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Antioxidant and Anti-inflammatory Properties of Peptide Fractions of *Morinda lucida* and *Alstonia boonei* and Protective Effects against Lead-induced Toxicity in *Drosophila melanogaster*

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Abstract

Morinda lucida and *Alstonia boonei* are widely used in ethnomedicine for treating and managing various ailments and have been validated for several biological activities. This study investigated the antioxidant and anti-inflammatory properties of partially purified peptide fractions of *Morinda lucida* (MLP) and *Alstonia boonei* (ABP) and their protective effect against lead (Pb)-induced toxicity on wild-type *Drosophila melanogaster*. Peptide fractions were partially purified using solid phase extraction and evaluated for antioxidant activities using 2,2-diphenyl-1-picrylhyhdrazyl (DPPH) radical scavenging activity and ferric reducing antioxidant power (FRAP) assays. Anti-inflammatory activity was assessed via protein denaturation and membrane stabilisation assays. Flies were treated with Pb (10 mM), peptide fractions (50 and 100 µg/10g diet), and co-treatment of Pb plus the fractions. After treatment, homogenized flies were analysed for total thiol (TSH) and non-protein thiol (NPSH) content, glutathione-S-transferase (GST) activity, nitric oxide (NO) (nitrite/nitrate) and hydroperoxide levels. The peptide fractions showed significant *in vitro* antioxidant and anti-inflammatory properties. Both fractions (50 and 100 µg/10g diet) maintained a balanced redox status of flies. Pb exposure reduced survival rates and increased oxidative stress markers compared to control untreated flies. Co-treatment with MLP and ABP (50 and 100 µg/10g diet) improved antioxidant enzyme activities (GST, NPSH and Total thiol) and accumulation of NO and hydroperoxide in Pb-treated flies. Therefore, the peptide fractions from *M. lucida* and *A. boonei* may be sources of bioactive agents with therapeutic potential against oxidative stress and inflammation associated with lead toxicity.

Keywords: *Alstonia boonei*; *Drosophila melanogaster*; Bioactive peptides; Lead toxicity, *Morinda lucida*.

INTRODUCTION

Oxidative stress and inflammation play destructive roles in the pathogenesis of many chronic diseases such as arthritis, cancer, diabetes, bowel, cardiovascular and neurodegenerative diseases (Libby 2007; Sharifi-Rad et al. 2020). The relentless pace of industrialisation and agricultural expansion has resulted in a significant increase in environmental pollution, leading to the accumulation of toxic heavy metals in water, food, and air. Lead, a toxic heavy metal, is prevalent in the environment, particularly in mining and industrial areas. Human exposure occurs mainly through the inhalation of dust particles and the ingestion of contaminated water from streams, rivers, and wells (Balali-Mood et al. 2021). Once absorbed, lead disrupts cellular function, producing free radicals that damage nucleic acids and compromise the integrity of the cellular membrane (Chandimali et al. 2025), hence accelerating the onset of degenerative diseases, including neurodegeneration in both adults and children (Collin et al. 2022).

The fruit fly *Drosophila melanogaster* is widely used model for toxicological and pharmacological studies involving chemicals and natural products (Rand et al. 2010; Siddique et al. 2005; Zemolin et al. 2014). It shares many fundamental biological, biochemical, physiological and neurological similarities with mammals, with approximately 70% of human disease-causing genes having functional homologues present in *D. melanogaster*. Its genetic composition and high sensitivity to toxic substances further enhance its suitability for studying toxicities and disease mechanisms (Bezerra et al. 2017).

Medicinal plants are rich sources of bioactive compounds with numerous significant biological and pharmacological importance. Plant-derived bioactive peptides are currently the new generation of biologically active regulators. They improve the treatment of various illnesses and disorders, thereby increasing the quality of life (Sánchez & Vázquez 2017). Bioactive peptides have been confirmed in different plant families including Rubiaceae (Attah et al. 2023; Gruber et al. 2008), Apocyanaceae (Alade & Attah 2023; Gruber 2010), Violaceae (Plan et al. 2007), Curcubitaceae (Hernandez et al. 2000), Fabaceae (Nguyen et al. 2011), Poaceae (Nguyen et al. 2013), Euphorbiaceae (Akano et al. 2024) etc.

Morinda lucida Benth. of the Rubiaceae family, is an evergreen shrub, growing up to 18- 25 m tall with grey bark, short bent branches and shining green foliage (Kwofie et al. 2016). *M. lucida*, known as *Oruwo* by the Yoruba tribe in Nigeria, is valued for its therapeutic and economic value in Nigeria and West Africa (Saalu 2016). This plant has been used traditionally for different ailments, including the treatment of malaria, dysentery, wound infections, fever, leprosy, ulcers, stomach ache and gonorrhoea (Adeleye et al. 2018; Bamisaye et al. 2013). *Morinda lucida* is rich in peptides and several reports have confirmed the biological activities of the peptide extracts including antimicrobial, antioxidant, antiplasmodial, antidiabetic, antilipidemic and anti-inflammatory (Adebayo et al. 2017; Adewole et al. 2018).

Alstonia boonei De Wild, a member of the Apocynaceae family, is a large deciduous tree that can grow up to 40 m in height and approximately 1.2 m in diameter (Burkill 1985). It is known as *Ahun* by the Yorubas in Nigeria (Majekodunmi et al. 2008). This plant has been used ethnomedicinally to treat malaria, jaundice, fever, skin conditions, sore throat, arthritis and rheumatic pain (Asase & Peterson 2019; Asuzu & Anaga 1991; Gbadamosi et al. 2011). The leaf, stem and root bark extracts of *A. boonei* possess anti-inflammatory, antioxidant, analgesic, antipyretic, antimicrobial and antidiabetic properties (Akinmoladun et al. 2007; Akinnawo et al. 2017; Olajide et al. 2000; Opoku & Akoto 2015; Oyebode et al. 2019). While there are reports of the biological activities of the peptide fractions of *M. lucida,* there are limited or no scientific validations of peptide fractions from *A. boonei* to the best of our knowledge. Therefore, this study aimed to evaluate the antioxidative and anti-inflammatory properties of partially purified peptide fractions from *Morinda lucida* and *Alstonia boonei in vitro* and their protective potential in *Drosophila melanogaster*.

MATERIALS AND METHODS

Preparation of Peptide-rich fraction

Fresh leaves of *M. lucida* and *A. boonei* were collected from the premises of the University of Ibadan. The plants were identified and authenticated at the Forestry Research Institute of Nigeria. The herbarium specimen was deposited with voucher numbers; FHI 113275 and 113284.

Extraction of Peptides from Morinda lucida and Alstonia boonei leaves

The leaves of *M. lucida* and *A. boonei* were air-dried and pulverised into powder before extraction. The pre-purified peptide fractions were prepared as described by Koehbach et al. (2013) as previously reported by Akano et al. (2024) and Ogbole et al. (2021) with slight modifications. The extraction involved macerating powdered plant samples in a 1:1:0.5 ratio of Dichloromethane (DCM): methanol (MeOH): distilled water mixture. Initially, the powdered plant samples were macerated for 24 h in DCM and MeOH (1:1) with intermittent agitation, followed by the addition of distilled water and further extraction for 24 h. The resulting aqueous-rich supernatants were concentrated using a rotary evaporator to remove methanol. Subsequent purification involved loading the aqueous-rich extracts onto a conditioned C18 column and eluting the fractions with increasing concentrations of acetonitrile and trifluoroacetic acid mixture in double distilled water (20%, 80%, and 100%). The 80% eluent; partially purified peptide fractions of *A. boonei* (ABP) and *M. lucida* (MLP) were concentrated, freeze-dried, and stored at -20 °C for further analysis.

Chemicals

The following chemicals and reagents; ascorbic acid, methanol, phosphate buffer saline (pH = 7.4), 2, 2-diphenyl-1-picrylhyhdrazy (DPPH), sodium nitrite, sodium nitroprusside, Griess reagent (containing 1% sulfanilamide in 5% phosphoric acid and 0.1% naphthyl ethylenediamine dihydrochloride), 1-chloro-2,4-dinitrobenzene (CDNB), 5,5'-dithiobis-2-nitro-benzoicacid (DTNB), 2',7' dichlorofluorescein diacetate (DCFH-DA), acetylthiocholine iodide (Sigma Aldrich (St. Louis, MO)) were used. Lead acetate was obtained from A K Scientific, USA. All chemicals were of analytical grade and freshly prepared before each experiment**.**

In vitro antioxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The free radical scavenging activity of the fractions was assessed with DPPH assay following the procedure of Mensor et al. (2001) using the synthetic antioxidant butylated hydroxytoluene (BHT) as the reference standard. To the plant samples or standard of varying concentrations (7.8125 to 1000 μg/ml), 1 ml DPPH solution was added followed by a 30 min incubation in the dark at room temperature. Absorbance was measured at 517 nm. The DPPH free radical scavenging ability of the fractions and BHT was subsequently calculated as the percentage of control.

Ferric reducing antioxidant potential (FRAP)
The ferric ion reducing ability of both fractions was assessed using a modified method of Benzie and Strain (1996). FRAP reagent was prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl and 20 mM ferric chloride in a ratio of 10:1:1 (v/v/v). Samples and BHT (standard) were allowed to react with FRAP reagent in the dark for 90 min at 37 °C. Absorbance was measured at 593 nm. The assay was performed in triplicate. The antioxidant activity of both fractions and standard was quantified and reported as mg of ascorbic acid equivalent per gram of extract (mg AAE/g).

***In vitro* anti-inflammatory activity**
Inhibition of protein denaturation
The protein denaturation assay was carried out following the protocol described by Padmanabhan and Jangle (2012). The reaction mixture consisting of 2 mL of either plant sample or Diclofenac (standard NSAID) at concentrations ranging from 7.8125 to 1000 µg/ml and 2.8 mL of phosphate-buffered saline (pH 6.4) was combined with 1% bovine serum albumin. Following incubation at 27 ± 1 °C for 15 min, protein denaturation was induced by heating the reaction mixture in a water bath at 70 °C for 10 min. After cooling, the absorbance was measured at 660 nm and the percentage inhibition of protein denaturation was calculated.

Membrane stabilisation assay
The membrane stabilisation assay was performed following the procedure of Sadique et al. (1989) with slight modifications. Briefly, 50 µL of each sample (7.8125 to 1000 µg/mL) was mixed with 100 µL phosphate buffer, 200 µL hyposaline solution (0.36% NaCl), and 50 µL RBC (2% v/v). The mixture was incubated at 37 °C for 30 min and centrifuged at 8,000 rpm for 3 min. Diclofenac sodium (standard NSAID) was used as the reference drug. The control consisted of 50 µL RBC and 200µL hyposaline solution. Absorbance was measured at 560 nm and percentage inhibition of haemolysis was calculated.

***Drosophila melanogaster* stock and culture**
Wild-type *Drosophila melanogaster* (Canton S strain) flies were maintained at the University of Ibadan's Biochemistry Department *Drosophila* Laboratory, Nigeria. The flies were cultured on a cornmeal-based diet containing 6% cornmeal, 1% brewer's yeast, 2% sucrose, 1% powdered milk, agar 1% and 0.08% nipagin. Environmental conditions were controlled at a temperature of 24 ±1 °C, with a relative humidity of 60–70% and a 12 h light-dark cycle.

Survival studies
Drosophila melanogaster (1–3 days old), were assigned to different groups, with each group consisting of five replicates, each containing 30 flies. Three concentrations of MLP or ABP (10, 50 and 100 µg/10 g diet) were compared to the control (0 µg/10 g diet). The survival rate was determined by recording the daily mortality of flies for 14 days, and the data were analysed (Abolaji et al. 2014). Survival studies were also carried out by exposing flies to (0, 1, 5 and 10 mM) of lead acetate for 7 days.

Lead (Pb) exposure and treatment with peptide fractions of *M. lucida* (MLP) and *A. boonei* (ABP)
For the ameliorative study, Pb and MLP/ABP were added to the medium at final doses of Pb (10 mM) and/or MLP/ABP (50 and 100 µg/10g diet) respectively. Flies were divided into groups of 50 flies each; (A) control (0 µg/10 g diet); (B) MLP/ABP (50 µg/10g diet); (C) MLP/ABP (100 µg/10g diet); (D) Pb (10 mM); (E) Pb (10 mM) + MLP/ABP (50 µg/10g diet); (F) Pb (10 mM) + MLP/ABP (100 µg/10g diet).

The (10 mM) dose selected for Pb for the ameliorative studies was determined based on a survival curve and biochemical assays presented later in this study. Studies by Venkareddy (2015) also guided the selection of (1, 5 and 10 mM) Pb doses. The doses of MLP and ABP (50 and 100 µg/10g diet) were chosen based on the absence of overt toxicity in the flies, as observed by the survival curve and biochemical assays conducted in this study.

Preparation of tissue homogenate
The treated flies were collected after anaesthetizing, weighed and homogenized in 0.1 M phosphate buffer at a pH of 7.4 (ratio of 1 mg:10 µL). The resulting homogenates were centrifuged at 4000 × *g* and 4 °C for 10 min in a Mikro 220R microlitre centrifuge (Tuttlingen, Germany). Subsequently, the supernatants (samples) were separated from the pellets into tubes for various biochemical assays.

Biochemical Assays
Determination of protein concentration
Total protein concentrations were measured following the modified method described by Lowry et al. (1951).

Determination of total thiol levels
Total thiol content was determined according to the method of Ellman (1959). The reaction mixture was made up of 510 µL of 0.1 M of potassium phosphate buffer (pH 7.4), 25 µL of sample, and 30 µL of DTNB (1 mM) and 35 µL distilled H_2O. Incubation was carried out for 30 min at room temperature, the absorbance was measured at a wavelength of 412 nm.

Determination of catalase activity
Catalase activity was determined according to the method described by (Aebi 1984). The assay was conducted in a reaction mixture consisting of 1.8 mL of potassium phosphate buffer (pH 7.0), 180 µL of 300 mM hydrogen peroxide, and 20 µL of sample diluted 1:50.

The clearance of hydrogen peroxide was monitored spectrophotometrically at 240 nm over a 2-min period, with absorbance readings taken at 10-s intervals using a UV-visible spectrophotometer. The resulting catalase activity was expressed in micromoles of hydrogen peroxide consumed per minute per milligram of protein (μmol/min/mg).

Determination of total hydroperoxide level

The total hydroperoxide level was determined using the method described by Wolff (1994). Briefly, a reaction mixture containing FOX 1 (10 ml of 100 mM xylenol orange, 10 ml of 100 mM sorbitol, 50 ml of 250 mM ferrous sulphate, 5 ml of 25 mM H_2SO_4 and 30 ml distilled water) was reacted with the sample homogenate. This mixture was incubated at room temperature for 30 min and the absorbance was measured at 560 nm. The total hydroperoxide concentration was calculated using a standard curve and expressed in μmol/mg protein.

Determination of nitric oxide (nitrate/nitrite) levels

The level of nitrites was quantified following the protocol by Green et al. (1982) using the Griess reaction. Samples were mixed with Griess reagent at a ratio of 1:1 and incubated at room temperature for 20 min and nitrite level was quantified from sodium nitrite standard curve measured at 550 nm.

Determination of glutathione S-transferase (GST) activity

The activity of GST was evaluated using the method of Habig and Jakoby (1981). In summary, the reaction mixture contained 270 μL of solution A containing (20 mL of 0.25 M potassium phosphate buffer (pH 7.0), 2.5 mM EDTA, 10.5 mL of distilled water, and 500 mL of GSH (0.1 M) at 25 °C), 20 μL of sample (1:5 dilution), and 10 μL of 25 mM CDNB as substrate. The reaction mixture was then monitored for 5 min (10-s intervals, at 340 nm).

Determination of the non-protein thiol content

The non-protein thiol (NPSH) content was estimated following the method described by (Jollow et al. 1974). The supernatants were precipitated with sulphosalicylic acid (4%) at a 1:1 ratio kept at 4 °C for 1 h. The mixture was then centrifuged at 5000 rpm for 10 min at 4 °C. The assay mixture consisted of 550 μL of 0.1 M phosphate buffer, 100 μL of supernatant and 100 μL of DTNB. The OD was read at 412 nm and the result was expressed as μmol of GSH per mg protein.

Data analysis

Graphs were prepared using Ms office Excel, and Prism 6 for Windows, version 6.01 (GraphPad Software, Inc). Data from the survival assay were evaluated using the Kaplan-Meier survival plot method, with group comparisons assessed by log-rank tests. Biochemical assay results were presented as mean ± standard error of the means (SEM). For multiple treatment group comparisons, one-way analysis of variance (ANOVA) was employed, followed by Dunnett's *post hoc* test. Statistical differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

***In vitro* antioxidant assays**

The antioxidant potential of MLP and ABP was evaluated using the DPPH free radical scavenging assay and the results are presented in Figure 1A. Concentration-dependent percentage radical scavenging activity was observed for the tested samples. The results revealed that ABP demonstrated a higher DPPH scavenging activity than MLP but not as high as the standard BHT. The IC_{50} values are reported in Table 1. The result of the ferric reducing antioxidant property (FRAP) of ABP and MLP was evaluated and expressed as ascorbic acid equivalent and presented in Figure 1B. The result revealed that ABP had greater reducing power than MLP although the reducing power of BHT was significantly greater ($p < 0.05$).

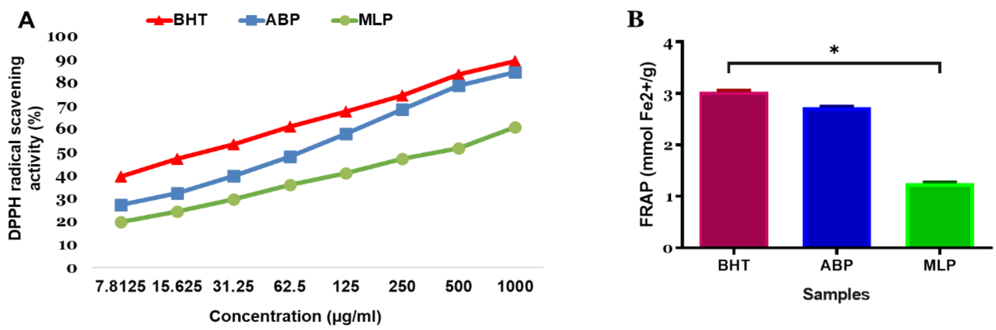

Figure 1. Percentage DPPH radical scavenging activity **(A)** Ferric reducing antioxidant power **(B)** of partially purified peptide fractions of A. boonei (ABP) and M. lucida (MLP).

Table 1. IC_{50} values of samples in antioxidant and anti-inflammatory assays.

Sample	DPPH	Inhibition of protein denaturation	Inhibition of haemolysis
	IC_{50} ± S.E.M (µg/ml)		
ABP	103.10 ± 0.74	47.47 ± 0.59	63.60 ± 0.41
MLP	110.60 ± 0.39	78.72 ± 0.39	204.90 ± 1.79
BHT	89.30 ± 1.22	-	-
Diclofenac	-	19.04 ± 0.23	19.82 ± 0.07

Anti-inflammatory assays

The protein denaturation inhibition assay was evaluated and the percentage inhibition was calculated and presented in Figure 2A. The results showed that the samples inhibited heat-induced protein denaturation in a concentration-dependent manner. ABP showed a stronger ability to inhibit protein albumin denaturation than MLP, although it was not as strong as the reference drug, diclofenac. The RBC membrane stabilisation assay was also carried out, and the percentage of haemolysis inhibition was calculated, Figure 2B. A dose-dependent increase in the percentage of haemolysis inhibition was observed. Diclofenac sodium displayed the most potent inhibition, followed by ABP displaying a stronger haemolysis inhibition than MLP. Table 1 shows the IC_{50} values for the assays.

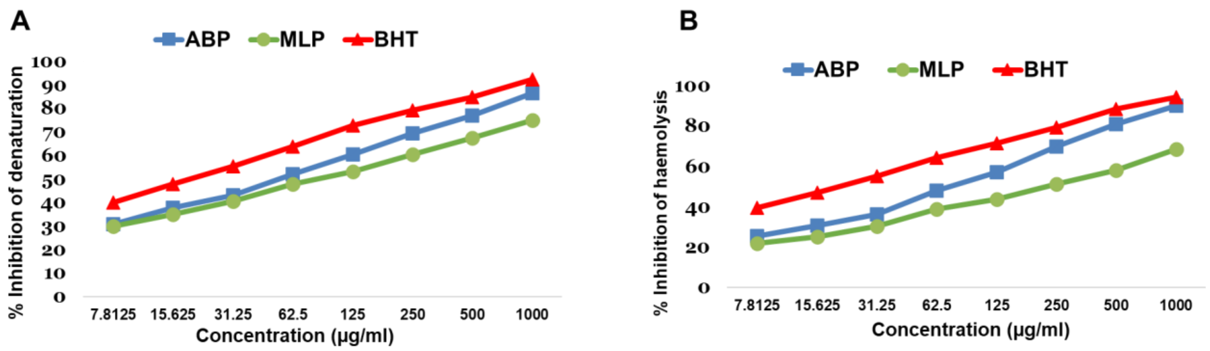

Figure 2. Effect of ABP and MLP on inhibition of albumin denaturation **(A)** inhibition of RBC haemolysis **(B)**.

ABP and MLP maintained the life span of flies and the redox status of flies.

The survival study showed that the survival of the flies treated with a 10, 50, and 100 µg/g diet of ABP and MLP was not significantly different from control flies (Figure 3 A & B). The three concentrations maintained the life span of the flies, indicating that the fractions did not exert any toxic effects on the flies. The effect of ABP and MLP on the biochemical markers of the flies was concentration-dependent, and better activities were observed with the 50 and 100 µg/g diet of both fractions (Figure 4 and 5). These concentrations were therefore selected for the ameliorative studies by co-exposing with Pb (10 mM).

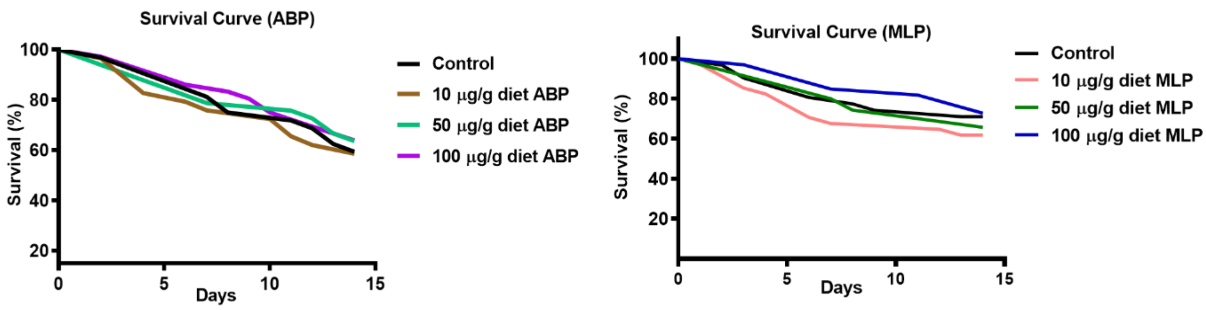

Figure 3. Effects of ABP **(A)** and MLP **(B)** on survival rate in *Drosophila* flies after treatment for 14 days.

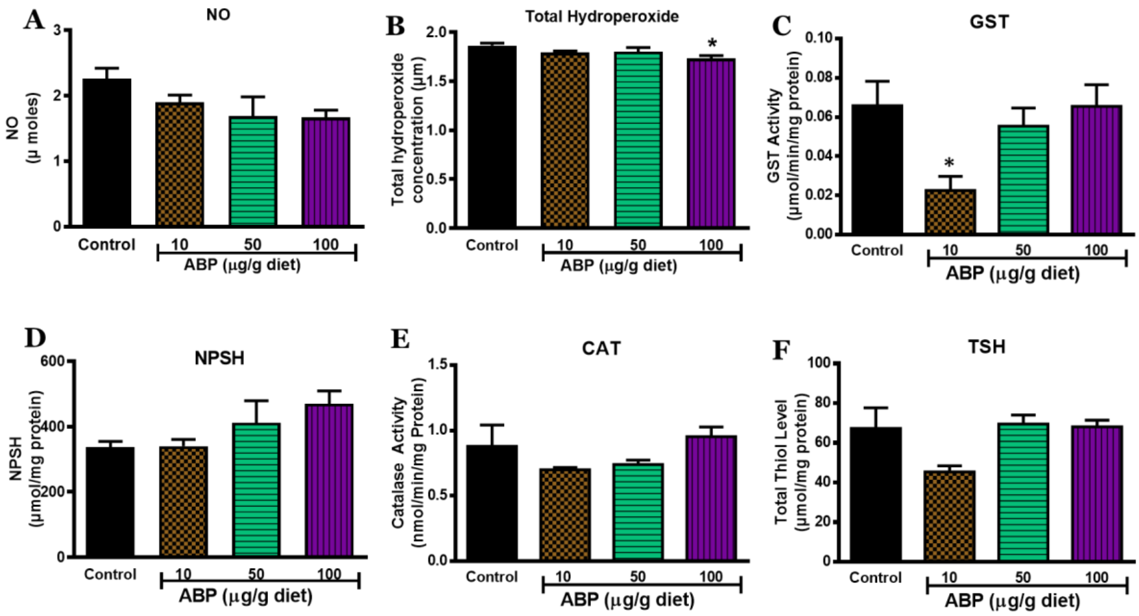

Figure 4. Effects of ABP on Nitric oxide level **(A),** Total hydroperoxide level **(B),** GST activity **(C)**, NPSH level (**D),** CAT activity **(E)** and TSH level **(F)** in *Drosophila* flies after treatment with ABP for 7 days. Values represent mean ± SEM of 50 flies/vial with 5 replicates per treatment group. Mean values are significantly different at *(p < 0.05) compared to control.

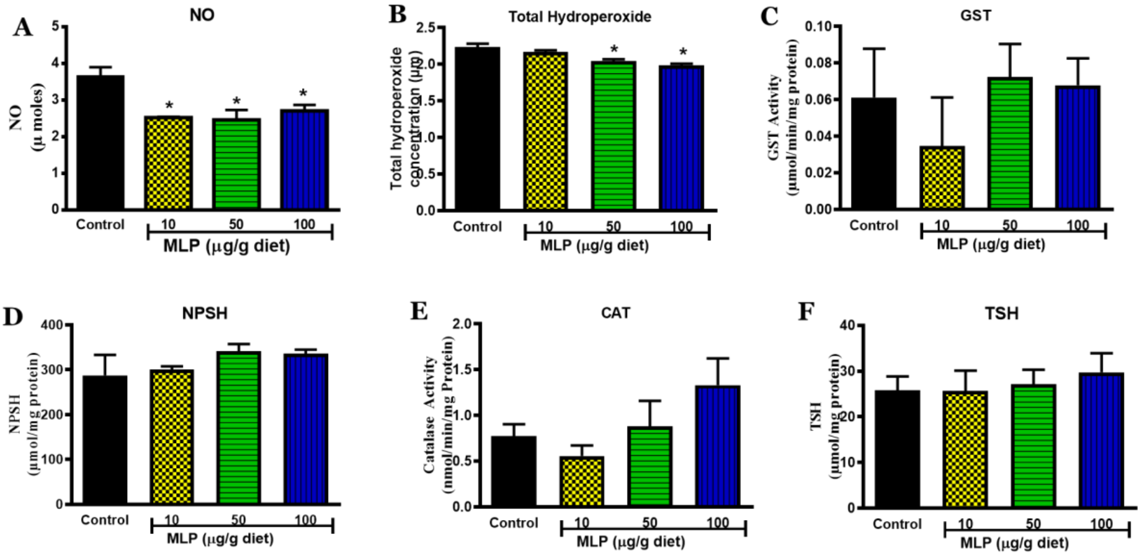

Figure 5. Effects of MLP on Nitric oxide level **(A),** Total hydroperoxide level **(B),** GST activity **(C)**, NPSH level (**D),** CAT activity **(E)** and TSH level **(F)** in *Drosophila* flies after treatment with MLP for 7 days. Values represent mean ± SEM of 50 flies/vial with 5 replicates per treatment group. Mean values are significantly different at *(p < 0.05) compared to control.

Effect of Pb exposure on survival rate and oxidant levels in flies

The daily exposure of flies to Pb (1, 5, 10 mM) for 7 days reduced their survival rate (Figure 6A). Fewer than 40% of the flies exposed to 10 mM Pb survived Pb-induced toxicity at day 7. Moreover, Pb significantly ($p < 0.05$) increased the levels of oxidant molecules including nitrites and total hydroperoxide while causing a notable depletion in thiol levels (Figure 6B-D).

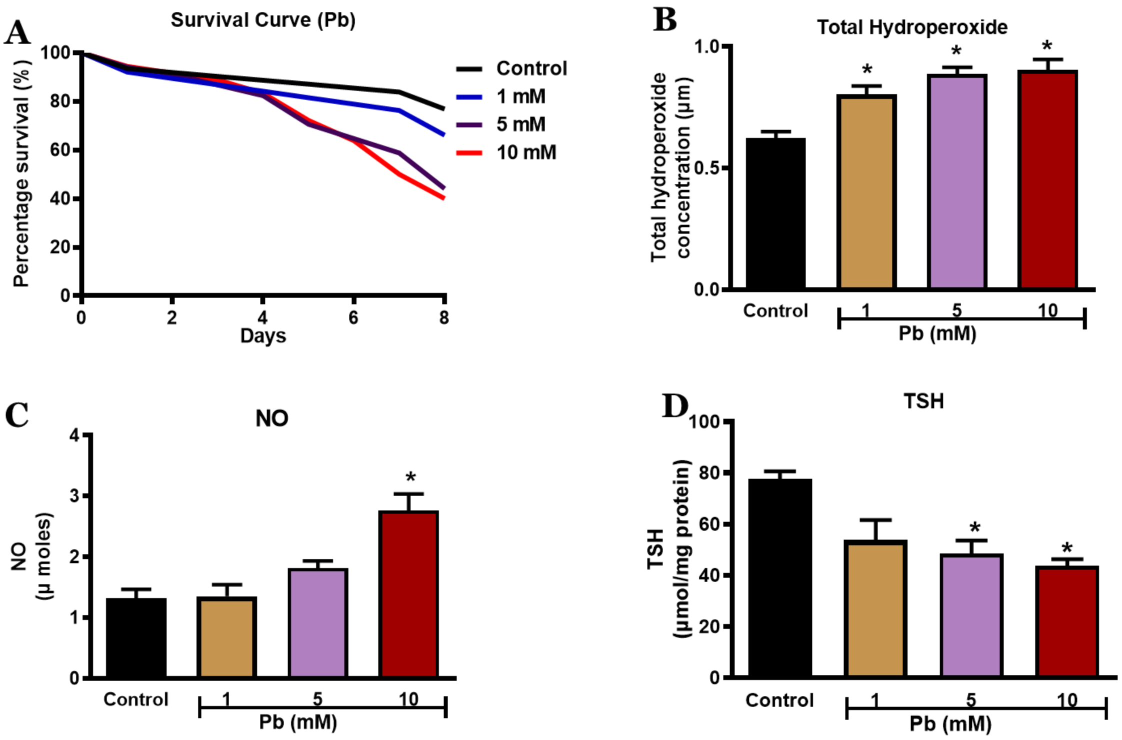

Figure 6. Effects of Pb on a 7-day Survival **(A),** Total hydroperoxide level **(B),** Nitric oxide level **(C)**, and Total thiol level **(D)** in *Drosophila* flies after treatment with Pb for 7 days. Values represent mean ± SEM of 50 flies/vial with 5 replicates per treatment group. Mean values are significantly different at *($p < 0.05$) compared to control.

ABP and MLP reduced nitric oxide and total hydroperoxide levels in *Drosophila* flies with Pb-induced toxicity

As depicted in Figure 7, Pb exposure caused a significant ($p < 0.05$) increase in nitric oxide (NO) (Figure 7A & B) and total hydroperoxide levels (7C & D) in flies when compared with the control (basal diet). However, ABP and MLP (50 and 100 µg/g diet) significantly reduced ($p < 0.05$) the levels of nitric oxide and hydroperoxide when compared to the Pb (10 mM) treated group (Figure 7A - D).

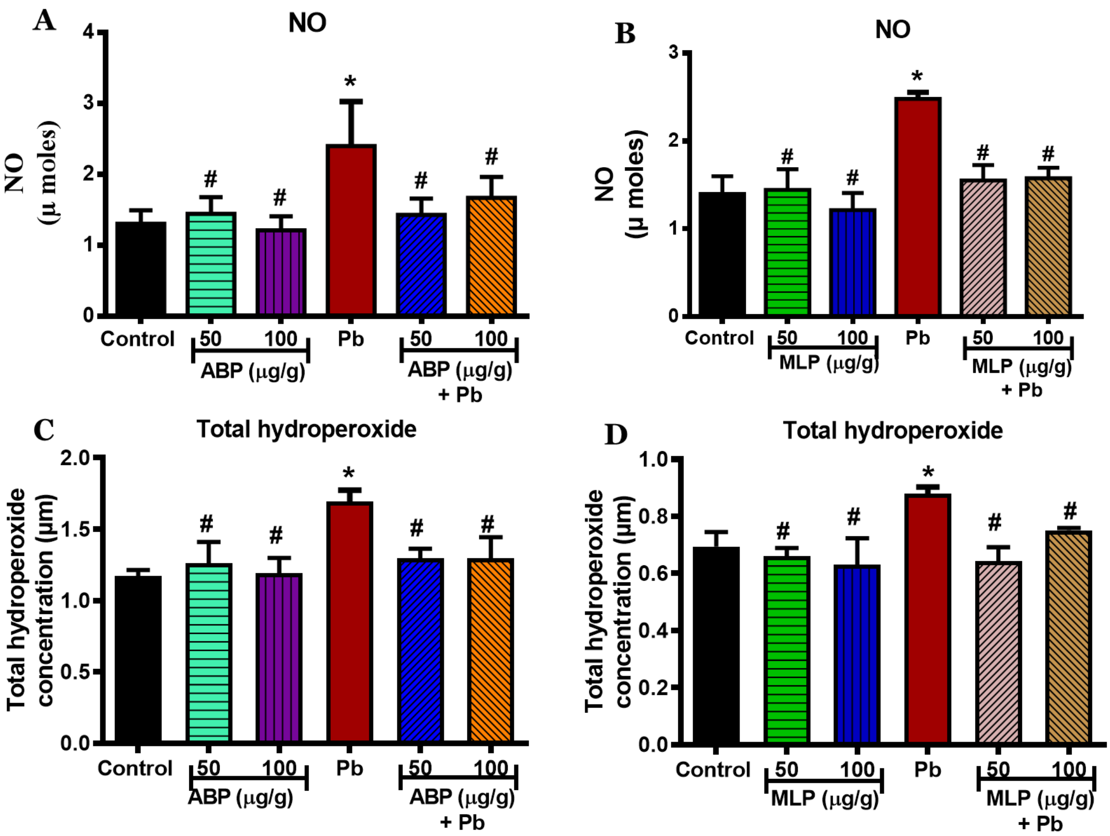

Figure 7. Effects of ABP/MLP and Pb on Nitric oxide **(A & B),** Total hydroperoxide levels **(C & D)** in *Drosophila* flies after treatment with Pb for 7 days. Values represent mean ± SEM of 50 flies/vial with 5 replicates per treatment group. Mean values are significantly different at $^{*}(p < 0.05)$ compared to control and $^{\#}(p < 0.05)$ relative to Pb.

ABP and MLP reversed Pb-induced depletion of total thiol and non-protein thiol levels in *Drosophila* flies

As shown in Figure 8, exposure to Pb caused a significant decrease ($p < 0.05$) in total thiol (Figure 8 A & B) and NPSH (Figure 8 C & D) contents in treated flies in comparison with those of control flies. However, ABP (100 µg/g diet) and MLP (50 and 100 µg/g diet) significantly increased ($p < 0.05$) the total thiol content, while ABP (50 and 100 µg/g diet) and MLP (100 µg/g diet) significantly ($p < 0.05$) increased the non-protein thiol content relative to the Pb treated flies (Figure 8 A - D).

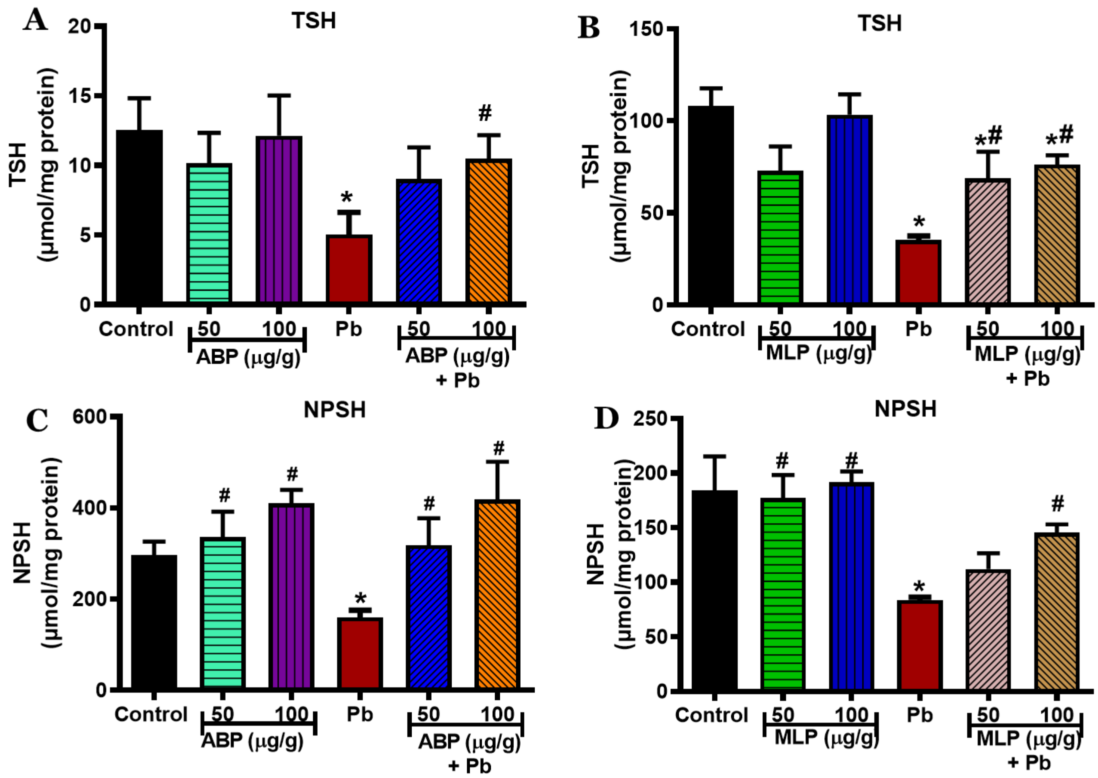

Figure 8. Effects of ABP/MLP and Pb on Total thiol **(A & B),** Nonprotein thiol contents **(C & D)** in *Drosophila* flies after treatment with Pb for 7 days. Values represent mean ± *SE*M of 50 flies/vial with 5 replicates per treatment group. Mean values are significantly different at *(p < 0.05) compared to control and #(p < 0.05) relative to Pb.

ABP and MLP improved glutathione-S-transferase activity in *Drosophila* flies exposed to Pb

As illustrated in Figure 9, the GST activity of *Drosophila* flies exposed to Pb (10 mM) was significantly ($p < 0.05$) lower than control flies. However, ABP and MLP treatment enhanced the activity of the GST enzymes in comparison to Pb treated group (Figure 9A & B).

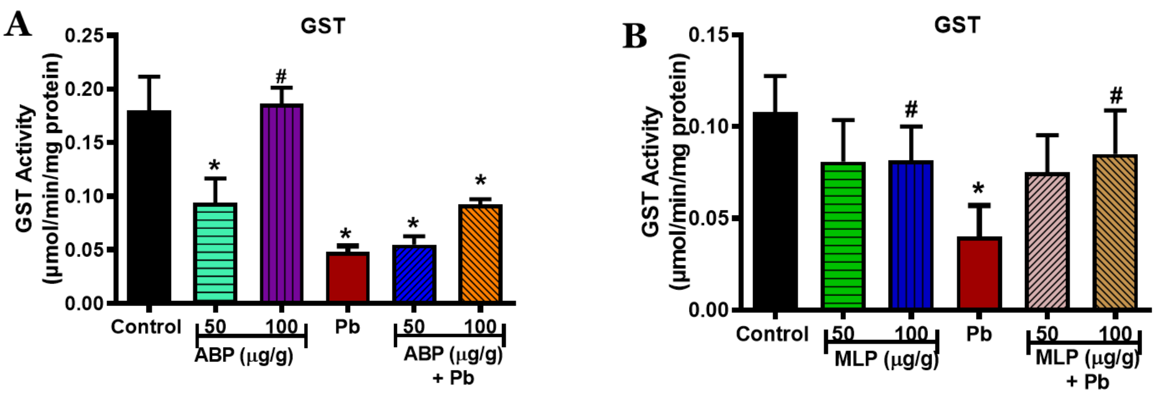

Figure 9. Effects of ABP/MLP and Pb on Glutathione S-transferase activity **(A & B)** in *Drosophila* flies after treatment with Pb for 7 days. Values represent mean ± SEM of 50 flies/vial with 5 replicates per treatment group. Mean values are significantly different at *(p < 0.05) compared to control and #(p < 0.05) relative to Pb.

Discussion

Bioactive peptides from plant sources have been identified as potent antioxidants that neutralize free radicals and oxidation initiators (Zhu et al. 2024). They have also been confirmed to resist excessive inflammatory responses by modulating inflammatory signalling pathways and inhibiting the secretion of inflammatory factors (Zhang et al. 2020). The DPPH radical serves as a reliable and stable radical model widely used to assess the antioxidant and free radical-scavenging ability of plant extracts. Antioxidant peptides can exert their effects directly by scavenging free radicals through hydrogen atoms or electron donation. Their effectiveness depends on the specific properties of their amino acid residues (Zhang et al. 2022). This study revealed that both peptide fractions (ABP and MLP) exhibited free radical scavenging capacity and were concentration-dependent, suggesting that their potent antioxidant properties may be attributed to specific amino acids present in the fractions. The antioxidant properties of synthetic and natural antioxidants play crucial roles in their ability to mitigate inflammation and provide therapeutic benefits (Bhol et al. 2024). The amino acid composition of both fractions is likely to contribute to their free radical scavenging activity by reducing the stable DPPH radical to its yellow diphenylpicrylhydrazine derivative. The FRAP assay measures the reducing ability of antioxidants by reacting with a ferric TPTZ complex and producing a blue-coloured ferrous TPTZ complex by a reductant in low pH media (Benzie & Strain 1996). In this study, both ABP and MLP fractions exhibited reducing potential. This reduction is likely associated with the presence of reducing amino acids in the peptide fractions, which exert their action by breaking the free radical chains through donating atoms. Hence, the amino acid compositions of ABP and MLP may enable them to disrupt the free radical chains through electron donation. The cysteine-stabilised peptide fraction of *M. lucida* and extracts of *A. boonei* have been reported to have anti-DPPH activity (Adewole et al. 2018; Akinmoladun et al. 2007; Akinnawo et al. 2017; Oyebode et al. 2019). Xie et al. (2008) reported that peptides isolated from Alfafa leaf protein showed a chelating effect on ferrous ions.

Inflammatory responses in tissues are initiated by injuries, microbial infections, and irritants which have been implicated in the pathogenesis of various chronic diseases including arthritis, cancer and stroke. Protein denaturation is a key feature of inflammatory responses in various diseases. Tissue injury can be characterised by the denaturation of cellular and tissue proteins (Opie 1962). Hence, inhibiting protein denaturation suggests anti-inflammatory properties of extracts. This study revealed that ABP and MLP demonstrated protective effects against heat-induced protein denaturation in a dose-independent manner comparable to the standard drug; diclofenac. To ascertain the membrane stabilising activity of ABP and MLP, the experiment involved erythrocyte membranes. The lysosomal enzymes released during the inflammatory process result in various disorders and since RBC membranes share similarities with lysosomal membranes, evaluating the protective effects of drugs and plant extracts against hypotonicity-induced RBC membrane lysis serves as a valuable indicator of their antiinflammatory activity (Mounnissamy et al. 2007; Umapathy et al. 2010). These results indicated that ABP and MLP exhibited protective effects against hypotonicity-induced erythrocyte lysis comparable to Diclofenac. The study by Akinnawo et al. (2017) demonstrated that an aqueous extract of *A. boonei* significantly inhibited heat-induced protein denaturation and stabilised hypotonicity-induced hemolysis of HRBC *in vitro*. Similarly, the aqueous extract of *M. lucida* presented the highest protection against albumin denaturation with the lowest IC_{50} among other screened extracts (Dah-Nouvlessounon et al. 2023). Plant-derived bioactive peptides may exhibit anti-inflammatory effects by interacting with cell membranes, stabilising them and disrupting the inflammatory cascade. Their hydrophobic nature enhances membrane binding and modulates cellular responses to inflammation (Liu et al. 2022).

The results of this study revealed that ABP and MLP had no adverse effects on the survival or biochemical indices of the flies and the results were comparable to the untreated control flies. The ability of the fractions to maintain the life span and biochemical parameters of the treated flies may be attributed to their potent antioxidative and anti-inflammatory properties. Existing research has indicated a positive association between increased intake of dietary supplements high in antioxidants and reduced risk for chronic inflammatory diseases and oxidative damage to cells (Roy et al. 2022; Wang et al. 2017). Additionally, it has been reported that varied intake of functional foods and nutraceuticals helps maintain healthy and strong tissues, decreases inflammatory factors and prevents diseases (Garza-Juárez et al. 2023).

Pb exposure has been associated with generating excessive amounts of ROS and inhibiting antioxidant defence systems (Gurer & Ercal 2000). Given the established link between Pb exposure, oxidative stress and inflammatory processes, attention has focused on compounds with antioxidant and anti-inflammatory properties to mitigate Pb-induced toxicity (Gurer & Ercal 2000; Mumtaz et al. 2020). However, relatively few studies have examined the protective role of medicinal plants, particularly peptide-rich plant extracts in addressing Pb-induced toxicity responses in experimental models. Evidence from epidemiological studies reveals the occurrence of adverse health effects even at low-level exposure to lead (Rees & Fuller 2020; Rinsky et al. 2018). Oxidative stress arising from chronic lead exposure can trigger subsequent events contributing to the onset of hypertension and cardiovascular diseases (Yan et al. 2022). Studies have shown that lead has a deleterious impact on the immune system leading to autoimmune disorders (Dou et al. 2022; Harshitha et al. 2024; Zheng et al. 2023). Lead toxicity was assessed in *Drosophila* flies and it was observed that Pb had adverse effects on the survival and biochemical indices of the treated flies. The lethal effects of Pb are attributed to oxidative damage to cellular components that trigger inflammation and cell death (Virgolini & Aschner 2021). Lead is a known neurotoxin, that has been demonstrated to cause mortality in *Drosophila* flies (Venkareddy 2015) and adverse effects on biochemical parameters of fruit flies (Abdulazeez et al. 2024).

Lead exposure can increase the generation of nitric oxide and hydroperoxides which can contribute to the development of various diseases (Dobrakowski et al. 2017; Fiorim et al. 2020; Ni et al. 2004). Nitric oxide (NO) is a key signalling molecule with a dual role in inflammation. Under normal physiological conditions, NO exhibits anti-inflammatory properties whereas, excessive NO production is considered as a pro-inflammatory mediator that induces inflammatory processes (Sharma et al. 2007). This study showed that both fractions (ABP and MLP) significantly ameliorated the increased levels of NO and hydroperoxide induced by Pb in flies. Pb exposure triggers NO production primarily by inducing oxidative stress caused by an imbalance between the production and scavenging of ROS in the tissues and components of flies (Patra et al. 2011; Shilpa et al. 2021). Increased production of hydroperoxide levels from lead exposure can result in membrane degradation and lipid peroxidation. A recent study reported that Pb exposure leads to free radical generation which resulted in increased NO levels (Osunbor & Orobor 2023). The ability of ABP and MLP to mitigate the effects of Pb-induced elevated levels of NO and hydroperoxide is consistent with their *in vitro* antioxidant and anti-inflammatory properties which are typified by their free radical scavenging abilities, reducing properties, membrane stabilising and protein denaturation inhibitory properties. A study reported the protective effect of the alkaloid-rich extract of *Morinda lucida* against manganese chloride-induced toxicity, significantly reducing ROS levels (Nwanna 2021). The aqueous extract of *A. boonei* also demonstrated potent antioxidant activity by scavenging NO and H_2O_2 radicals *in vitro* (Akinnawo et al. 2017).

Thiols make up a major component of the body's antioxidant defence system, protecting against ROS (Kumar et al. 2009). As reducing agents, thiols maintain cellular redox homeostasis protecting cells from oxidative damage. Consequently, decreased thiol levels have been linked to various medical disorders including kidney disorders, diabetes mellitus and other neurological disorders (Erel & Neselioglu 2014; Kundi et al. 2015). Glutathione (GSH) is one of the most abundant endogenous antioxidants in the body and plays a vital role in protecting cells from oxidative damage caused by ROS, free radicals, peroxides and heavy metals and helps maintain redox homeostasis (Forman et al. 2009; Lushchak 2012). The reduction in TSH and NPSH levels following Pb treatment is an indicator of oxidative stress which could result from Pb binding with sulfhydryl group of GSH or impairment of GSH synthesis (Flora et al. 2012). This result is comparable with data obtained by Olakkaran et al. (2018) who reported a Pb-induced depletion of GSH content in *Drosophila* flies. However, the observed protective effects of ABP and MLP in mitigating Pb-induced decreases in TSH and NPSH levels in flies suggest their potential against diseases associated with oxidative stress. The Glutathione-S-transferases (GSTs) are a diverse family of enzymes that are involved in many physiological processes including protection against oxidative damage caused by toxic compounds by catalyzing their conjugation to reduced glutathione, biosynthesis of hormones, intracellular transport, and are also responsible for the detoxification of both endogenous and xenobiotic compounds (Ściskalska & Milnerowicz 2020; Shi et al. 2012). Thus, the deleterious effect of Pb on flies may be a result of reduced ability to detoxify Pb and its reactive species leading to oxidative stress and this is attributed to decreased glutathione-S-transferase activity. However, ABP and MLP were able to reverse the inhibitory effect of Pb indicating their antioxidant potentials. Bioactive peptides have been reported to enhance antioxidant defences by modulating key enzymes involved in redox homeostasis (Xu et al. 2024). These peptides can protect cells from oxidative damage by restoring GST activity and facilitating the detoxification of ROS and toxic compounds (Okagu & Udenigwe 2022). Plant extracts and their active fractions can counteract heavy metal-induced oxidative stress by boosting GST activity and increasing glutathione levels, thereby mitigating oxidative damage and cellular function (Sheweita et al. 2016).

CONCLUSIONS

This study highlights the antioxidant and anti-inflammatory properties of partially purified peptide fractions of *Morinda lucida* and *Alstonia boonei.* These peptide fractions demonstrated significant free radical scavenging abilities, reducing properties, membrane stabilising ability and protein denaturation inhibitory properties. The dietary inclusion of lead-induced toxicity in *Drosophila* flies primarily disrupts the redox balance. However, both peptide fractions effectively mitigated lead-induced toxicity by reducing inflammatory markers and restoring the activities of key antioxidant enzymes. The protective effects of the peptide fractions of *M. lucida and A. boonei* could be attributed to the presence of bioactive peptides present in the plants. This study highlights the potential of these peptide fractions as a source of natural antioxidant and anti-inflammatory agents. Further study is necessary to isolate and identify the individual peptide(s) responsible for these activities.

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REFERENCES

Abdulazeez, R., Highab, S., Onyawole, U., Jeje, M., Musa, H., Shehu, D., & Ndams, I. (2024). Co-administration of resveratrol rescued lead-induced toxicity in Drosophila melanogaster. *Environmental Toxicology and Pharmacology*, *109*, 104470.

Abolaji, A. O., Kamdem, J. P., Lugokenski, T. H., Nascimento, T. K., Waczuk, E. P., Farombi, E. O., . . . Rocha, J. B. T. (2014). Involvement of oxidative stress in 4-vinylcyclohexene-induced toxicity in *Drosophila melanogaster*. *Free Radical Biology and Medicine*, *71*, 99-108.

Adebayo, J. O., Adewole, K. E., & Krettli, A. U. (2017). Cysteine-stabilised peptide extract of *Morinda lucida* (Benth) leaf exhibits antimalarial activity and augments antioxidant defense system in *P. berghei*-infected mice. *Journal of Ethnopharmacology*, *207*, 118-128. https://doi.org/10.1016/j.jep.2017.06.026

Adeleye, O., Ayeni, O., & Ajamu, M. (2018). Traditional and medicinal uses of *Morinda lucida*. *Journal of Med Plants Studies*, *6*(2), 249-254.

Adewole, K., Attah, A., Sonibare, M., & Adebayo, J. (2018). Identification of antioxidant cysteine-stabilised peptides of *Morinda lucida* Benth. leaf. *Indian Journal of Pharmaceutical Sciences*, *80*(1), 99-107.

Aebi, H. J. M. i. e. (1984). [13] Catalase in vitro. *105*, 121-126.

Akano, J.-J. M., Molik, Z. A., Abolaji, A. O., & Ogbole, O. O. (2024). Mitigating aluminum chloride-induced toxicity in Drosophila melanogaster with peptide fractions from Euphorbia species. *Drug and Chemical Toxicology*, 1-10.

Akinmoladun, A. C., Ibukun, E., Afor, E., Akinrinlola, B., Onibon, T., Akinboboye, A., . . . Farombi, E. (2007). Chemical constituents and antioxidant activity of Alstonia boonei. *African journal of biotechnology*, *6*(10).

Akinnawo, O. O., God'swill, N. A., & Osilesi, O. (2017). Aqueous fraction of Alstonia boonei de Wild leaves suppressed inflammatory responses in carrageenan and formaldehyde induced arthritic rats. *Biomedicine and Pharmacotherapy*, *86*, 95-101.

Alade, G., & Attah, F. A. (2023). Pharmacognostic study and peptidomic analysis of the leaves of Nigerian Rauvolfia vomitoria Wennberg (Apocynaceae). *Proceedings of the Nigerian Academy of Science*, *16*(1).

Asase, A., & Peterson, A. T. (2019). Predicted impacts of global climate change on the geographic distribution of an invaluable African medicinal plant resource, Alstonia boonei De Wild. *Journal of Applied Research on Medicinal and Aromatic Plants*, *14*, 100206.

Asuzu, I., & Anaga, A. (1991). Pharmacological screening of the aqueous extract of Alstonia boonei bark.

Attah, F. A., Mbanu, A. E., Chukwudulue, U. M., Jonah, U. J., & Njinga, N. S. (2023). Ethnopharmacology, phytochemistry and a new chemotaxonomic marker in Oldenlandia affinis (Roem. & Schult.) DC. Rubiaceae. *Physical Sciences Reviews*, *8*(11), 3939-3959.

Balali-Mood, M., Naseri, K., Tahergorabi, Z., Khazdair, M. R., & Sadeghi, M. (2021). Toxic Mechanisms of Five Heavy Metals: Mercury, Lead, Chromium, Cadmium, and Arsenic. *Frontiers in Pharmacology*, *12*, 643972. https://doi.org/10.3389/fphar.2021.643972

Bamisaye, F., Odutuga, A., Minari, J., Dairo, J., Oluba, O. M., & Babalola, L. (2013). Evaluation of hypoglycemic and toxicological effects of leaf extracts of *Morinda lucida* in hyperglycemic albino rats. *International Research Journal of Biochemistry Bioinformatics*, *3*(2), 37-43.

Benzie, I. F., & Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry*, *239*(1), 70-76.

Bezerra, J., Costa, A., da Silva, M., Rocha, M., Boligon, A., da Rocha, J., . . . Kamdem, J. J. S. A. J. o. B. (2017). Chemical composition and toxicological evaluation of Hyptis suaveolens (L.) Poiteau (LAMIACEAE) in Drosophila melanogaster and Artemia salina. *113*, 437-442.

Bhol, N. K., Bhanjadeo, M. M., Singh, A. K., Dash, U. C., Ojha, R. R., Majhi, S., . . . Jena, A. B. (2024). The interplay between cytokines, inflammation, and antioxidants: mechanistic insights and therapeutic potentials of various antioxidants and anti-cytokine compounds. *Biomedicine and Pharmacotherapy*, *178*, 117177. https://doi.org/https://doi.org/10.1016/j.biopha.2024.117177

Burkill, H. (1985). The useful plants of West Africa. *Royal Botanical Gardens, Kew*, *1*, p319.

Chandimali, N., Bak, S. G., Park, E. H., Lim, H.-J., Won, Y.-S., Kim, E.-K., . . . Lee, S. J. (2025). Free radicals and their impact on health and antioxidant defenses: a review. *Cell Death Discovery*, *11*(1), 19. https://doi.org/10.1038/s41420-024-02278-8

Collin, M. S., Venkatraman, S. K., Vijayakumar, N., Kanimozhi, V., Arbaaz, S. M., Stacey, R. G. S., . . . Swamiappan, S. (2022). Bioaccumulation of lead (Pb) and its effects on human: A review. *Journal of Hazardous Materials Advances*, *7*, 100094. https://doi.org/https://doi.org/10.1016/j.hazadv.2022.100094

Dah-Nouvlessounon, D., Chokki, M., Noumavo, A. D. P., Cârâc, G., Furdui, B., Sina, H., . . . Baba-Moussa, F. (2023). Ethnopharmacological Value and Biological Activities via Antioxidant and Anti-Protein Denaturation Activity of Morinda lucida Benth and Momordica charantia L. Leaves Extracts from Benin. *Plants*, *12*(6), 1228. https://www.mdpi.com/2223-7747/12/6/1228

Dobrakowski, M., Boroń, M., Birkner, E., Kasperczyk, A., Chwalińska, E., Lisowska, G., & Kasperczyk, S. (2017). The Effect of a Short-Term Exposure to Lead on the Levels of Essential Metal Ions, Selected Proteins Related to Them, and Oxidative Stress Parameters in Humans. *Oxidative Medicine and Cellular Longevity*, *2017*, 8763793. https://doi.org/10.1155/2017/8763793

Dou, J., Zhou, L., Zhao, Y., Jin, W., Shen, H., & Zhang, F. (2022). Effects of long-term high-level lead exposure on the immune function of workers. *Archives of Environmental & Occupational Health*, *77*(4), 301-308.

Ellman, G. L. (1959). Tissue sulfhydryl groups. *Archives of Biochemistry and Biophysics*, *82*(1), 70-77. https://doi.org/10.1016/0003-9861(59)90090-6

Erel, O., & Neselioglu, S. (2014). A novel and automated assay for thiol/disulphide homeostasis. *Clinical Biochemistry*, *47*(18), 326-332.

Fiorim, J., Simões, M. R., de Azevedo, B. F., Ribeiro, R. F., dos Santos, L., Padilha, A. S., & Vassallo, D. V. (2020). Increased endothelial nitric oxide production after low level lead exposure in rats involves activation of angiotensin II receptors and PI3K/Akt pathway. *Toxicology*, *443*, 152557. https://doi.org/https://doi.org/10.1016/j.tox.2020.152557

Flora, G., Gupta, D., & Tiwari, A. (2012). Toxicity of lead: A review with recent updates. *Interdisciplinary Toxicology*, *5*(2), 47-58. https://doi.org/10.2478/v10102-012-0009-2

Forman, H. J., Zhang, H., & Rinna, A. (2009). Glutathione: overview of its protective roles, measurement, and biosynthesis. *Molecular Aspects of Medicine*, *30*(1-2), 1-12.

Garza-Juárez, A., Pérez-Carrillo, E., Arredondo-Espinoza, E. U., Islas, J. F., Benítez-Chao, D. F., & Escamilla-García, E. (2023). Nutraceuticals and Their Contribution to Preventing Noncommunicable Diseases. *Foods*, *12*(17), 3262. https://www.mdpi.com/2304-8158/12/17/3262

Gbadamosi, I., Moody, J., & Lawal, A. (2011). Phytochemical screening and proximate analysis of eight ethnobotanicals used as antimalaria remedies in Ibadan, Nigeria. *J Appl Biosci*, *44*, 2967-2971.

Green, L. C., Wagner, D. A., Glogowski, J., Skipper, P. L., Wishnok, J. S., & Tannenbaum, S. R. (1982). Analysis of nitrate, nitrite, and [15N]nitrate in biological fluids. *Analytical Biochemistry*, *126*(1), 131-138. https://doi.org/10.1016/0003-2697(82)90118-x

Gruber, C. W. (2010). Global cyclotide adventure: a journey dedicated to the discovery of circular peptides from flowering plants. *Biopolymers*, *94*(5), 565-572. https://doi.org/10.1002/bip.21414

Gruber, C. W., Elliott, A. G., Ireland, D. C., Delprete, P. G., Dessein, S., Goransson, U., . . . Robbrecht, E. (2008). Distribution and evolution of circular miniproteins in flowering plants. *The Plant Cell*, *20*(9), 2471-2483.

Gurer, H., & Ercal, N. (2000). Can antioxidants be beneficial in the treatment of lead poisoning? *Free Radical Biology and Medicine*, *29*(10), 927-945. https://doi.org/https://doi.org/10.1016/S0891-5849(00)00413-5

Habig, W. H., & Jakoby, W. B. (1981). Assays for differentiation of glutathione S-Transferases. In *Methods in Enzymology* (Vol. 77, pp. 398-405). Elsevier.

Harshitha, P., Bose, K., & Dsouza, H. S. (2024). Influence of lead-induced toxicity on the inflammatory cytokines. *Toxicology*, *503*, 153771. https://doi.org/https://doi.org/10.1016/j.tox.2024.153771

Hernandez, J.-F., Gagnon, J., Chiche, L., Nguyen, T. M., Andrieu, J.-P., Heitz, A., . . . Le Nguyen, D. (2000). Squash trypsin inhibitors from Momordica cochinchinensis exhibit an atypical macrocyclic structure. *Biochemistry*, *39*(19), 5722-5730.

Jollow, D., Mitchell, J., Zampaglione, N., & Gillette, J. J. P. (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. *11*(3), 151-169.

Koehbach, J., Attah, A. F., Berger, A., Hellinger, R., Kutchan, T. M., Carpenter, E. J., . . . Wong, G. K. S. (2013). Cyclotide discovery in Gentianales revisited—identification and characterization of cyclic cystine-knot peptides and their phylogenetic distribution in Rubiaceae plants. *Peptide Science*, *100*(5), 438-452.

Kumar, A., Dogra, S., & Prakash, A. (2009). Protective effect of curcumin (Curcuma longa), against aluminium toxicity: Possible behavioral and biochemical alterations in rats. *Behavioural Brain Research*, *205*(2), 384-390.

Kundi, H., Ates, I., Kiziltunc, E., Cetin, M., Cicekcioglu, H., Neselioglu, S., . . . Ornek, E. (2015). A novel oxidative stress marker in acute myocardial infarction; thiol/disulphide homeostasis. *The American journal of emergency medicine*, *33*(11), 1567-1571.

Kwofie, K. D., Tung, N. H., Suzuki-Ohashi, M., Amoa-Bosompem, M., Adegle, R., Sakyiamah, M. M., . . . Atchoglo, P. (2016). Antitrypanosomal activities and mechanisms of action of novel tetracyclic iridoids from Morinda lucida Benth. *Antimicrobial Agents and Chemotherapy*, *60*(6), 3283-3290.

Libby, P. (2007). Inflammatory mechanisms: the molecular basis of inflammation and disease. *Nutrition Reviews*, *65*(suppl_3), S140-S146.

Liu, W., Chen, X., Li, H., Zhang, J., An, J., & Liu, X. (2022). Anti-Inflammatory Function of Plant-Derived Bioactive Peptides: A Review. *Foods*, *11*(15). https://doi.org/10.3390/foods11152361

Lowry, O. H., Rosebrough, N. J., Farr, A. L., & Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. *Journal of Biological Chemistry*, *193*(1), 265-275. https://www.ncbi.nlm.nih.gov/pubmed/14907713

Lushchak, V. I. (2012). Glutathione homeostasis and functions: potential targets for medical interventions. *Journal of Amino Acids*, *2012*, 736837. https://doi.org/10.1155/2012/736837

Majekodunmi, S., Adegoke, O., & Odeku, O. (2008). Formulation of the extract of the stem bark of Alstonia boonei as tablet dosage form. *Tropical Journal of Pharmaceutical Research*, *7*(2), 987-994.

Mensor, L. L., Menezes, F. S., Leitão, G. G., Reis, A. S., Santos, T. C. d., Coube, C. S., & Leitão, S. G. (2001). Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. *Phytotherapy Research*, *15*(2), 127-130.

Mounnissamy, V. M., Kavimani, S., Balu, V., & Quine, S. D. (2007). Evaluation of Anti-inflammatory and Membrane stabilizing property of Ethanol Extract of Cansjera rheedii J. Gmelin (Opiliaceae). *Iranian Journal of Pharmacology and Therapeutics*, *6*(2), 235-230.

Mumtaz, S., Ali, S., Khan, R., Shakir, H. A., Tahir, H. M., Mumtaz, S., & Andleeb, S. (2020). Therapeutic role of garlic and vitamins C and E against toxicity induced by lead on various organs. *Environmental Science and Pollution Research*, *27*, 8953-8964.

Nguyen, G. K. T., Lian, Y., Pang, E. W. H., Nguyen, P. Q. T., Tran, T. D., & Tam, J. P. (2013). Discovery of linear cyclotides in monocot plant Panicum laxum of Poaceae family provides new insights into evolution and distribution of cyclotides in plants. *Journal of Biological Chemistry*, *288*(5), 3370-3380.

Nguyen, G. K. T., Zhang, S., Nguyen, N. T. K., Nguyen, P. Q. T., Chiu, M. S., Hardjojo, A., & Tam, J. P. (2011). Discovery and characterization of novel cyclotides originated from chimeric precursors consisting of albumin-1 chain a and cyclotide domains in the Fabaceae family. *Journal of Biological Chemistry*, *286*(27), 24275-24287.

Ni, Z., Hou, S., Barton, C. H., & Vaziri, N. D. (2004). Lead exposure raises superoxide and hydrogen peroxide in human endothelial and vascular smooth muscle cells. *Kidney International*, *66*(6), 2329-2336. https://doi.org/https://doi.org/10.1111/j.1523-1755.2004.66032.x

Nwanna, E. (2021). Protective effect of alkaloid-rich extract of Brimstone tree (Morinda lucida) on Neurotoxicity in the fruit-fly (Drosophila melanogaster) model. *African Journal of Biomedical Research*, *24*(2), 257-263.

Ogbole, O. O., Akinleye, T. E., Nkumah, A. O., Awogun, A. O., Attah, A. F., Adewumi, M. O., & Adeniji, A. J. (2021). In vitro antiviral activity of peptide-rich extracts from seven Nigerian plants against three non-polio enterovirus species C serotypes. *Virology Journal*, *18*, 1-7.

Okagu, I. U., & Udenigwe, C. C. (2022). Transepithelial transport and cellular mechanisms of food-derived antioxidant peptides. *Heliyon*, *8*(10), e10861. https://doi.org/https://doi.org/10.1016/j.heliyon.2022.e10861

Olajide, O. A., Awe, S. O., Makinde, J. M., Ekhelar, A. I., Olusola, A., Morebise, O., & Okpako, D. T. (2000). Studies on the anti-inflammatory, antipyretic and analgesic properties of Alstonia boonei stem bark. *Journal of Ethnopharmacology*, *71*(1-2), 179-186.

Olakkaran, S., Antony, A., Kizhakke Purayil, A., Tilagul Kumbar, S., & Hunasanahally Puttaswamygowda, G. (2018). Lead modulated Heme synthesis inducing oxidative stress mediated Genotoxicity in Drosophila melanogaster. *Science of the Total Environment*, *634*, 628-639. https://doi.org/https://doi.org/10.1016/j.scitotenv.2018.04.004

Opie, E. L. (1962). On the relation of necrosis and inflammation to denaturation of proteins. *The Journal of experimental medicine*, *115*(3), 597.

Opoku, F., & Akoto, O. (2015). Antimicrobial and phytochemical properties of Alstonia boonei extracts. *Organic Chemistry: Current Research*, *4*(1), 137.

Osunbor, J., & Orobor, A. (2023). Investigation of Some Oxidative Stress Parameters in Drosophila Melanogaster Exposed to Lead and Treated with Picralima Nitida. *Journal of Applied Sciences and Environmental Management*, *27*(5), 905-910.

Oyebode, O. A., Erukainure, O. L., Ibeji, C. U., Koorbanally, N. A., & Islam, M. S. (2019). Phytochemical constituents, antioxidant and antidiabetic activities of different extracts of the leaves, stem and root barks of Alstonia boonei: an in vitro and in silico study. *Botany letters*, *166*(4), 444-456.

Padmanabhan, P., & Jangle, S. (2012). Evaluation of in-vitro anti-inflammatory activity of herbal preparation, a combination of four medicinal plants. *International journal of basic and applied medical sciences*, *2*(1), 109-116.

Patra, R. C., Rautray, A. K., & Swarup, D. (2011). Oxidative stress in lead and cadmium toxicity and its amelioration. *Veterinary Medicine International*, *2011*, 457327. https://doi.org/10.4061/2011/457327

Plan, M. R. R., Göransson, U., Clark, R. J., Daly, N. L., Colgrave, M. L., & Craik, D. J. (2007). The cyclotide fingerprint in Oldenlandia affinis: elucidation of chemically modified, linear and novel macrocyclic peptides. *ChemBioChem*, *8*(9), 1001-1011.

Rand, M. D., Kearney, A. L., Dao, J., & Clason, T. (2010). Permeabilization of Drosophila embryos for introduction of small molecules. *Insect Biochemistry and Molecular Biology*, *40*(11), 792-804. https://doi.org/10.1016/j.ibmb.2010.07.007

Rees, N., & Fuller, R. (2020). *The toxic truth: children's exposure to lead pollution undermines a generation of future potential*. Unicef.

Rinsky, J. L., Higgins, S., Angelon-Gaetz, K., Hogan, D., Lauffer, P., Davies, M., . . . MacFarquhar, J. (2018). Occupational and take-home lead exposure among lead oxide manufacturing employees, North Carolina, 2016. *Public Health Reports*, *133*(6), 700-706.

Roy, A., Das, S., Chatterjee, I., Roy, S., & Chakraborty, R. (2022). Anti-inflammatory Effects of Different Dietary Antioxidants. In H. M. Ekiert, K. G. Ramawat, & J. Arora (Eds.), *Plant Antioxidants and Health* (pp. 573-597). Springer International Publishing. https://doi.org/10.1007/978-3-030-78160-6_20

Saalu, L. C. (2016). Nigerian folklore medicinal plants with potential antifertility activity in males: a scientific appraisal.

Sadique, J., Al-Rqobah, W., Bughaith, M., & El-Gindy, A. (1989). The bio-activity of certain medicinal plants on the stabilization of RBC membrane system.

Sánchez, A., & Vázquez, A. (2017). Bioactive peptides: A review. *Food quality and safety*, *1*(1), 29-46.

Ściskalska, M., & Milnerowicz, H. (2020). The role of GSTπ isoform in the cells signalling and anticancer therapy. *European Review for Medical and Pharmacological Sciences*, *24*(16).

Sharifi-Rad, M., Anil Kumar, N. V., Zucca, P., Varoni, E. M., Dini, L., Panzarini, E., . . . Sharifi-Rad, J. (2020). Lifestyle, Oxidative Stress, and Antioxidants: Back and Forth in the Pathophysiology of Chronic Diseases [Review]. *Frontiers in Physiology*, *11*. https://doi.org/10.3389/fphys.2020.00694

Sharma, J., Al-Omran, A., & Parvathy, S. (2007). Role of nitric oxide in inflammatory diseases. *Inflammopharmacology*, *15*, 252-259.

Sheweita, S., Mashaly, S., Newairy, A., Abdou, H., & Eweda, S. (2016). Changes in oxidative stress and antioxidant enzyme activities in streptozotocin-induced diabetes mellitus in rats: Role of Alhagi maurorum extracts. *Oxidative Medicine and Cellular Longevity*, *2016*(1), 5264064.

Shi, H., Pei, L., Gu, S., Zhu, S., Wang, Y., Zhang, Y., & Li, B. (2012). Glutathione S-transferase (GST) genes in the red flour beetle, Tribolium castaneum, and comparative analysis with five additional insects. *Genomics*, *100*(5), 327-335. https://doi.org/https://doi.org/10.1016/j.ygeno.2012.07.010

Shilpa, O., Anupama, K. P., Antony, A., & Gurushankara, H. P. (2021). Lead (Pb) induced Oxidative Stress as a Mechanism to Cause Neurotoxicity in Drosophila melanogaster. *Toxicology*, *462*, 152959. https://doi.org/10.1016/j.tox.2021.152959

Siddique, H. R., Gupta, S. C., Dhawan, A., Murthy, R. C., Saxena, D. K., & Chowdhuri, D. K. (2005). Genotoxicity of industrial solid waste leachates in *Drosophila melanogaster*. *Environmental and Molecular Mutagenesis*, *46*(3), 189-197. https://doi.org/10.1002/em.20149

Umapathy, E., Ndebia, E., Meeme, A., Adam, B., Menziwa, P., Nkeh-Chungag, B., & Iputo, J. (2010). An experimental evaluation of Albuca setosa aqueous extract on membrane stabilization, protein denaturation and white blood cell migration during acute inflammation. *J Med Plants Res*, *4*(9), 789-795.

Venkareddy, L. K. (2015). Potential of casein as a nutrient intervention to alleviate lead (Pb) acetate-mediated oxidative stress and neurotoxicity: first evidence in Drosophila melanogaster. *Neurotoxicology*, *48*, 142-151.

Virgolini, M. B., & Aschner, M. (2021). Molecular mechanisms of Lead neurotoxicity. *Adv Neurotoxicol*, *5*, 159-213. https://doi.org/10.1016/bs.ant.2020.11.002

Wang, H. l., Sun, Z. o., Rehman, R. u., Wang, H., Wang, Y. f., & Wang, H. (2017). Rosemary extract-mediated lifespan extension and attenuated oxidative damage in drosophila melanogaster fed on high-fat diet. *Journal of Food Science*, *82*(4), 1006-1011.

Wolff, S. P. (1994). Ferrous ion oxidation in presence of ferric ion indicator xylenol orange for measurement of hydroperoxides. *Methods in Enzymology*, *233*, 182-189.

Xie, Z., Huang, J., Xu, X., & Jin, Z. (2008). Antioxidant activity of peptides isolated from alfalfa leaf protein hydrolysate. *Food Chemistry*, *111*(2), 370-376. https://doi.org/https://doi.org/10.1016/j.foodchem.2008.03.078

Xu, B., Dong, Q., Yu, C., Chen, H., Zhao, Y., Zhang, B., . . . Chen, M. (2024). Advances in Research on the Activity Evaluation, Mechanism and Structure-Activity Relationships of Natural Antioxidant Peptides. *Antioxidants*, *13*(4), 479. https://www.mdpi.com/2076-3921/13/4/479

Yan, L. D., Rouzier, V., Pierre, J. L., Lee, M. H., Muntner, P., Parsons, P. J., . . . Pierre, G. (2022). High lead exposure associated with higher blood pressure in Haiti: a warning sign for low-income countries. *Hypertension*, *79*(1), 283-290.

Zemolin, A. P., Cruz, L. C., Paula, M. T., Pereira, B. K., Albuquerque, M. P., Victoria, F. C., . . . Franco, J. L. (2014). Toxicity induced by Prasiola crispa to fruit fly Drosophila melanogaster and cockroach Nauphoeta cinerea: evidence for bioinsecticide action. *Journal of Toxicology and Environmental Health. Part A*, *77*(1-3), 115-124. https://doi.org/10.1080/15287394.2014.866927

Zhang, H.-Y., Li, H.-Z., Zhang, T.-W., & Zhang, Z.-J. (2022). Research progress on the mechanism of antioxidant peptides.

Zhang, J., Li, W., Ying, Z., Zhao, D., Yi, G., Li, H., & Liu, X. (2020). Soybean protein-derived peptide nutriment increases negative nitrogen balance in burn injury-induced inflammatory stress response in aged rats through the modulation of white blood cells and immune factors. *Food & Nutrition Research*, *64*.

Zheng, K., Zeng, Z., Tian, Q., Huang, J., Zhong, Q., & Huo, X. (2023). Epidemiological evidence for the effect of environmental heavy metal exposure on the immune system in children. *Science of the Total Environment*, *868*, 161691.

Zhu, Z., Xu, Z., Li, Y., Fan, Y., Zhou, Y., Song, K., & Meng, L. (2024). Antioxidant Function and Application of Plant-Derived Peptides. *Antioxidants*, *13*(10), 1203. https://www.mdpi.com/2076-3921/13/10/1203