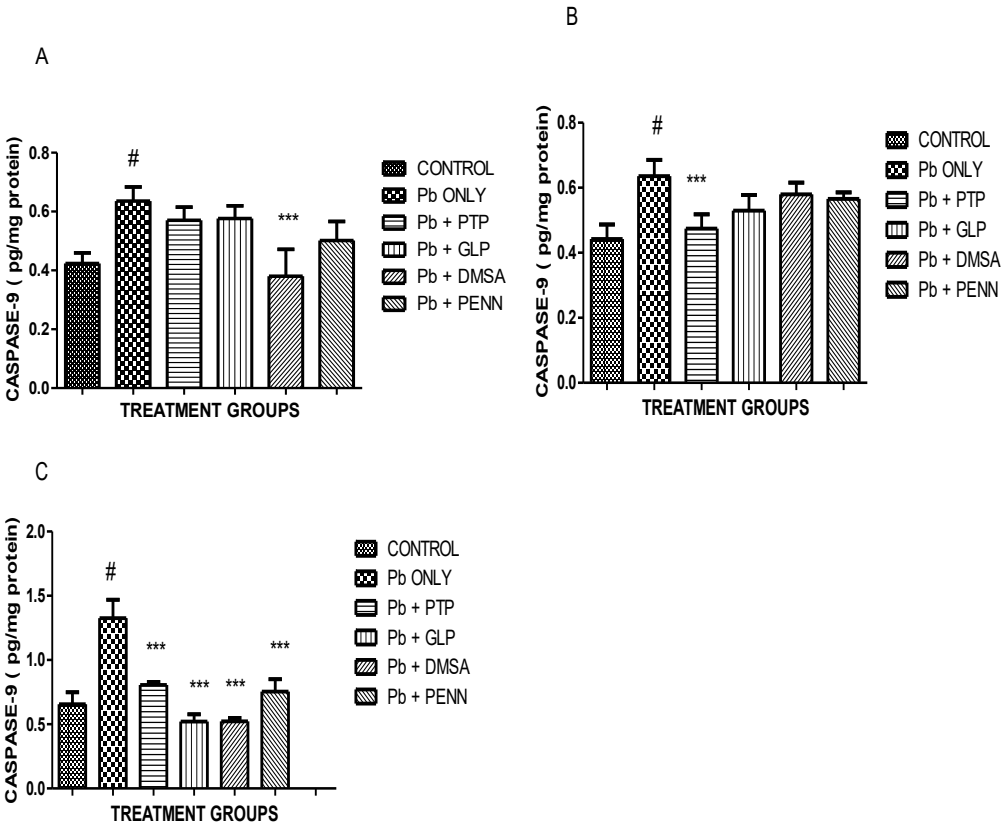

Figure 14: Effects of *G. lucidum* (GLP) and *P. tuberregium* (PTP) on Neurosignalling molecules (A) Caspase 9 STR (B) Caspase 9 PFC (C) Caspase 9 HPC. Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and ***p<0.001 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN-Pennicillamine

The effects of *Pleurotus tuberregium* polysaccharides and *Ganoderma lucidum* polysaccharide on apoptotic level in caspase 3

There was significant (#p< 0.05) increased in the expression of caspase 3 in the group treated with Pb only when compared with control group following exposure to lead acetate. However, treatment with PTP (100 mg/kg) and GLP (100 mg/kg) significantly downregulated caspase 3 in HPC region of the brain but not in STR and PFC when compared with lead treated group. Also, DMSA (50 mg/kg) and PENN (30 mg/kg) also significantly (***p< 0.001) increased the level of serotonin when compared with the lead acetate (25 mg/kg) only.

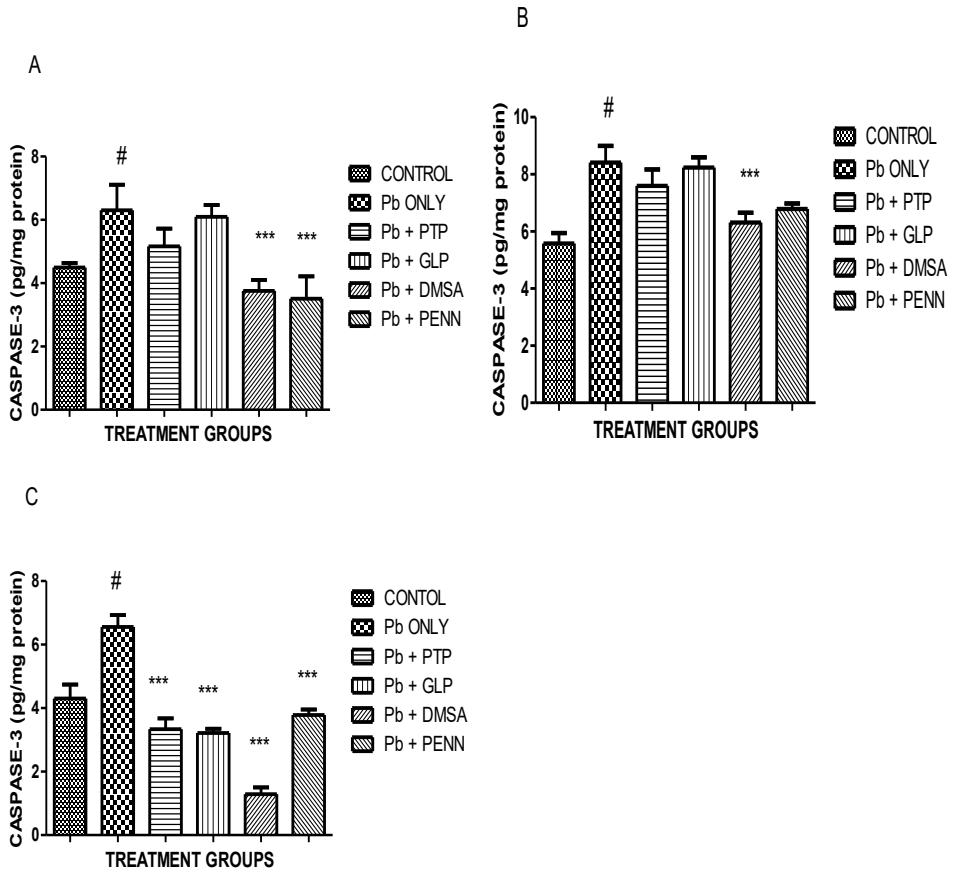

Figure 15: Effects of GLP and PTP on Neurosignalling molecules (A) Caspase 3 STR (B) Caspase 3 PFC (C) Caspase 3 HPC. Values are represented as Mean ± SEM (n=5). #p<0.05 compared with control and ***p<0.05 compared with Pb-only group. DMSA- 2, 3-dimercaptosuccinic acid, PENN- Pennicillamine .

Effects of *Pleurotus tuberregium* polysaccharides and *Ganoderma lucidum* polysaccharide on histoarchitectural alteration of the striatum in lead induced neurodegeneration in rats.

As depicted in figure 16, rat repeatedly exposed to lead acetate only (25 mg/kg) exhibit abnormal histoachitectural changes with some blotted and degenerated neuronal cells (black arrow), following staining with cresyl violet relative to non-lead control. However, administration of PTP (100mg/kg) and GLP (100 mg/kg) restored alteration caused by lead acetate in the striatum which shows normal neuronal cells including medium spiny neurons (blue arrow). Also, DMSA (50 mg/kg) and PENN (30mg/kg) reversed the effect of lead toxicity which shows normal neurofibrillary network (slender arrow)

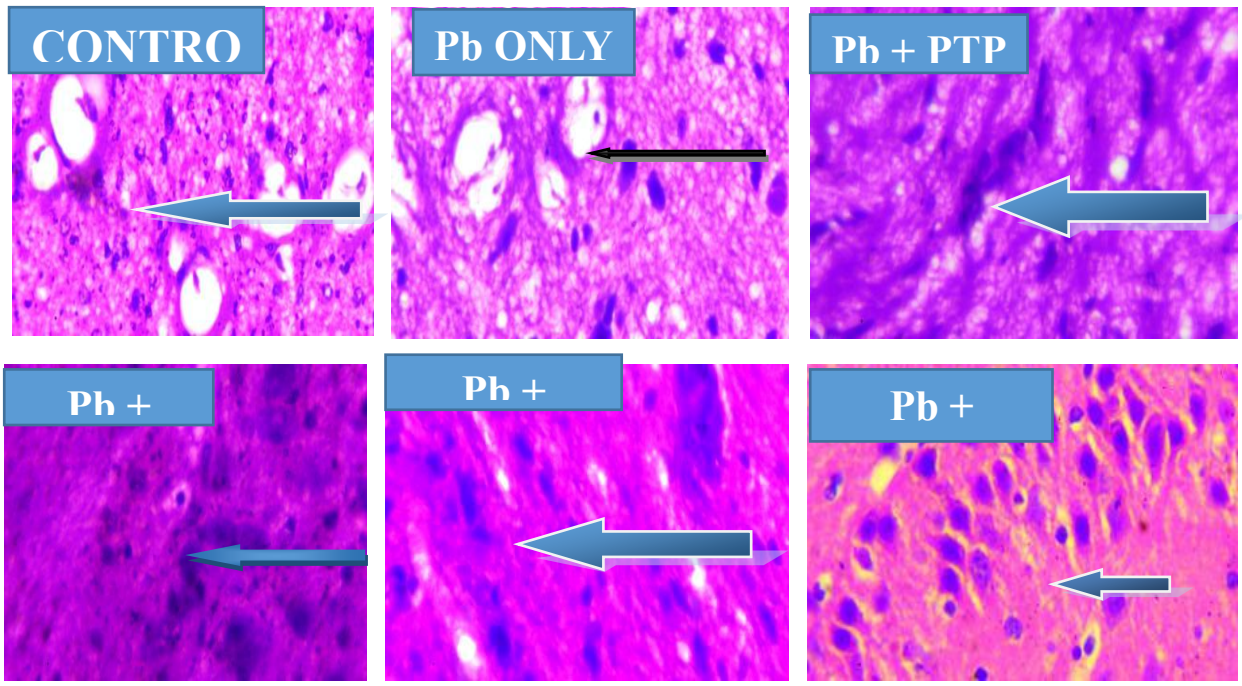

Figure 16. Effects of PTP and GLP on Pb-treated histoarchitectural degeneration in the striatum section of the brain. Hematoxylin-eosin stain, original magnification × 400. Black arrows indicate significant neuronal alteration caused by Pb and blue arrows indicate amelioration of the neuronal damage by PTP and GLP.

Effects of Pleurotus tuberregium polysaccharides and Ganoderma lucidum polysaccharide on histoarchitectural alteration of the hippocampus in lead induced neurodegeneration in rats.

As depicted in figure 17, rat repeatedly exposed to lead acetate only (25 mg/kg) exhibit abnormal histoachitectural changes with poor structural organization of the cornu Amonis Ca3 seen with severe depletion of neuronal cells (black arrow) on the hippocampus following staining with cresyl violet relative to non-lead control group. However, administration of PTP (100 mg/kg) and GLP (100 mg/kg) did not reversed histoarchitectural alteration caused by lead induced toxicity to the hippocampus because of the presence of the poor structural organization of the cornu ammonis including Ca3 (blue arrow) and mildy depleted pyramidal cell. Also, DMSA (50 mg/kg) and PENN (30mg/kg) could not reversed the abnormal structural changes (blue arrow) induced by lead acetate.

HIPPOCAMPUS SECTION OF A BRAIN

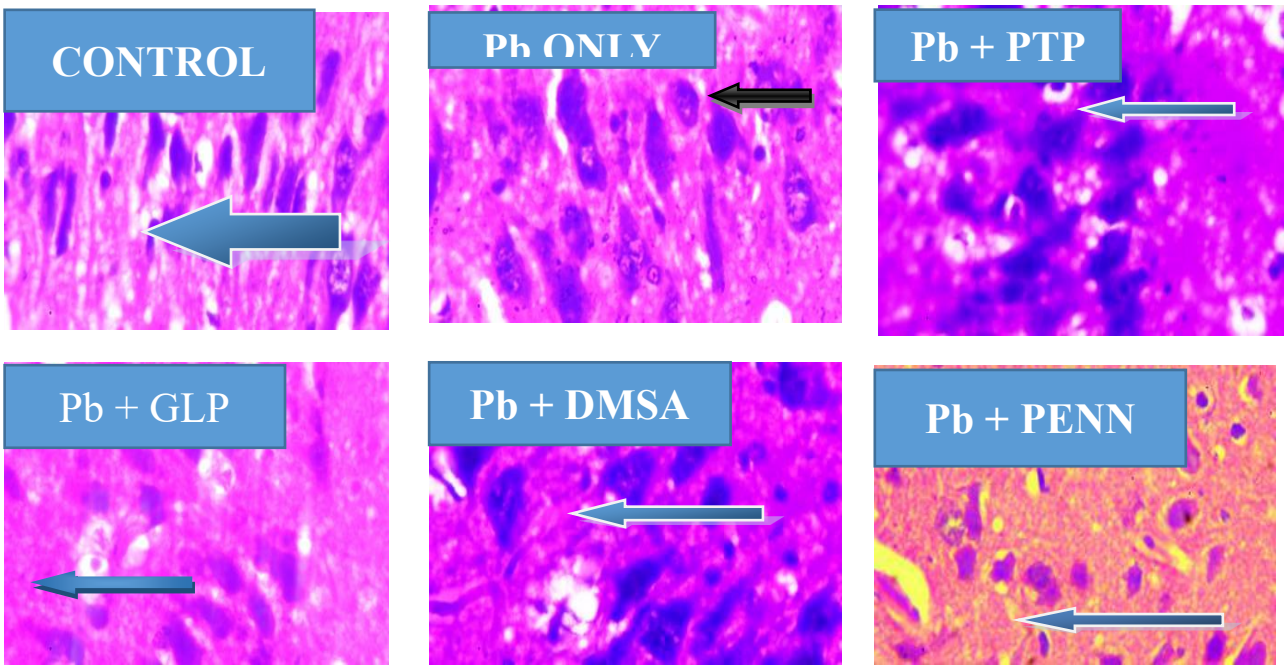

Figure 17. Effects of PTP and GLP on Pb-induced histoarchitectural alteration in the hippocampus. Hematoxylin-eosin stain, original magnification × 400. Black arrows indicate significant neuronal alteration caused by Pb and blue arrows indicate amelioration of the neuronal damage by PTP and GLP.

Effects of Pleurotus tuberregium polysaccharides and Ganoderma lucidum polysaccharide on histoarchitectural alteration of the prefrontal cortex in lead induced neurodegeneration in rats. As shown in figure 18, rat repeatedly exposed to lead acetate only (25 mg/kg) exhibit abnormal histoachitectural changes with some necrotic necrons with hyalinization (black arrow), following staining with cresyl violet relative to non-lead control. However, administration of PTP (100 mg/kg) and GLP (100 mg/kg) was able to restore alteration caused by lead acetate in the prefrontal cortex which shows normal laminae (blue arrow). Also, DMSA (50 mg/kg) and PENN (30 mg/kg) was able to reverse the effect of lead toxicity which shows normal necronal cells ((blue arrow).

PREFONTAL CORTEX OF BRAIN

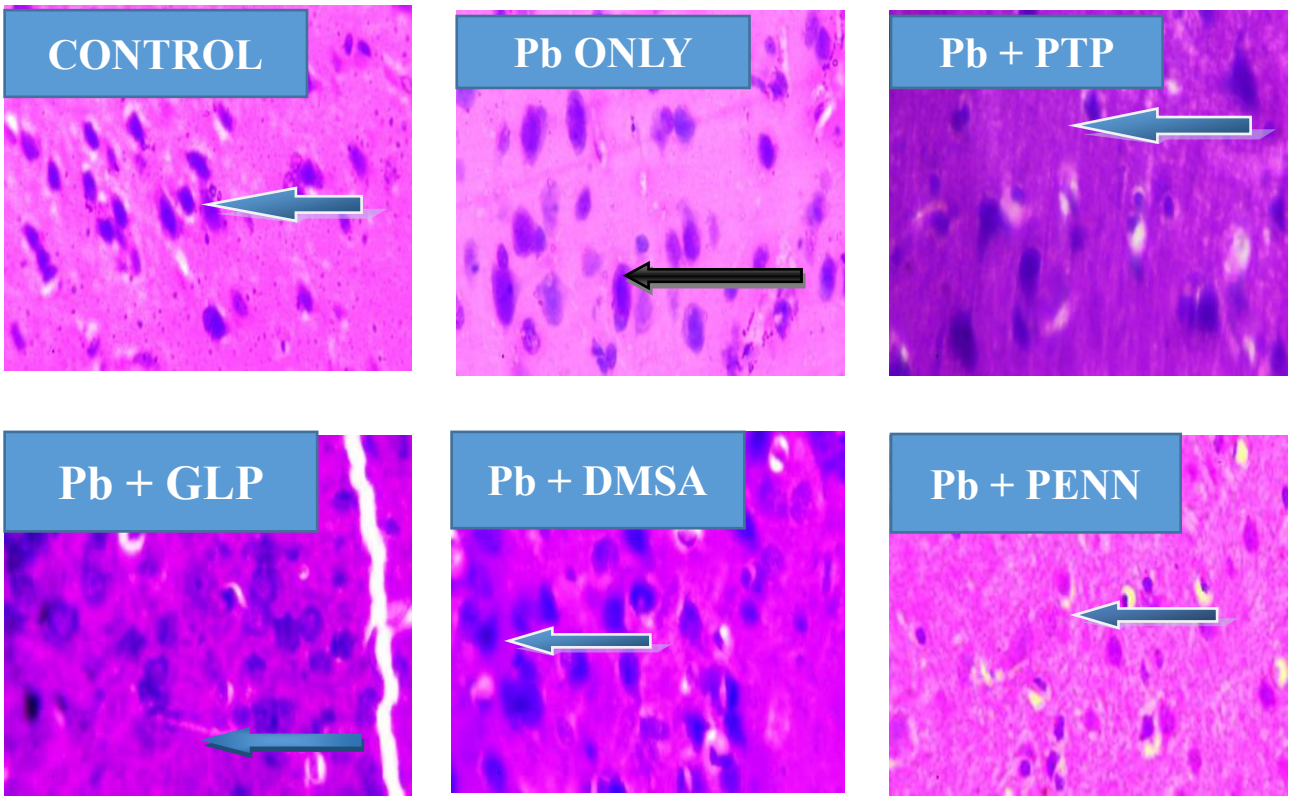

Figure 18. Effects of PTP and GLP on Pb-treated histoarchitectural degeneration in the prefrontal cortex of the brain. Hematoxylin-eosin stain, original magnification × 400. Black arrows indicate significant neuronal alteration caused by Pb and blue arrows indicate amelioration of the neuronal damage by PTP and GLP.

DISCUSSION

Circumstances and life challenges can alter brain functions and behavior, which may impact brain activities negatively. This study was designed to investigate the protective effects of *Plerotus tuberregium* polysaccharide (PTP) and *Ganoderma lucidum* polysaccharide (GLP) on lead induced neurotoxicity and neuroinflammatory on wistar rat, coupled with its underlying mechanism of action. Our investigation from this study showed rats exposed to lead toxicant, the group treated with lead acetate only, compared with the control group, displayed anxiety- and depression-like behaviors and poor cognitive performance in all the behavioral tests and showed significant increase in pro-inflammatory cytokines in the cerebral regions of the striatum, prefrontal cortex and hippocampus in which there were relationship between abnormal behavioral performances and the microglial activation. This cerebral inflammatory process was inhibited by the administration of PTP and GLP, and the lead-induced behavioral abnormalities were attenuated [26]. The results from this study revealed that, bioactive compounds present in PTP and GLP attenuate the poor cognitive performance, the anxiety and symptoms related to depressive-like behavior in rats. Oxidative stress tends to be the underlying mechanism of neuronal toxicity associated with lead exposure. This occurs, when the amount of reactive oxygen species (ROS) produced in the body system overwhelm the antioxidant defense system. Lead toxicity results in oxidative stress in biological membrane by releasing ROS such as superoxide radical, hydroxyl radical, lipid peroxide and hydrogen peroxide [27] According to the previous research, exposure to lead in rats resulted in neurodegeneration which occurs due to increase in lipid peroxidation product in NO and MDA levels, as well as decreased in neuronal SOD, CAT and enzymatic antioxidant GST activities, implying the induction of neuronal oxidative stress in striatum, prefrontal cortex and hippocampus. Increased in NO and MDA in lead treated animals may have occurred due to decrease in the level of SOD, CAT and GST, which resulted in the breakdown of lipid membranes due to accumulation of lipid peroxidation[28]. Administration of PTP and GLP resulted in a great drop of NO and MDA levels, but a considerable increased in SOD, CAT and GST levels, indicating

that they can boost the antioxidant defense system in the brain regions of striatum, prefrontal cortex and hippocampus. Increased in the level of anti-oxidant which occurred as a result of administration of PTP and GLP could lend credence to the improved neuronal cells. Also, the convectional drugs, DMSA and Penicillamine used as chelating agent in the management of lead toxicity down regulate the effect of lead acetate in NO and MDA level but up regulated the level of SOD, CAT and GST in the striatum, prefrontal cortex and hippocampus of the brain regions of the rats.

Lead acetate induced memory impairment and oxidative stress in the brain regions, especially in the striatum, prefrontal cortex and hippocampus region. More so, it is believed that, Pb has tendency to hinder the neurogenesis process of the brain which can result into cognition impairment, loss of memory and learning disabilities [27,28].. Natural substances which have the potential of increasing the cholinergic neuronal functions and learning, are effective against memory loss. PTP and GLP, a compound obtained from *Plerotus tuberregium* and *Ganoderma lucidum*, are examples of natural substances which increases cholinergic neuronal activities in the brain and can attenuate the memory impairment. Acetylcholinesterase (ACHE), glutamic acid decarboxylase (GAD) and monoamine (MOA) activities were analyzed in the striatum, prefrontal cortex and hippocampus region of the brain. These marker enzymes are essential for detecting the cholinergic status of the brain[29.] The acetylcholinesterase determination has supported the results obtained in NO and MDA activities of the rat brain in which the statistical reduction in the ACHE activities have caused the increment in the acetylcholine level in the brain regions of the striatum, prefrontal cortex and hippocampus which resulted in increased function of memory. Also increased in the level of glutamic acid decarboxylase and monoamine in the group treated with PTP and GLP have lent credence to the results obtained in SOD, CAT and GST of this study. Therefore, the current study suggests that extract of PTP and GLP have potential to increase cholinergic activities of the neuronal system and this resulted in increase in glutamic acid decarboxylase, monoamine oxidase and decrease in brain acetylcholineesterase level, which enhance improvement in memory functions[30.] Also, DMSA and Penicillamine which were used as standard drugs to compare the effects of PTP and GLP on lead induced toxicity ameliorated the effects of lead acetate in the striatum, prefrontal cortex and hippocampus.

The presence of oxidative stress in the brain results in neuroinflammation. In this study, we investigated the neuronal level of some pro-inflammatory cytokines i.e. IL-6 and TNF-α to ascertain the possibility of neuronal inflammation following exposure to lead acetate. When compared with the control animal, the presence of pro-inflammatory cytokines in lead treated only group is higher than the control group, showing that lead produced neuronal inflammation. Increase in the level of NO found in the brain of rats exposed to lead acetate could be the basis for development of neuro-inflammation. In this study, exposure to lead acetate increased the activity of IL-6 and TNF-α in the rat striatum, prefrontal cortex and hippocampus. More so, one-way ANOVA revealed the significant increase in brain concentration of IL-6 and TNF-α positively related to the molecular and clinical features following continuous exposure to neuro-toxicant which is lead acetate. However, administration of PTP and GLP significantly inhibit or suppress IL-6 and TNF-α release in a comparable manner to DMSA and Penicillamine in the striatum, prefrontal cortex and hippocampus which might be the reason for possible mechanism involved in anti-depressive effects as well as improved motor behavior. Furthermore, it has been suggested that, strong bioactive flavonoid and chelating agents could ameliorate pro-inflammatory cytokines release after exposure to lead acetate, and this may serve as a major contributing factor behind the clinical efficacy of DMSA and Penicillamine boosting the antioxidant defense system and anti-inflammatory properties of the brain in striatum, prefrontal cortex and hippocampus[31]. Serotonin (5 – HT) and dopamine are central nervous system neurotransmitters which involve in a range of neurological functions. Serotonin 5 – HT has been associated with mood changes and cognition, as well as other neurological function. Dopamine helps in reward function and controls locomotion[32]. Any changes in dopamine system functions, have been observed to be as result of response to chemically induced oxidative insult, that is, the genetic alteration that model oxidative stress and neurodegenerative disease. To our best understanding, this is the first study that explored the effects of PTP and GLP on serotonin and dopamine against lead toxicity. We found impairment in striatum, prefrontal cortex and hippocampus dopamine release and uptake in response to lead induced toxicity in the group treated with lead acetate only. Also, lead acetate inhibit the expression of serotonin in the group treated with lead acetate alone [33]. However, administration of PTP and GLP increase the level of dopamine in striatum, prefrontal cortex and hippocampus. Also, there was a significant increase in the level of serotonin in striatum, prefrontal cortex and hippocampus when compared with the group treated with lead acetate only. DMSA and penicillamine also ameliorated the effect of lead toxicity across the brain regions of the

rat in striatum, prefrontal cortex and hippocampus both in dopamine and in serotonin. Thus, administration of PTP and GLP increase the level of serotonin and dopamine in striatum, prefrontal cortex and hippocampus which enhance cognitive function, learning and memory [34].

Caspases belong to the family of endoproteases which provide critical links in cell regulatory networks controlling inflammation and cell death. Inadequate caspase activation enhances infection or tumorigenesis and hyper activation of caspases enhance neurodegeneration and inflammatory diseases[35]. The result of this study showed that expression of apoptosis related protein, caspase 3 and caspase 9 increased in the lead only group and decrease in the group treated with PTP and GLP, indicating that PTP and GLP can activate autophagy, reduce neuroinflammation and produce antiapoptotic effects [37]. DMSA and Penicillamine reduced the expression of caspase 3 and caspase 9 in striatum and hippocampus but not in prefrontal cortex.

To affirm the credibility and the potential effects of PTP and GLP, histoarctectural staining using cresyl violet staining techniques in striatum, prefrontal cortex and hippocampus in the rat brain were carried out. Neurodegenerative changes in lead only group were characterized with some blotted and degenerated neuronal cells following staining with cresyl violet relative to non-lead control group. PTP and GLP restore alteration caused by lead acetate in the striatum which show normal neuronal cells including medium spiny neurons. Also, DMSA and penicillamine reversed the effect of lead toxicity which shows normal neurofibrillary network., In hippocampus, lead acetate caused poor structural organization of the cornu amonis Ca1, Ca2, Ca3 with severe depletion of neuronal cell. However, PTP and GLP did not restore histoarchitectural alteration caused by lead induced toxicity to the hippocampus because of the poor structural organization of the crnu ammonis including Ca 3 and mild depleted pyramidal cell. Also, DMSA and penicillamine did not reverse the abnormal structural changes induced by lead acetate. In prefrontal cortex, the rat brain exposed to lead-induced neuroinflammation showed abnormal histoarchitectural alteration as depicted with some necrotic neurons and hyalinization, following staining with cresyl violet. However, administration of PTP and GLP restore the alteration caused by lead acetate in the prefrontal cortex which shows normal laminae. Also, DMSA and Penicillamine reverse the effects of lead toxicity which shows normal necronal cells. These results align and further confirmed the neuroprotective and anti-inflammatory effects of PTP and GLP as seen in this study.

CONCLUSSION

In conclusion, this comprehensive study has revealed the neuroprotective effects of PTP and GLP and its ability to ameliorate mechanism related to the released of oxidative stress, proinflammatory cytokines, apoptotic marker enzymes and histoarchitectural alteration in the rat striatum, prefrontal cortex and hippocampus. Our findings therefore suggest that PTP and GLP are promising natural substances with strong antioxidant, antineuroinflammatory and anti-apoptotic properties. Taking all the evidences into consideration, GLP and PTP might be taken as supplement and could have a promising effects in the treatment of neurological disorder induced by lead

REFERENCES

1. Lane CA, Hardy J, Schott JM. Alzheimer's disease. Eur J Neurol. 2018 Jan;25(1):59-70. doi: 10.1111/ene.13439.
2. Adebayo OG, Wopara I, Ben-Azu B, Aduema W, Adebayo OR, Nicodemus OU. Acute lead (II) acetate 3-hydrate neurotoxicity alters neurocognition and induced depressive-like behavior via 5-hydroxytryphtamine, neurohormone melatonin and (Na+, K+) ATPase activity in female wistar rats. Asian Journal of Biotechnology and Genetic Engineering (AJBGE). 2020;3(1):31-40. Asian J. Biotechnol. Gen. Eng
3. Ahmad M, Butt MA, Zhang G, Sultana S, Tariq A, Zafar M. Bergenia ciliata: A comprehensive review of its traditional uses, phytochemistry, pharmacology and safety. Biomed Pharmacother. 2018 Jan;97:708-721. doi: 10.1016/j.biopha.2017.10.141.
4. Imosemi IO, Owoeye O, Ao M. Lead-induced oxidative stress in postnatal developing cerebellum of Wistar rats: Role of aqueous extract of Cucumis sativus Linn and vitamin. MOJ Anat. Physiol. 2020;7:104-13.
5. Okwulehie IC, Ogoke JA. Bioactive, nutritional and heavy metal constituents of some edible mushrooms found in Abia State of Nigeria. International Journal of Applied Microbiology and Biotechnology Research. 2013 Jul;1(2):7-15. Int J App Microbio Biotech Res.
6. Lee CH, Lee YY, Chang YC, Pon WL, Lee SP, Wali N, Nakazawa T, Honda Y, Shie JJ, Hsueh YP. A carnivorous mushroom paralyzes and kills nematodes via a volatile ketone. Sci Adv. 2023 Jan 18;9(3):eade4809. doi: 10.1126/sciadv.ade4809.

7. Abdullah E, Abidin NZ, Abdullah N. Cytotoxic Proteins from King Tuber Oyster Medicinal Mushroom, Pleurotus tuber-regium (Agaricomycetes), Sclerotium against Human MDA-MB-231 Breast Cancer Cells. Int J Med Mushrooms. 2022;24(1):27-40. doi: 10.1615/IntJMedMushrooms.2021041548.
8. Agoreyo BO, Oseghale EI. Effect of post-harvest storage at ambient temperature on nutritional constituents, in vitro antioxidant activity and gc-ms profile of king tuber mushroom (pleurotus tuber-regium). Eur. J. Food Sci. Technol. 2019 Aug;7:24-46.
9. Huang HY, Korivi M, Chaing YY, Chien TY, Tsai YC. Pleurotus tuber-regium Polysaccharides Attenuate Hyperglycemia and Oxidative Stress in Experimental Diabetic Rats. Evid Based Complement Alternat Med. 2012;2012:856381. doi: 10.1155/2012/856381.
10. Ohiri RC, Amadi BA, Isoje FE. Phytochemical confinement in sections of pleurotus tuber-regium (king tuber mushroom) basidocarp. Am. J. Biomed. Sci. 2018 Oct 1;10(4):195-201
11. Oni JO, Akomaye FA, Markson AA, Egwu AC. GC-MS analysis of bioactive compounds in some wild-edible mushrooms from Calabar, Southern Nigeria. European Journal of Biology and Biotechnology. 2020 Dec 18;1(6). doi: 10.24018/ejbio.2020.1.6.129
12. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979 Jun;95(2):351-8. doi: 10.1016/0003-2697(79)90738-3.
13. Zhao C, Zhang C, Xing Z, Ahmad Z, Li JS, Chang MW. Pharmacological effects of natural Ganoderma and its extracts on neurological diseases: A comprehensive review. Int J Biol Macromol. 2019 Jan;121:1160-1178. doi: 10.1016/j.ijbiomac.2018.10.076.
14. Zhang Y, Luo J, Huang NH. Effects of Ganoderma lucidum triterpenoids on apoptosis of brain tissue of Alzheimer disease model rat. J Guiyang Med Coll. 2016;41(5):532-4.
15. Cai Q, Li Y, Pei G. Polysaccharides from Ganoderma lucidum attenuate microglia-mediated neuroinflammation and modulate microglial phagocytosis and behavioural response. J Neuroinflammation. 2017 Mar 24;14(1):63. doi: 10.1186/s12974-017-0839-0.
16. Özevren H, İrtegün S, Deveci E, Aşır F, Pektanç G, Deveci Ş. Ganoderma Lucidum Protects Rat Brain Tissue Against Trauma-Induced Oxidative Stress. Korean J Neurotrauma. 2017 Oct;13(2):76-84. doi: 10.13004/kjnt.2017.13.2.76.
17. Izídio GS, Lopes DM, Spricigo L Jr, Ramos A. Common variations in the pretest environment influence genotypic comparisons in models of anxiety. Genes Brain Behav. 2005 Oct;4(7):412-9. doi: 10.1111/j.1601-183X.2005.00121.x.
18. El-Tantawy WH. Antioxidant effects of Spirulina supplement against lead acetate-induced hepatic injury in rats. J Tradit Complement Med. 2015 Mar 27;6(4):327-331. doi: 10.1016/j.jtcme.2015.02.001.
19. Flora SJ, Saxena G, Gautam P, Kaur P, Gill KD. Response of lead-induced oxidative stress and alterations in biogenic amines in different rat brain regions to combined administration of DMSA and MiADMSA. Chem Biol Interact. 2007 Dec 15;170(3):209-20. doi: 10.1016/j.cbi.2007.08.003.
20. Ferrero ME. Rationale for the Successful Management of EDTA Chelation Therapy in Human Burden by Toxic Metals. Biomed Res Int. 2016;2016:8274504. doi: 10.1155/2016/8274504.
21. Kim HC, Jang TW, Chae HJ, Choi WJ, Ha MN, Ye BJ, Kim BG, Jeon MJ, Kim SY, Hong YS. Evaluation and management of lead exposure. Ann Occup Environ Med. 2015 Dec 15;27:30. doi: 10.1186/s40557-015-0085-9.
22. Gürağaç Dereli FT, Ilhan M, Küpeli Akkol E. Discovery of new antidepressant agents: In vivo study on Anthemis wiedemanniana Fisch. & Mey. J Ethnopharmacol. 2018 Nov 15;226:11-16. doi: 10.1016/j.jep.2018.07.019.
23. Mingorance C, Andriantsitohaina R, Alvarez de Sotomayor M. Cedrelopsis grevei improves endothelial vasodilatation in aged rats through an increase of NO participation. J Ethnopharmacol. 2008 Apr 17;117(1):76-83. doi: 10.1016/j.jep.2008.01.019.
24. Beers RF Jr, Sizer IW. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem. 1952 Mar;195(1):133-40.
25. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem. 1969 Nov 25;244(22):6049-55.
26. Asiwe JN, Asiwe N, Onuh JE. The Effect of High Dietary Salt Consumption on Renal Function in Streptozotocin-Induced Diabetic Male Wistar Rats. Int J Nutr Sci. 2021;6(4):201-207. doi: 10.30476/IJNS.2021.92685.1153.

27. Cao P, Chen C, Liu A, Shan Q, Zhu X, Jia C, Peng X, Zhang M, Farzinpour Z, Zhou W, Wang H, Zhou JN, Song X, Wang L, Tao W, Zheng C, Zhang Y, Ding YQ, Jin Y, Xu L, Zhang Z. Early-life inflammation promotes depressive symptoms in adolescence via microglial engulfment of dendritic spines. Neuron. 2021 Aug 18;109(16):2573-2589.e9. doi: 10.1016/j.neuron.2021.06.012.
28. Li Y, Liu H, Qi H, Tang W, Zhang C, Liu Z, Liu Y, Wei X, Kong Z, Jia S, Du B, Yuan J, Wang C, Li M. Probiotic fermentation of Ganoderma lucidum fruiting body extracts promoted its immunostimulatory activity in mice with dexamethasone-induced immunosuppression. Biomed Pharmacother. 2021 Sep; 141:111909. doi: 10.1016/j.biopha.2021.111909.
29. Shaban El-Neweshy M, Said El-Sayed Y. Influence of vitamin C supplementation on lead-induced histopathological alterations in male rats. Exp Toxicol Pathol. 2011 Mar;63(3):221-7. doi: 10.1016/j.etp.2009.12.003.
30. Aprioku JS. Pharmacology of free radicals and the impact of reactive oxygen species on the testis. J Reprod Infertil. 2013 Oct;14(4):158-72.
31. Kouémou NE, Taiwe GS, Moto FCO, Pale S, Ngoupaye GT, Njapdounke JSK, Nkantchoua GCN, Pahaye DB, Bum EN. Nootropic and Neuroprotective Effects of *Dichrocephala integrifolia* on Scopolamine Mouse Model of Alzheimer's Disease. Front Pharmacol. 2017 Nov 21;8:847. doi: 10.3389/fphar.2017.00847.
32. Agarwal NB, Agarwal NK, Mediratta PK, Sharma KK. Effect of lamotrigine, oxcarbazepine and topiramate on cognitive functions and oxidative stress in PTZ-kindled mice. Seizure. 2011 Apr;20(3):257-62. doi: 10.1016/j.seizure.2010.12.006.
33. Ortiz AN, Oien DB, Moskovitz J, Johnson MA. Quantification of reserve pool dopamine in methionine sulfoxide reductase A null mice. Neuroscience. 2011 Mar 17; 177:223-9. doi: 10.1016/j.neuroscience.2011.01.001.
34. Ortiz AN, Kurth BJ, Osterhaus GL, Johnson MA. Dysregulation of intracellular dopamine stores revealed in the R6/2 mouse striatum. J Neurochem. 2010 Feb;112(3):755-61. doi: 10.1111/j.1471-4159.2009.06501.x.
35. Radi E, Formichi P, Battisti C, Federico A. Apoptosis and oxidative stress in neurodegenerative diseases. J Alzheimers Dis. 2014;42 Suppl 3:S125-52. doi: 10.3233/JAD-132738.
36. Ren D, Tu HC, Kim H, Wang GX, Bean GR, Takeuchi O, Jeffers JR, Zambetti GP, Hsieh JJ, Cheng EH. BID, BIM, and PUMA are essential for activation of the BAX- and BAK-dependent cell death program. Science. 2010 Dec 3;330(6009):1390-3. doi:10.1126/science.1190217.