



Moringa oleifera* Leaf Extract Promotes Antioxidant, Survival, Fecundity, and Locomotor Activities in *Drosophila melanogaster

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Authors' contributions

This work was carried out in collaboration with all authors. Author WMI designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors WMI, SO, SSG and ETA managed the analyses of the study. Author GDB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: The study was designed to investigate antioxidant, survivability, fecundity, and locomotor activity of *Moringa oleifera* leaf (MOL) extract in *Drosophila melanogaster*.

Materials and Methods: The study was conducted at the Africa Centre of Excellence in phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria, in August 2019 - March 2020. In the first place, *in vitro* analysis of the antioxidant activity of extracts of *M. oleifera* in various solvents (Aqueous, Methanol-Aqueous co-solvent (80 % v/v) and n-Hexane) were evaluated using DPPH (1,1-Diphenyl-2-Picrylhydrazyl) assay. Based on the *in vitro* result, the methanol extract with the best free radical scavenging activity was used in graded doses for conducting the *in vivo* studies, and the observations were recorded. Distilled water (1000 µL) was

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used alone in 10 g fly food (as negative control) or as a solvent to dissolve MOL extract or ascorbic acid (positive control) separately before mixing with the fly food. Statistical significance was taken at $P < 0.05$

Results: The methanol extract of *M. oleifera* leaf (MEMOL) showed significantly ($P < 0.05$) higher free radical scavenging ability ($IC_{50} = 60 \mu\text{g/ml}$) compared with the aqueous ($IC_{50} = 100 \mu\text{g/ml}$) and n-hexane ($IC_{50} = 250 \mu\text{g/ml}$) extracts respectively. The median lethal dose (LD_{50}) of MEMOL was $>2000 \text{ mg}$. Supplementation with MEMOL non-significantly ($P > 0.05$) improved movement, significantly ($P < 0.05$) increased survivability, fecundity, and total thiol level. The activities of glutathione-S-transferase (GST) and catalase (CAT) significantly ($P < 0.05$) increased. The superoxide dismutase (SOD) activity non-significantly ($P > 0.05$) decreased, while malondialdehyde (MDA) concentration decreased significantly ($P < 0.05$) compared with controls, respectively.

Conclusion: *In vitro* study suggested better antioxidant activity of MEMOL. *In vivo* study also revealed that MEMOL was relatively safe in *D. melanogaster*, supported by high LD_{50} , increased survivability, fecundity, locomotor ability, antioxidant enzyme activities, total thiol level, along with a concomitant decrease in MDA content.

Keywords: Antioxidants; Malondialdehyde; *Moringa oleifera*; superoxide dismutase; total thiols.

1. INTRODUCTION

Medicinal plants are alternatives for the treatment of various diseases in man and animals. They contain bioactive constituents that are responsible for their therapeutic action [1], and these phytoconstituents are lead compounds for the manufacture of modern medicines [2]. Several indices are used for evaluating the medicinal values of plants, of which antioxidant activities are among the most important [3]. The genus *Moringa* (Family: Moringaceae) comprises of thirteen (13) species [4] of which the species *Moringa oleifera* Lam. is more commonly cultivated and used in the tropical or sub-tropical countries including Nigeria [5,6]. *M. oleifera* is well known for its antioxidant effects [6,7]. The plant contains a high concentration of vitamins A and C [8,9] as well as tannins, phenols, and flavonoids [9,10,11,12]. Several research works have reported the *in vitro* free radical scavenging and *in vivo* antioxidant properties of *M. oleifera* leaves, stem, bark, and seed oil, respectively [7, 8, 9]. However, the leaves of *M. oleifera* remains the most extensively used part of the plant [5]. Ezejindu et al. [13] described the protective effect of *M. oleifera* leaf extract against mercury-induced kidney toxicity in rats. Maduka et al. [14] demonstrated the ameliorative efficacy of *M. oleifera* leaf against acetaminophen-induced cytotoxicity. Sathya et al. [15] reported that *M. oleifera* leaves prevented cyclophosphamide-induced DNA damage in mice.

Reactive oxygen and nitrogen species (RONS) are highly reactive free radicals produced in a biosystem during oxidative phosphorylation or xenobiotic exposure [16]. At low concentrations, RONS may act as second messengers in

signaling pathways [17]. However, at high concentrations, free radicals may attack biomolecules and damage lipids, protein, and nucleic acids. [18]. The body fights these free radicals through enzymatic (e.g., Superoxide dismutase, Catalase, Glutathione peroxidases) [19,20] or non-enzymatic antioxidants (e.g., Vitamins C & E, albumin, thiols) [20]. Thus, oxidative stress occurs when the rate of RONS production supersedes the natural antioxidant mechanisms of the body [21]. Oxidative stress is implicated in human diseases (e.g., Diabetes mellitus) [21] or xenobiotic exposures (e.g., highly active antiretroviral therapy (HAART) drugs) [22]. In diabetes mellitus, the state of enhanced glycolysis promotes pyruvate generation and increased mitochondrial membrane potentials. These events result in severe mitochondrial dysfunction that culminates into high free radical generation at complex II. [16]. Nucleoside reverse transcriptase inhibitors (NRTIs) competes with polymerase gamma (pol- γ), causing a profound reduction in mitochondria DNA (mtDNA) quantity and value [23] and results in mitochondrial dysfunctions, including decreased energy production and escalation in RONS generation [23]. Therefore, the search for safe and efficiently sourced plant-based antioxidants to ameliorate the menace of RONS shall tremendously contribute to general health and wellbeing.

Drosophila melanogaster is a well-known model for studying oxidative stress [24,25] and the evaluation of plants for antioxidant properties [26]. Most importantly, 60-75% of genes implicated in human diseases are very similar to *D. melanogaster* genes [25]. Precisely, *D. melanogaster* possesses a well-defined

antioxidant defense system that is comparable to mammals [24]. It has two superoxide dismutase (Sod) genes, a thioredoxin reductase (TrxR-1) gene, and thioredoxin peroxidase (TPxs) gene. Another crucial antioxidant gene in *D. melanogaster* is glutathione peroxidase (GTPx1) that is similar in activity to thioredoxin peroxidase [24]. Survivability, fecundity, and locomotor assays are common end-points of toxicity in *D. melanogaster* [25,27]. Some of the many advantages of *D. melanogaster* as a research tool include its highly conserved aging pathway [28], short developmental cycle [29], and the similarity of neurotransmitters (e.g., acetylcholine) implicated in skeletal muscle movement disorders [30]. Therefore, the present study was designed to investigate the antioxidant activity and safety of *M. oleifera* leaf in *D. melanogaster*. As regards the safety profile, behavioral and biochemical end-points of toxic exposures in *D. melanogaster*, namely survivability, locomotor, fecundity, and some oxidative stress biomarkers (total thiols, SOD, CAT, GST, and MDA) were studied.

2. MATERIALS AND METHODS

2.1 Plant Material: Collection, Identification, and Extraction

The leaves of *M. oleifera* Lam. were collected from Dogo Village, Benue State of Nigeria, and authenticated at the Federal College of Forestry, Jos, Plateau state of Nigeria. The specimen with voucher number FHJ244 was deposited at the college herbarium. The leaves were washed and shade dried. The extraction was carried out as described by Luqman et al. [7] with some modifications. 250 g of *M. oleifera* leaf powder was weighed and soaked in different solvent systems, namely, distilled water (100%), methanol/distilled water co-solvent system (80% v/v), and n-hexane (100%) by maceration (sample/solvent mass ratio 1:10) for 72 hours shaking the extraction bottles intermittently. With the aid of a Whatman filter paper (No.1), the macerates were filtered, and alcohol contents recovered on a rotary evaporator (Buchi, Flavil, Switzerland) at 45°C, and then freeze-dried at -4°C. The dried pellets were weighed and kept in amber airtight bottles in the refrigerator until further use.

2.2 Drosophila Melanogaster Strain and Culture

The Drosophila research laboratory of ACEPRED University of Jos, Nigeria, provided

the fly stock (*D. melanogaster* Harwich strain) used for this study. Flies were fed on standard fly food and maintained under suitable environmental conditions as prescribed by Abolaji et al. [31].

2.3 Free Radical Scavenging Ability (DPPH Assay)

The free radical scavenging activity of *M. oleifera* leaf extract with different solvents: Viz aqueous (100%), methanol (80%v/v), n-hexane (100%) were investigated using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method [7]. The stable dark violet color of the DPPH radical in ethanol is breached upon reaction with a hydrogen donor [7]. Different concentrations each of *M. oleifera* solvent extracts (10, 20, 30, 60, 100, 150, 250, 300 µg/ml or Ascorbic acid 60 µg/ml or distilled water) were mixed with 100 mM TRIS-HCL buffer (pH 7.4) and 100 µM of DPPH. The mixture was incubated in the dark for 30 min, after which the absorbances were read at 515 nm against a blank.

2.4 Determination of Median Lethal Dose (LD₅₀)

The LD₅₀, defined as the amount of a substance in standard fly media that kills 50% of flies in seven days [32], was determined as described by lorjiim et al. [33] with little modifications. Sixty (60) flies (1- 3 day old) were anesthetized with ice, counted into plastic vials (Length 15 cm x width 1.5 cm), and fed with series of different concentrations of *M. oleifera* (50, 100, 150, 200, 300, 400, 500, 1000, 1500, 2000 mg separately dissolved with 1000 µL distilled water) or 1000 µL distilled water only (as control) each per 10 g fly food respectively for seven days. Each treatment was replicated into five group of sixty flies each. Deaths were counted and recorded every 24 hrs during this period. The cumulative number of fly death was subjected to a dose-response simulation in Graphpad prism 8.0.2 to calculate the LD₅₀.

2.5 Survival Assay

To perform the 28-day survival assay, sixty (60) flies of both genders (1-3 days old) were taken per treatment group. The flies were exposed to 50, 200, 350, 500 mg methanol extract of *M. oleifera* leaf or 25 mM Ascorbic acid previously dissolved in 1000 µL distilled water, respectively, or 1000 µL distilled water alone each mixed with 10 g food in five replicates for 28 days [33]. The mortality and survival rates were scored daily for

28 days, and the survival rate calculated as a percentage of live flies.

2.6 Seven-day Treatment for Biochemical Tests, Fecundity Assay, and Negative Geotaxis

Sixty (60) flies were fed with food supplemented with different concentrations of methanol extract of *M. oleifera* leaves or Ascorbic acid 25 mM (positive control) prepared as described above (section 2.5) in five replicates for seven (7) days [34]. After the seven-day exposure period, ten (10) (5 males, 5 females) were randomly selected from each group and used for determination of locomotor ability and fecundity assay. After that, the flies from the *M. oleifera* supplemented groups or controls were immobilized under ice, weighed, and homogenized using 0.1 M phosphate buffer saline (PBS) (pH 7.0, fly to buffer ratio = 1 mg: 10 μ L). The homogenates were centrifuged using Eppendorf centrifuge (Model No.: AG 5227 R, Germany, temperature 4°C) for 10 min at 4000 rpm. The resultant supernatants were used for the evaluation of Total thiol content, the activities of antioxidant enzymes [Catalase (CAT), Glutathione-S-transferase (GST), Superoxide dismutase (SOD)], and Malondialdehyde (MDA) levels.

2.6.1 Negative geotaxis assay

The negative geotaxis assay (climbing ability) of both *M. oleifera* exposed or control groups was evaluated using the method described by Adedara et al. [34].

2.6.2 Fecundity assay (reproductive ability)

After the 7-day treatment as described above, flies were assayed for reproductive ability [35] with slight changes in the method. Ten flies (5 males and 5 females) from each treatment group were paired in vials containing standard fly food, labeled and kept for 24 hours where they mated and lay eggs, after which the adult flies were removed. The vials (with eggs therein) were observed daily for 14 days for the emergence of adult flies. The cumulative number of flies that emerged during this period of 14 days represents a measure of fecundity or reproductive ability.

2.6.3 Total thiol determination

The Total thiol content was evaluated as described by Abolaji et al. [31]. 510 μ L potassium

phosphate buffer (0.1 M, pH 7.4) and 25 μ L of the sample were added to 30 μ L of DTNB (10 mM) and incubated for 30 min at room temperature. After that, the absorbance was read at 412 nm, and then total thiol levels were calculated (in mmol/mg protein) using 35 μ L of GSH as standard.

2.6.4 Glutathione-S-transferase (GST) activity

The activity of Glutathione-S-transferase (GST; EC 2.5.1.18) was evaluated as described by Omale et al. [21] utilizing 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. The reaction mixture was made to 600 μ L (solution A) by adding 20 μ L of 0.25 M potassium phosphate buffer (pH 7.0 with 2.5 mM EDTA), 510 μ L of 0.1 M GSH at 25°C, 60 μ L of the dilute sample (distilled water to sample ratio 1:5), and 10 μ L of 25 mM CDNB. The absorbance was read at 340 nm (for 2 min at 10 s interval) with the aid of a spectrophotometer (Jenway Model No.7315). The data were expressed in mmol/min/mg of protein using the molar extinction coefficient (ϵ) of 9.6 mM⁻¹ cm⁻¹ of the coloured GST derived GS-DNB conjugate.

2.6.5 Catalase (CAT) activity

The Catalase (CAT; EC 1.11.1.6) activity was determined, as described by Abolaji et al. [31]. The reaction mixture containing 100 μ L of potassium phosphate buffer, pH 7.0, and 194 μ L of 300 mM H₂O₂ was freshly prepared and labeled as solution A. Then 10 μ L of whole fly homogenate was mixed with 590 μ L of the solution A. The clearance of H₂O₂ then measured at 240 nm (2 min at 10 s interval) at room temperature using a spectrophotometer (Jenway Model No. 7315). The activity of CAT was expressed as mmol of H₂O₂ consumed/min/mg of protein.

2.6.6 Superoxide dismutase (SOD) activity

The SOD activity was investigated using the method described by Iorjiim et al. [22]. A 1:5 dilution of 200 μ L of whole fly homogenate in 800 μ L of distilled water was freshly prepared. Then, 200 μ L of the diluted sample was mixed with 2500 μ L of 0.05 M carbonate buffer (pH 10.2). This mixture was allowed to equilibrate in the spectrophotometer, then reaction initiated by adding 300 μ L newly prepared 0.3 M adrenaline. The cuvette containing 2500 μ L buffer, 300 μ L adrenaline, and 200 μ L of water was used as a standard reference. The absorbance was

observed at 480 nm every 30 seconds for 150 seconds.

2.6.7 Malondialdehyde (MDA) content

Malondialdehyde (MDA) level was evaluated using the method of Varshney and Kale [36]. A sample volume of 400 μL was mixed with 1600 μL of a tris-KCl buffer, then 500 μL of 30% TCA was added. Then 500 μL of 0.75% TBA was added in a water bath and allowed for 45 minutes at 80°C. The reaction mixture was gradually cooled in ice and centrifuged at 3000 g for 5 minutes. The supernatant collected was used to read the absorbance at 532 nm against a blank of distilled water as a reference. MDA content was calculated using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$, and the result expressed as MDA formed/mg protein.

3. RESULTS

3.1 Free Radical Scavenging (DPPH) Assay

The ability of different solvent extract of *M. oleifera* leaf to scavenge free radical using DPPH

assay (Fig. 1) showed that the methanol extract of *M. oleifera* was significantly ($P < 0.05$) superior free radical scavenger ($\text{IC}_{50} = 60 \mu\text{g/ml}$) compared with Aqueous ($\text{IC}_{50} = 100 \mu\text{g/ml}$) or n-hexane extracts ($\text{IC}_{50} = 250 \mu\text{g/ml}$) respectively, but was comparable ($P > 0.05$) to that of Ascorbic acid ($\text{IC}_{50} = 43.5 \mu\text{g/ml}$).

3.2 Lethal Dose (LD_{50}) Determination

Series of survival experiments with different concentrations of *M. oleifera* leaf extract were conducted to determine the safe concentrations to use and the duration of exposure. The result of LD_{50} calculated from the survival experiment showed a 90% survival rate in *D. melanogaster* exposed to a maximum of 2000 mg *M. oleifera* extract for seven (7) days (Fig. 2). By extrapolation, the LD_{50} of *M. oleifera* in *D. melanogaster* was $>2000 \text{ mg}$. However, it was observed that supplementation with *M. oleifera* leaf extract above 500 mg resulted in rapid fly food deterioration within 48 hours and hence the risk of non-experimental fly deaths. Thus concentrations from 50 to 500 mg of *M. oleifera* leaf extract were used in this study.

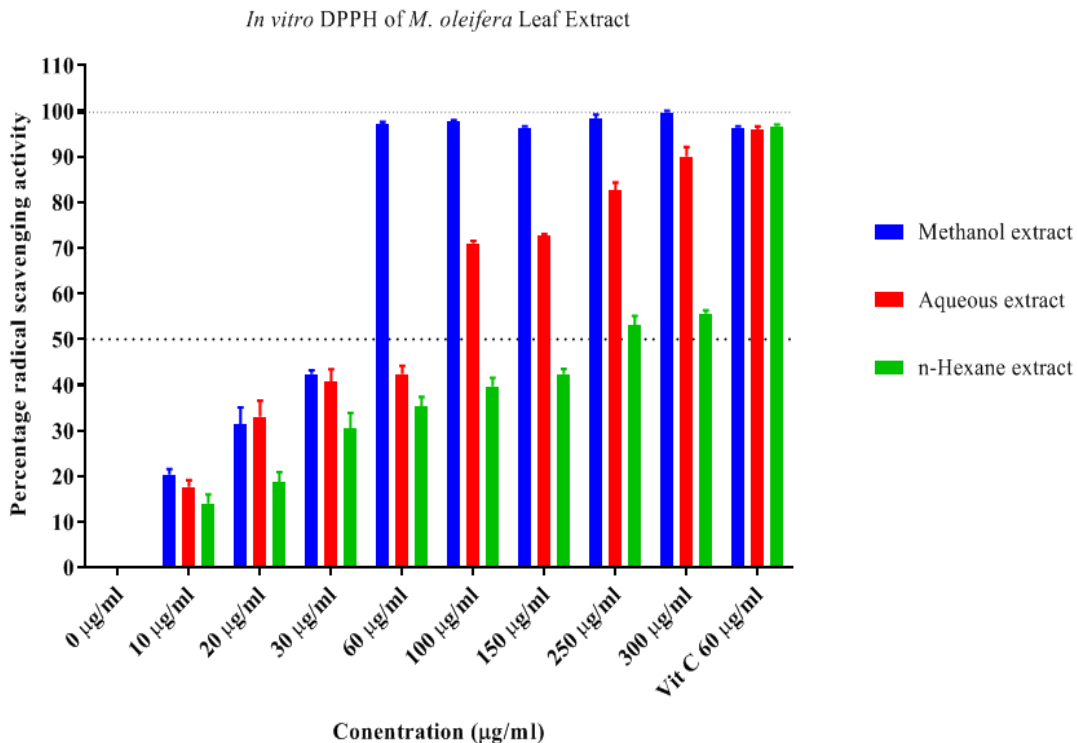


Fig. 1. *In vitro* antioxidant test of *M. oleifera* leaf extract showed significantly ($p < 0.05$) higher antioxidant activity in the methanol extract compare to the aqueous and n-hexane, respectively

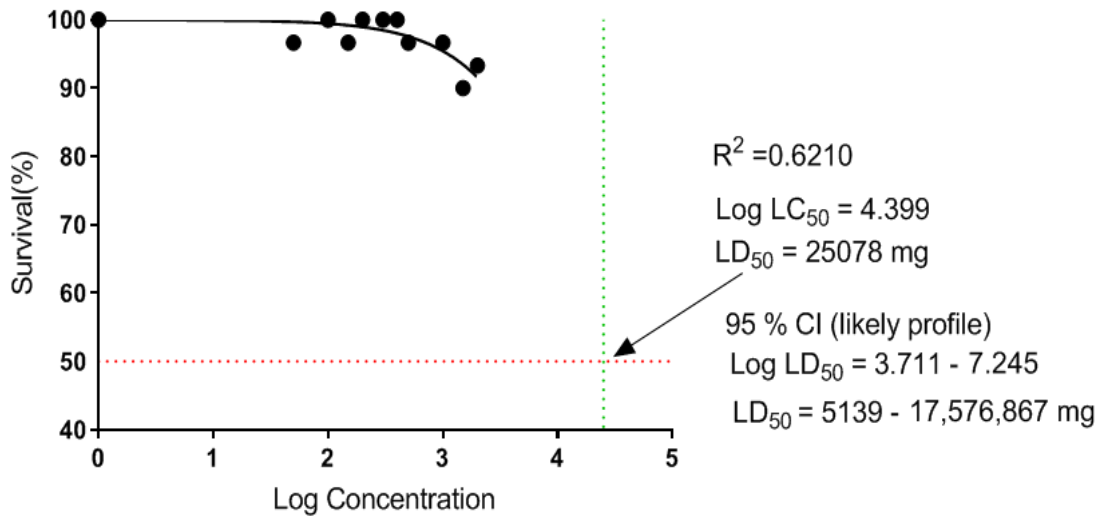


Fig. 2. The LD₅₀ of *M. oleifera* calculated from sigmoid percentage normalized survival curves after 7 days exposure in *Drosophila melanogaster*

3.3 Survival Assay

The result of our survival assay showed significantly ($P=0.001$) increased the survival rate in all the *M. oleifera* supplemented groups compared to Ascorbic acid or distilled water. The mean survival time (MS) of all the experimental

groups were undefined. Calculated hazard ratios were: *M. oleifera* 500 mg = 0.28 ± 0.21 , 350 mg = 0.35 ± 0.20 , 200 mg = 0.50 ± 0.30 , 50 mg = 0.7 ± 0.50 , Ascorbic acid 25 mM = 0.79 ± 0.61 , and distilled water 1.27 ± 0.9 . Based on the hazard ratio and mortality rate, the best concentration was 500 mg, as shown (Fig. 3).

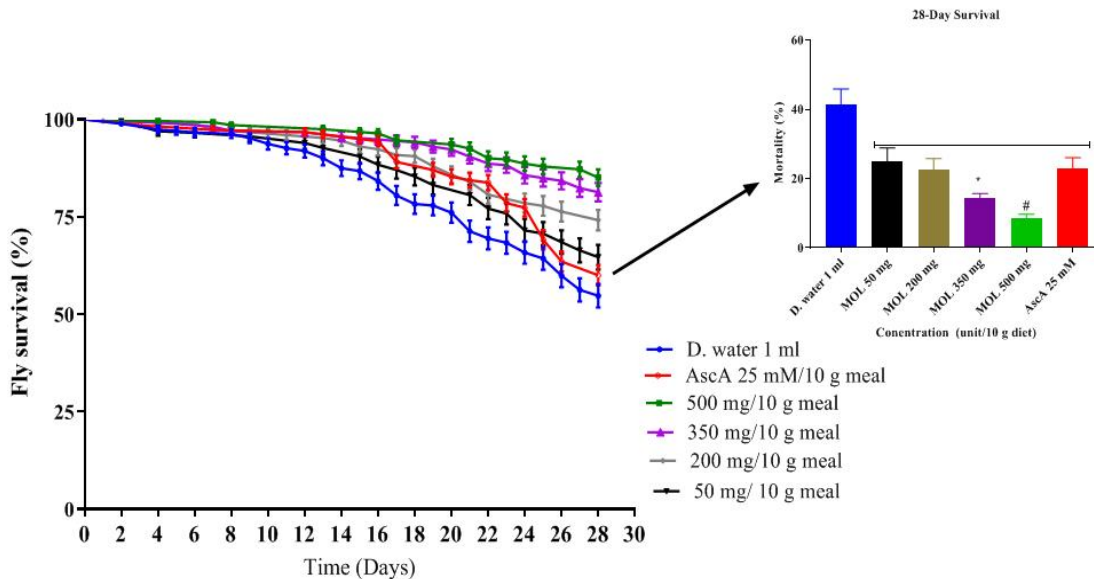


Fig. 3. *Moringa oleifera* leaf (MOL) extract reduced the mortality rate of *D. melanogaster* after 28 days. (A) Survival curve analysis and (B) chart of survival (%) of flies (both sexes) after 28 days of exposure of *D. melanogaster* to MOL

Data are presented as mean \pm SEM of five independent biological replicates carried out in two separate experiments. * $p < 0.05$ vs. control, # $p < 0.05$ vs. ascorbic acid (AscA)

3.4 Negative Geotaxis and Fecundity Assay

The result of negative geotaxis (Fig. 4A) showed a slight but non-significant ($P>0.05$) increase in climbing ability in all the groups supplemented with *M. oleifera* leaf extract (200 mg, 350 mg or 500 mg respectively) compared with control or Ascorbic acid. Fecundity assay (Fig. 4B) showed that supplementation with *M. oleifera* leaf 500 mg significantly ($P<0.05$) increased fly emergence compared to control or Ascorbic acid supplementation. Lower *M. oleifera* concentrations (50 mg, 200 mg, or 350 mg) showed no effect on fly emergence.

3.5 Total Thiol Content and Glutathione-S-Transferase Activity

Total thiol contents (Fig. 5A) was significantly ($P = 0.0001$ or 0.0003) higher in the fly group supplemented with *M. oleifera* 500 mg compared with distilled water or ascorbic acid groups, respectively. Similarly, the activity of GST significantly ($P = 0.0002$ or 0.0001) increased in the experimental group supplemented with 500 mg *M. oleifera* leaf extract compared to either of the controls. The slight increase in GST activity was non-significant ($P=0.88$ or 0.68) at 350 mg *M. oleifera* supplemented group compared to either distilled water or Ascorbic acid group as shown (Fig. 5B).

3.6 Superoxide Dismutase and Catalase Activities

The result of SOD activity (Fig. 6A) showed a non-significant ($P>0.05$) decrease in the treated groups compared with controls. Conversely, the result of Catalase activity significantly ($P < 0.05$) increased in the fly groups supplemented with 350 mg or 500 mg *M. oleifera* leaf extract compared with both control groups. However, supplementation with 200 mg or 50 mg *M. oleifera* leaf extract increased Catalase activity non-significantly ($P > 0.05$) when compared to the control groups as shown (Fig. 6B).

3.7 Malondialdehyde (MDA) Concentration

The MDA result showed a consistent dose-dependent decrease in all *M. oleifera* leaf supplemented groups. The difference was significant ($P = 0.011$) at 500 mg concentrations compared to the control groups, as shown (Fig. 7).

4. DISCUSSION

M. oleifera leaves are used in traditional medicine for the treatment of many disease conditions such as hypertension, diabetes mellitus, asthma, skin diseases, antibacterial, antiviral, scorpion stings, and snakebite [7,8,37].

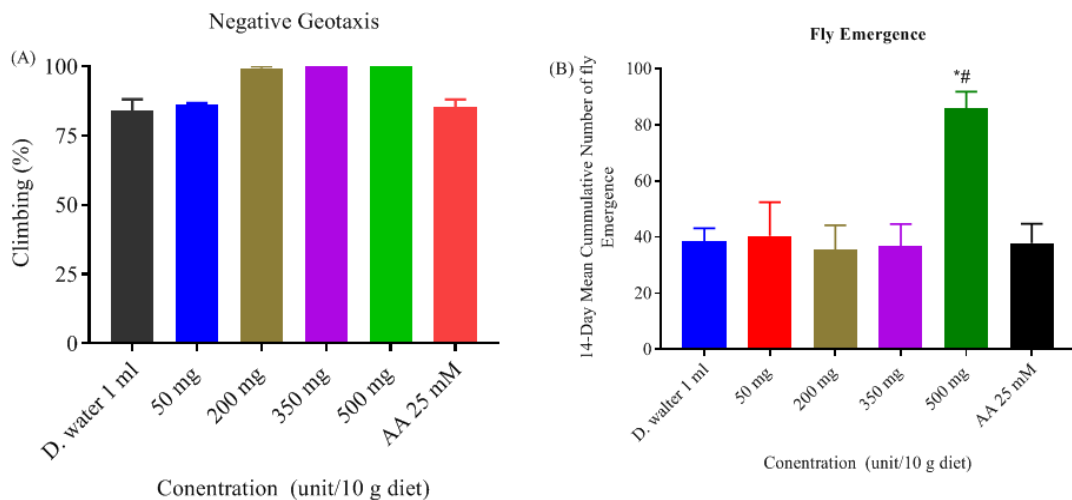


Fig. 4 (A-B). Seven-day *Moringa oleifera* leaf extract exposure increased climbing ability and fly emergence in *D. melanogaster*. (A) Negative geotaxis (B) Fly emergence, and (C) 7 day fly survival

Data are presented as mean±SEM of five independent biological replicates carried out in two separate experiments. * $P<0.05$ vs. control

It is also supplemented in HIV-positive patients receiving HAART [38]. However, data on safety and antioxidant activities are scarcely known.

Current findings showed that the methanolic extract of *M. oleifera* leaf was a better free radical scavenger compared to the aqueous and n-hexane extract, respectively (Fig. 1). It agreed with the work of Fitriana et al. [39], who reported

that methanolic extract of *M. oleifera* leaf showed significantly ($P < 0.001$) higher scavenging ability ($IC_{50} = 49.3 \mu\text{g/ml}$) compared to n-hexane extract ($IC_{50} = 750 \mu\text{g/ml}$). The methanol extract showed the highest antioxidant activity, probably due to a higher concentration of antioxidant compounds available to react with the relatively less reactive DPPH radical. This result was the basis to use the methanol extract of *M. oleifera* for subsequent experiments in this study.

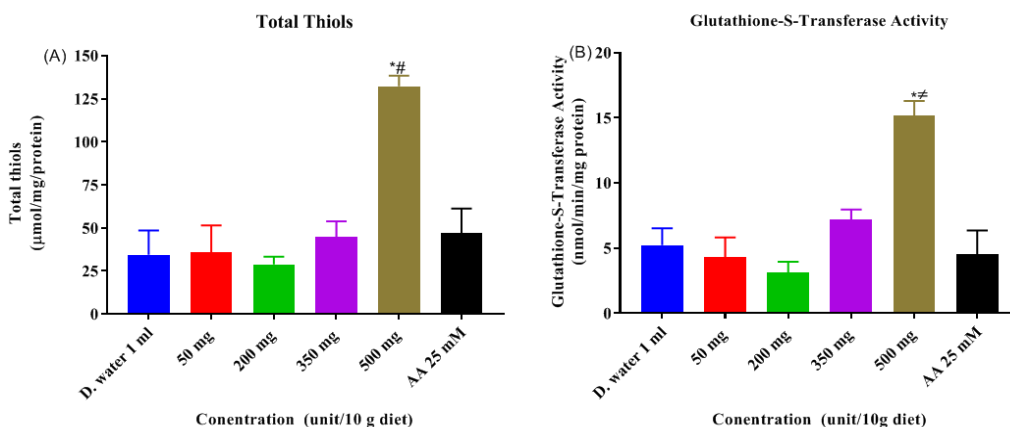


Fig. 5 (A-B). *Moringa oleifera* leaf extract increased total thiol content and Glutathione-S-Transferase activity in *D. melanogaster*. (A) Total thiol content in the whole fly homogenate of *D. melanogaster* exposed to *M. oleifera* after 7 days. (B) Glutathione-S-Transferase activity in the whole fly homogenate of *D. melanogaster* exposed to *M. oleifera* after 7 days

Data presented as mean±SME of five independent biological replicates for each drug concentration (n=60). * $P < 0.05$ vs. control. # $P > 0.05$ vs. ascorbic acid (AA)

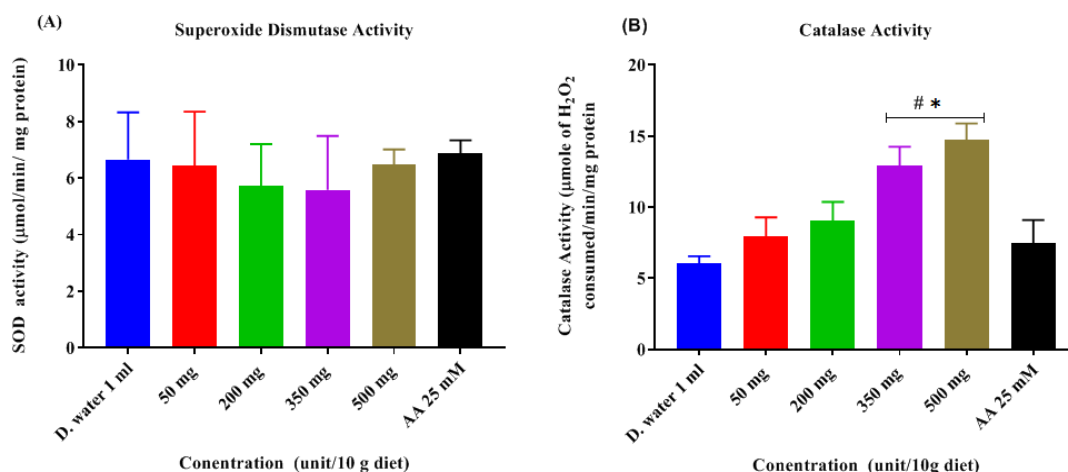


Fig. 6 (A-B). *Moringa oleifera* leaf extract increased superoxide dismutase and catalase activities in *D. melanogaster* (A) Effect of *M. oleifera* leaf on SOD activity in the whole fly homogenate of *D. melanogaster* after 7 days exposure. (B) Effect of *M. oleifera* leaf on SOD activity in the whole fly homogenate of *D. melanogaster* after 7 days exposure

Data presented as mean±SME of five independent biological replicates for each drug concentration (n=60). * $P < 0.05$ vs. control., # $P > 0.05$ vs. ascorbic acid (AA)

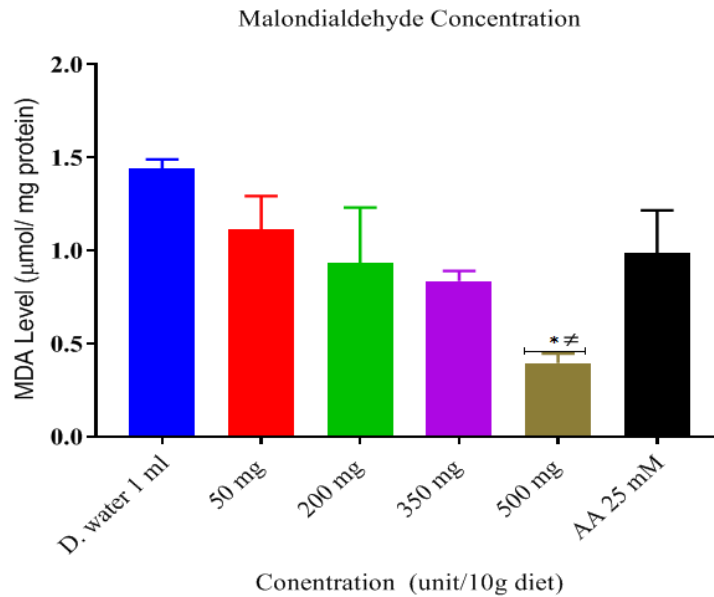


Fig. 7. *Moringa oleifera* leaf extract significantly ($P>0.05$) decreased the MDA level in the whole fly homogenate of *D. melanogaster* after 7 days of exposure

Data presented as mean±SME of five independent biological replicates for each drug concentration (n=60).

* $P<0.05$ vs. control

The LD₅₀ of *M. oleifera* in *D. melanogaster* was above 2000 mg per 10 g fly food. Verma et al. [40] reported that 2000 mg methanol extract of *M. oleifera* caused no death in rats. The survival assay showed a significant ($P = 0.001$) increase in the survival rate of *M. oleifera* exposed flies (Fig. 2) with undefined mean survival time and low hazard ratios (range 0.28±0.21 to 0.7±0.5) compared to ascorbic acid (0.79±0.61) or distilled water (1.27±0.9). The hazard ratios consistently decreased as the concentration of *M. oleifera* leaf extract increased, further demonstrating the relative safety of this extract. The free radical theory of aging proposed that RONS generated during metabolic processes or environmental insults results in loss of functionality in biosystems leading to aging and death [41]. The present LD₅₀ and survival assay put together confirms the relative safety of *M. oleifera* leaf extract and suggests that the extract may have reduced some factors (e.g., decreased RONS generation) that promotes aging in *D. melanogaster*.

The result of negative geotaxis in the current study (Fig. 4A) showed a slight, non-significant ($P>0.05$) improvement in climbing ability. In contrast, the fecundity assay (Fig. 4B) significantly ($P<0.05$) increased fly emergence at the 500 mg *M. oleifera* leaf supplemented group compared to both controls, respectively. Studies

have reported climbing deficits and reproductive decline in states of increased oxidative stress in *D. melanogaster* [34,33]. Locomotor deficits in *D. melanogaster* has been linked to declined acetylcholinesterase activity [27], increased acetylcholine concentrations, and RONS generation [27,42]. Also, oxidative stress has been associated with reduced quantity and quality of sperm and deficits in the human oocyte maturation and fertilization [43,44]. The increased climbing performance and reproductive ability in our present study might be due to the ameliorative effect of *M. oleifera* leaf against the factors mentioned above that could influence locomotor deficits or decreased fecundity.

The role of *M. oleifera* leaf on the antioxidant system in *D. melanogaster* could be best explained by the state of the augmented antioxidant system after seven (7) day supplementations with *M. oleifera* leaf extract evidenced by significant ($P<0.05$) increase in total thiols and GST activity (Fig. 5 A-B). Similarly, relative stability in SOD and significant ($P<0.05$) increase in CAT activities were observed (Fig. 6A-B). The result agrees with an earlier report by Oyewole and Olabiyi [45] wherein *M. oleifera* leaf extract significantly increased Chlorpyrifos-induced decrease in SOD, CAT, and GST activities in a rat model. In

the same context, increased total thiol level in the present study agrees with the work of Ahmed et al. [46]. They recently reported that *M. oleifera* leaf extract significantly elevated sodium fluoride-induced decrease in glutathione (GSH, a thiol-containing antioxidant) in *Nile tilapia*.

The first antioxidant enzymatic defense system involves SOD and CAT activities [19,47,48]. Total thiols are sulfhydryl (-SH) containing compounds (e.g., GSH) that constitute the greater portion of the entire body antioxidants [49]. In a state of severe oxidative stress, thiol groups are the first to be oxidized. Thus, total thiol concentration represents a useful index of the body's antioxidant status [49]. Glutathione-S-transferase is the antioxidant enzyme that plays a catalytic role in the chemical transfer of an active -SH group from thiol antioxidants (e.g., GSH) onto oxidant molecules [50], thereby neutralizing them. The augmented antioxidant system in the present study may be due to free radical scavenging activity, or antioxidant enzyme gene expression that ultimately enhanced total thiol content, as well as GST and CAT activities, respectively. It is pertinent to note that no exogenous inducer of oxidative stress was employed. Though there was an absence of a significant effect on SOD, there was increased total thiols, activities of GST, and CAT. A further impact of *M. oleifera* leaf supplementation on lipid peroxidation was evaluated to support this assertion.

The circulating level of MDA was evaluated to quantify the level of free radical-induced lipid peroxidation. The result (Fig. 7) showed a concentration-dependent (500 mg>350 mg>200 mg<50 mg) decrease in MDA levels. The decrease in MDA level was significantly ($P<0.05$) lower at 500 mg *M. oleifera* leaf supplemented groups compared to the controls (distilled water and 25 mM Ascorbic acid, respectively). The result agrees with the works of Luqman et al.[7] and Oyewole and Olabiyi [45], who independently reported that *M. oleifera* leaf supplementation significantly ($P<0.05$) decreased MDA concentrations. Polyunsaturated fatty acids are highly susceptible to RONS-induced lipid peroxidation [51]. The peroxidation of polyunsaturated fatty acids results in hydroperoxides, which then decomposes to unstable Malondialdehyde (MDA) [48,51]. Free radical-induced lipid peroxidation may change cell membrane flexibility leading to a loss in the functional integrity of membrane-bound enzymes

and apoptosis [52]. The decrease in MDA level, with concomitant improvement in antioxidant parameters, in the current study might be attributed to the free radical scavenging ability of *M. oleifera* leaf extract. However, the genetic involvement of this extract in augmenting antioxidant enzymes is feasible but not elucidated at this stage.

5. CONCLUSION

Methanolic extract of *M. oleifera* leaf has strong free radical scavenging ability. Supplementation with *M. oleifera* leaf increased survivability, locomotor performance, and reproductive capacity in *D. melanogaster*. It also augmented antioxidant system function evidenced by increased total thiol content, stable SOD activity, increased activities of GST, and CAT with a concomitantly decreased MDA concentration. Thus, *M. oleifera* leaf is relatively safe and may be employed in the management of conditions that predisposes to free radical insults.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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