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Monodora Myristica Protects Against Oxidative Stress in the Drosophila Melanogaster Model of Lead-Induced Neurotoxicity

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Abstract

Background to Study: Lead (Pb) is ubiquitous in the environment and toxic. It has many negative effects on many organs and induces biochemical, physiological, and behavioural alterations on the brain. *Monodora myristica* is a calabash nutmeg belonging to the family of Annonaceae.*It is* a rich source of flavonoids which have been shown to exhibit both antioxi-dant and anti-inflammatory properties. We evaluated the protective role of *Monodora myristica* on lead-induced toxicity in D. melanogaster in this study.

Materials and Methods: Wild-type D. Melanogaster of both genders (1–3 days old) was divided into four groups. group 1-Control was fed with diet only; Group 2-Pb (50mg/kg diet),Group 3 -*Monodora myristica* (100mg /kg diet) and Group 4 -Pb 50mg/kg diet& *Monodora myristica* (100mg /kg diet were exposed for 7 days, Biochemical assay of catalase CAT, super-oxide dismutase (SOD), glutathione S transferase (GST), reductase glutathione GSH, glutathione peroxidase (GPx) total an-tioxidants capacity (TAC), hydrogen peroxide (H_2O_2) and Malondialdehyde (MDA) were assayed using manufacturer proto-cols.

Results: It indicated that lead acetate significantly increased ($p < 0.05$) the lipid peroxidation marker (MDA), and nitric oxide while lowering levels of the antioxidant biomarkers (SOD, CAT, TAC, GST, GPx and GSH). Co-exposure of flies to lead acetate and *Monodora myristica* significantly restored antioxidant status and alleviated the accumulation of N_2O and MDA in the flies.

Conclusion: Our findings thus suggest that *Monodora myristica* ameliorated lead-induced neurotoxicity in D. melanogaster via its antioxidant properties.

Keywords: Antioxidants; Lead Acetate; Drosophila Melanogaster

Introduction

The widespread use of Lead (Pb) based paints, batteries, pipes, gasoline, petrol, cosmetic products, children's toys, food cans, ammunition, herbal medicines, vitrified ceramics, industrial processes such as smelting of Pb and its combustion, pottery, boat building, the printing of books etc. has caused extensive environmental contamination [1] Consequently, Pb is persistent in the environment and detected in soil in the range of >50 ppm to 200 ppm; in surface water and groundwater, 5 and 30 μg/L and air <0.05 $\mu g/m^3$ [2].

Humans are exposed to Pb through the air, water, and food in polluted areas [3]. Pb enters into the human body through inhalation, ingestion, or skin penetration. It is a risk factor for, neurodevelopmental and neurodegenerative diseases [4]; and is considered the 2nd most hazardous heavy metal, contributing to 0.6 % of public health complications [1]. Even though Pb-induced toxicity has been linked to human diseases, the exact mechanism is not fully understood.

The central nervous system (CNS)" is the primary target for Pb toxicity, and the developing brain is highly vulnerable to its toxic effects [5]. Pb mimics calcium ions (Ca^{+2}), and it crosses the BBB; as a result, it accumulated in the brain and disrupts the brain's structural components [6]. Pb in the developing brain interferes with critical developmental processes, i.e., cellular proliferation, migration, differentiation, synaptogenesis, myelination, and apoptosis. Increased neurodegeneration and the disruptions in neural networking result in brain damage, neurochemical dysfunction, impaired cognitive development and clinical brain dysfunction leading to several neurobehavioral and psychological anomalies [7].

The mechanisms of neurotoxicity caused by Pb are complex and are unknown. However, the primary evidence points to oxidative stress playing a significant role in Pb neurotoxicity [8]. This is strengthened by the observation of Shilpa et al. [9], which postulated that modulation of the heme synthesis pathway by Pb can induce oxidative stress. These oxidative free radicals damage biomolecules such as proteins, lipids, and nucleic acids and are implicated in the aetiology of cancers, and neurodevelopmental and neurodegenerative disorders [10]. Even though the potential role of antioxidants in protecting the brain against Pb-induced oxidative stress and neurotoxicity is known, the mechanism of Pb-induced oxidative stress to cause neurotoxicity is not well established.

Drosophila melanogaster is commonly used in toxicological studies as an alternative to mammalian models. The advantages of using the fly as a model include its small size, ease of maintenance, short generation time (9–12 days), very short lifespan (55–80 days), and ability to generate large homogeneous populations [11]. Although *Drosophila* and humans are distinct, the brain's basic neuronal units and support cells are similar. The mushroom bodies in the *Drosophila* brain and the vertebrate pallium or hippocampus share a common origin. Several essential synaptic proteins, many processes operating during neural development, and the BBB are evolutionarily conserved between mammals and flies [12]. The *Drosophila* genome is fully sequenced; extensive conservation of genes, biological functions, developmental processes, and disease progression obey evolutionarily conserved mechanisms between fly and humans Indeed, Drosophila demonstrated the neuronal changes with response to low doses of Pb, making a good model for elucidating Pb-induced neurotoxicity mechanisms [13].

The modern pharmaceutical industry is founded on compounds identified in medicinal plants .Numerous studies have shown how the antioxidant qualities of natural products serve to reduce the toxicity of heavy metal poisoning, and a variety of plant-based extracts and medications have shown promise in the treatment of a variety of neurological illnesses. Mondora myristica is a tropical plant that is a member of the Annonaceae family. It is also known by the name calabash, African nutmeg, and Jamaica. This widely widespread plant is found in Africa, Asia, Australia, Central America, and South America; it has received little research and usage [14]. It is used to treat a wide range of illnesses, including stomach aches and arthritis. The antioxidant qualities of M. myristica seeds have been documented in earlier research [15].

In this study, we investigated the antioxidant effects of *Monodora myristica* on lead neurotoxicity using the drosophilia model.

Materials and Methods

Drosophila melanogaster stock and culture

D. melanogaster wild-type (Harwich strain) flies were obtained from the Department of Anatomy University of Lagos Nigeria. The flies were maintained and reared in Neuro-Provictoire Drosophila Laboratory, Department of Anatomy, University of Port Harcourt, Nigeria on cornmeal medium containing 1 % w/v brewer's yeast, 2 % w/v sucrose, 1 % w/v agar, and 0.08 % v/w nipagin at constant temperature and humidity (22–24 °C; 60–70 % relative humidity) under 12 h dark/light cycle conditions.

Lead Exposure and *Monodora Myristica* Treatment

Wild-type D. Melanogaster of both genders (1–3 days old) was divided into four groups. Group 1- Control was fed with diet only; Group 2-Pb (50mg/kg diet), [16].Group 3- *Monodora myristica* (100mg /kg diet) [15].and Group 4- Pb 50mg/kg diet & *Mono-dora myristica* (100mg /kg diet were exposed for 7 days.

Preparation of Samples for Biochemical Assays

The fly heads were rapidly separated, homogenized, and Biochemical assay of catalase CAT, superoxide dismutase (SOD), glutathione S transferase (GST), reductase glutathione GSH, glutathione peroxidase (GPx) total antioxidants capacity (TAC), hydrogen peroxide (N2O) and Malondialdehyde (MDA) were assayed using manufacturer protocols all the assays were carried out in du-plicates for each of the 5 replicates of control and treated flies.

Superoxide Dismutase (SOD) Activity

Calculating the activity of superoxide dismutase (SOD). We measured superoxide dismutase activity using the protocol outlined by Misra and Fridovich17 [16]. This is primarily based on SOD's capacity to prevent adrenaline from autoxidizing at pH 10.2.

Catalase (CAT) Activity

The Clairborne method was somewhat modified to determine the catalase activity. The idea that catalase in sample preparation splits hydrogen peroxide, which can be detected spectrophotometrically at 240 nm, supports this method [18].

Determination of Nitric Oxide (Nitrate/Nitrite) Level

The method of Griess reaction was used to quantify the level of nitric oxide (nitrate and nitrite). 250 µL of sample was incubated at room temperature for 20 minutes with 250 µL of Griess reagent (0.1 % N-(1-naphthyl) ethylenediamine dihydrochloride; 1% sulfanilamide in 5% phosphoric acid; 1:1 ratio). By comparing the absorbance at 550 nm (OD 550) with the OD 550 of a standard solution containing known amounts of sodium nitrite, the nitrite concentration was determined using spectrophotometry [19].

Determination of Glutathione S-Transferase Activity

Using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate, the Habig and Jakoby [20] technique was utilised to assess the activity of GST. 270 µL of a solution (20 µL of 0.25 M potassium phosphate buffer, pH 7.0, with 2.5 mM EDTA, 10.5 µL of distilled water, and 500 µL of 0.1 M GSH at 25 °C) was included in the reaction mixture, along with 10 µL of 25 mM CDNB and 20 µL of sample (1:5 dilution). A spectro-photometer was used to track the mixture at 340 nm for five minutes at 10-second intervals.

Determination of Reduced Glutathione (GSH) Activity

Calculating the lowered amount of glutathione (GSH). Using the method outlined by Sedlak and Lindsay [21], the reduced glutathione level was calculated.

Determination of Hydrogen Peroxide Level

The level of hydrogen peroxide level was carried out using the method of Wolff [22]. The reaction mixture consisted of FOX 1 (10ml of 100 mM xylenol orange, 50ml of 250 mM ammonium ferrous sulfate, 10ml of 100 mM sorbitol, 5ml of 25 mM H_2SO_4 and 30ml of distilled water) mixed with the sample. After 30 minutes incubation at room temperature, the absorbance was measured at 560 nm. The values were extrapolated from the standard curve and expressed in micromole per milligram protein.

Determination of MDA level

Lipid peroxidation marker (MDA) activity The MDA level was assayed by using the procedure of Ohkawa and Ohishi [23]. Under an acidic medium, MDA reacts with the chromogenic reagent, 2-thiobarbituric acid (TBA), to form a pink-coloured complex at 532 nm absorbance.

Statistical Analyses

The biochemical data are presented as the Mean ± SEM. One-way Analysis of variance (ANOVA) was used to assess the significant differences among multiple groups under various treatments, followed by Dunett's posthoc test. In all the groups, differences were considered statistically significant among groups with $p < 0.05$, using the GraphPad Prism5.0 software.

Results

Figure 1:Effect of Pb on antioxidant enzymes.The activity of SOD,CAT,TAC,GST ,GPx and GSH were significantly increased in Monodora treated group and decreased in the lead acetate treated group which is shown in Figure 1.

Figure 2:Effect of Pb on markers of oxidative stress, It indicated that lead acetate significantly increased ($p < 0.05$) the lipid peroxidation marker (MDA), and nitric oxide while lowering levels of the antioxidant biomarkers (SOD, CAT, TAC, GST, GPx and GSH). Co-exposure of flies to lead acetate and *Monodora myristica* significantly restored antioxidant status and alleviated the accumulation of NO and MDA in the flies.

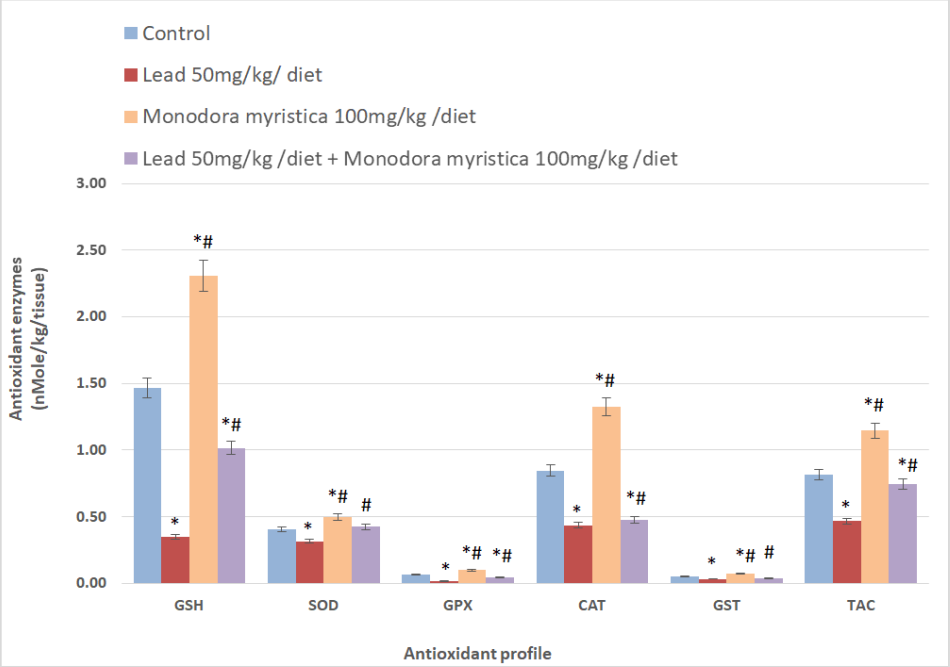

Figure 1: The effect of *Monodora myristica* on the antioxidant profile

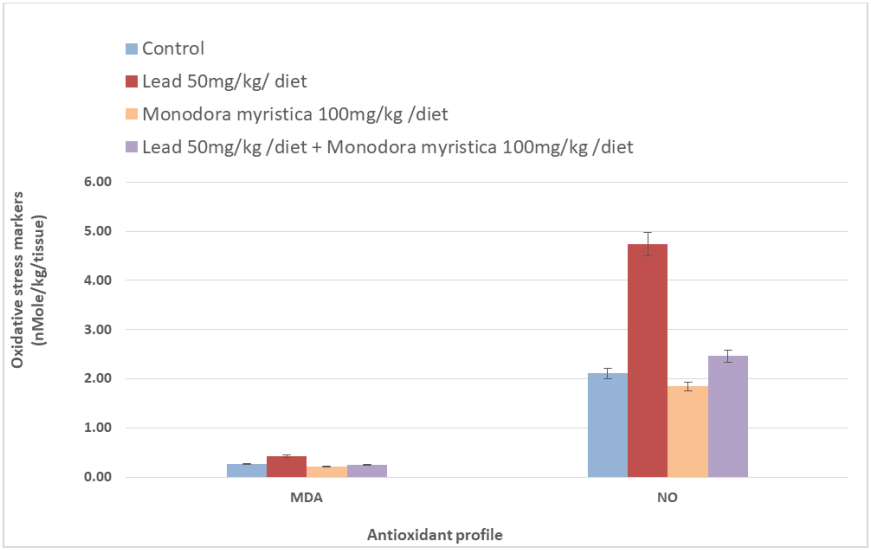

Figure 2: The effect of *Monodora myristica* on oxidative stress markers

Discussion

Drosophila melanogaster has become a model system in neurotoxicology and genetics due to its, short life span, and quick generation time, D. melanogaster has previously been utilized as an additional and alternative model for researching diseases that could be linked to lead acetate[24].This study assesed *Monodora myristica* attenuating effects on lead acetate-induced toxicity in Wild--type D. melanogaster. *Monodora myristica* has been demonstrated to have antioxidative effects using animal models [15].

Pb exposure during the developmental period is considered a significant risk factor as it markedly alters brain development resulting in biochemical, physiological, and anatomical changes [25]. Even though Pb-induced oxidative stress is the causative factor for neurotoxicity, its precise molecular mechanism is not evidenced [25].

There is a paucity of information on the antioxidant effect of *M myristica* on lead-induced neurotoxicity of the drosophila model. The present study showed that lead acetate significantly increased the lipid peroxidation marker (MDA), and nitric oxide while lowering levels of the oxidative biomarkers (SOD, CAT, TAC, GST, GPx and GSH). Co-exposure of flies to lead acetate and *M myristica* significantly restored antioxidant status and alleviated the accumulation of NO and MDA in the flies.Pb caused significant elevations in NO and MDS in the head when compared with control.

The activities of SOD, CAT and GST were significantly increased in the *M. myristica-treated* group and decreased in the lead acetate-treated group. This is in line with a study done by Venkareddy, 2015 areas [26] on the potential of casein as a nutrient inter-vention to alleviate lead (Pb) acetate-mediated oxidative stress and neurotoxicity: first evidence in Drosophila melanogaster. *Neu-rotoxicology*, showed that the activity of CAT,SOD and GST were significantly increased in casein treated group and decreased in the lead acetate-treated group and also increased the level of NO in the lead treated group.

There is a dearth of information on the antioxidant effect of *M myristica* on lead-induced neurotoxicity using the drosophila model however,related study on other natural antidotes showed an antioxidant effect which is similar to the above study.

Abolaji et al 2020 areas [27]. study on the topic 'Curcumin Attenuates Copper-induced Oxidative Stress and Neurotoxicity in *Drosophila melanogaster*' indicated that copper toxicity was also associated with a marked decrease in total thiol (T-SH), as well as catalase and glutathione S transferase activities, contemporaneous with an increased in nitric oxide (nitrate and nitrite). Co-exposure of flies to Cu2+ and Curcumin restored cellular antioxidant status, as well as alleviated the accumulation of nitric oxide levels in the flies. Curcumin ameliorated oxidative damage in the flies as evidenced by restoration of antioxidant status. Shilpa et al 2021 [28] in their study on (Pb) induced oxidative stress as a mechanism to cause neurotoxicity in Drosophila melanogaster revealed that Pb accumulation in the Drosophila brain induces oxidative stress by generating reactive oxygen species (ROS) and lipid peroxidation (LPO), depleting antioxidant enzymes which are in line with the present study.

Shilpa, O., Anupama et al 2021 research work on (Pb) induced oxidative stress as a mechanism to cause neurotoxicity in Drosophila melanogaster revealed that Pb accumulation in the Drosophila brain induces oxidative stress by generating reactive oxygen species (ROS) and lipid peroxidation (LPO), depleting antioxidant enzymes which is in line with the present study.[28].

Due to the ability of oxidative stress to induce several health problems, it activates mitochondrial and cellular dysfunction. When the generation of free radicals surpasses the counteracting effects of endogenous antioxidants, reactive oxygen species (ROS) become extremely noxious to the central nervous system. Lead acetate exposure to the brain causes oxidative stress by activating pro-oxidative pathways, including the stimulation of NADPH oxidase, an enzyme responsible for generating superoxide radicals. Lead--induced ROS, such as superoxide radicals and hydroxyl radicals, can initiate and propagate lipid peroxidation. Malondialdehyde (MDA), a marker of lipid peroxidation, is increased as a result.

The decreased activities of Superoxide Dismutase (SOD) and Catalase (CAT) levels may be attributed to the binding of lead acetate with the sulfhydryl groups of these enzymes. This binding and the subsequent substitution of endogenous redox metals alter the configurations of these enzymes, leading to their inhibition. This, in turn, reduces the cell's ability to neutralize and detoxify ROS.[29]. The depletion in glutathione (GSH) content may be a consequence of its utilization in scavenging the generated free radicals. GSH, a crucial antioxidant, becomes depleted as it is consumed in an attempt to counteract the increased oxidative stress. Superoxide Dismutase (SOD) is responsible for converting superoxide radicals (O2•-) into hydrogen peroxide (H_2O_2), and Catalase (CAT) further detoxifies hydrogen peroxide into water and oxygen. The combined effect of increased MDA and decreased SOD, CAT, and GSH in our present study suggests a potential pathway to neurodegeneration resulting from lead acetate exposure. This imbalance between increased oxidative damage and decreased antioxidant defence mechanisms contributes to cellular dysfunction and may contribute to the development of neurodegenerative conditions.

Perhaps one limitation of the present study which will be addressed in subsequent work is the use crude extract of *Monodora myristica* without characterization of active ingredients.

Conclusion

Taken together, our findings thus suggest that *Monodora myristica* ameliorated lead-induced neurotoxicity in *D. melanogaster* via its antioxidant properties. *Monodora myristica* may be considered an effective agent in the prevention and treatment of neurodegenerative and neurological disorders such as Alzheimer's and Parkinsonism where oxidative stress is implicated.

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