*Journal of Complementary and Alternative Medical Research*

*11(4): 33-46, 2020; Article no.JOCAMR.62136*
*ISSN: 2456-6276*

# *Moringa oleifera* Leaf Extract Extends Lifespan and Ameliorate HAART Drug-Induced Locomotor, Reproductive, and Antioxidant Deficits 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 WMI managed the literature searches. All authors read and approved the final manuscript.*

***Article Information***

DOI: 10.9734/JOCAMR/2020/v11i430194
Editor(s):
(1) Dr. Sachin Kumar Jain, IPS Academy College of Pharmacy, India.
Reviewers:
(1) Ioana Stancium, University of Bucharest, Romania.
(2) Malathi Challa, Ramaiah Institute of Technology, India.
Complete Peer review History: http://www.sdiarticle4.com/review-history/62136

***Original Research Article***

***Received 17 August 2020***
***Accepted 22 October 2020***
***Published 11 November 2020***

## ABSTRACT

**Aim:** To evaluate the longevity and ameliorative activities of *Moringa oleifera* leaf (MOL) extract against some HAART drug-induced toxicities in *Drosophila melanogaster*.
**Materials and Methods:** The research was conducted at the Drosophila laboratory, Africa Centre of Excellence in phytomedicine Research and Development (ACEPRD), University of Jos-Nigeria, between August 2019 - March 2020. *D. melanogaster* (1-3 day) were first exposed for life to different concentrations of MOL (50 – 500 mg) or 25 mM Ascorbic acid or 1000 µL distilled water to determine longevity. Secondly, flies were fed on 46.56 mg of HAART drugs (Efavirenz-based or

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Dolutegravir-based) alone or supplemented with MOL 250 mg or 500 mg per 10 g fly food in five replicates for seven days. Afterward, longevity, fecundity, and negative geotaxis were evaluated. Also, activities of Superoxide dismutase, Catalase, as well as Malondialdehyde content as biomarkers of oxidative stress were evaluated using whole fly homogenate. Statistical significance was taken at $P<0.05$ or ($P<0.006$) (Bonferroni adjusted $P$-value for multiple curve comparisons.

**Results:** The MOL extract significantly ($P<0.001$) increased fly longevity compared to control groups. Similarly, supplementation with 500 mg MOL extracts significantly ($P<0.05$) ameliorate HAART drug-induced deficits in climbing ability, fecundity, SOD, and CAT activities as well as MDA content compared to groups exposed to HAART drugs alone respectively.

**Conclusion:** The results suggest that *M. oleifera* leaf extract extends lifespan and ameliorate HAART drug-induced toxicities via its antioxidant activities. This was supported by improved locomotor and reproductive decline, and restoration of the deficits in the biomarkers of oxidative stress (SOD, CAT, and MDA) in *D. melanogaster*.

*Keywords: Moringa oleifera; antioxidants; oxidative stress; malondialdehyde; superoxide dismutase.*

## 1. INTRODUCTION

Highly active antiretroviral therapy (HAART)-related toxicities constitute a serious public health challenge among the human immunodeficiency virus (HIV) positive population [1,2]. Several studies have implicated these drug-induced adverse events as one of the causes of poor adherence to antiretroviral chemotherapy and treatment failures [3,4,5]. They also confound morbidities and mortalities primarily associated with HIV/AIDs infection [1,6,7]. HAART drugs have impacted positively on the lives of people living with HIV/AIDS; however, the life expectancy of these HAART-treated HIV positive population remains shorter compared to the uninfected population [1]. The molecular mechanisms of different antiretroviral toxic events in humans have been widely documented [1]. The toxicity of nucleoside reverse transcriptase inhibitors (NRTIs) involves mitochondrial dysfunctions[8]. It starts as antiretroviral drug-induced mitochondrial energy depletion, followed by oxidative stress induction that culminates into mutations of mitochondrial deoxynucleic acid (mtDNA) [1,8,9]. Precisely, NRTIs (e.g., Lamivudine, Tenofovir, Emtricitabine) contends with natural nucleosides for the binding site on polymerase gamma (pol-$\gamma$), leading to DNA chain termination, decreased energy production, increased inflammatory signals, reactive Oxygen and Nitrogen species (RONS) generation and cell death [1,9].

Several haematologic (e.g., anemias), metabolic (e.g., lactic acidosis), and skin (e.g., Stevens-Johnson's syndrome) toxicities associated with HAART drugs have been previously described [7,10]. Oxidative stress and reproductive deficits associated with HAART drug-induced toxicity in humans [11,12], as well as rodents [2,13,14], have been reported. In the same context, several investigations have reported locomotor deficits, increased mortality, oxidative stress, and reproductive declines in *Drosophila melanogaster* exposed to HAART drugs [15,16,17].

*Moringa oleifera* Lam. (Family: *moringaceae*) is a medicinal plant widely grown in tropical and sub-tropical regions of the world [18]. In Nigeria, the plant is identified by different local names as Zongale (Hausa), Okwe Oyibo (Igbo), Ewe Ile (Yoruba) [18], and Jegelegede (Tiv) [19]. Ethnopharmacological properties of *M. oleifera* include antistress /antioxidants [20, 21], anti-inflammatory/analgesic [22,18], anti-diabetic, antibiotic and anticancer activities [23]. Research has shown that *M. oleifera* leaf extract possesses protective activities against anti-tubercular drug-induced liver damage [24], acetaminophen-induced nephrotoxicity [25,26], and gamma radiation-induced oxidative stress in a rat model [22]. In traditional medicine, *M. oleifera* leaf (MOL) powder supplementation is common among HIV positive patients taking antiretroviral drugs [27]. Despite the anecdotal report of MOL powder supplementation among HIV patients on antiretroviral drugs therapy [27], there is no empirical data at the moment to support such claims as a standard pharmacological intervention against HAART drug-induced toxicities. Exploring the ameliorative effects of MOL against HAART drug-induced toxicities has thus become very imperative.

Reactive Oxygen and Nitrogen Species (RONS) are highly reactive molecules generated within biological systems during normal cellular respiration or xenobiotic exposure [28,29]. Oxidative stress occurs when the generation of

RONS molecules overwhelms the antioxidant mechanism of the body [30]. Clinically, oxidative stress has been implicated in the mechanism underlying the pathophysiology of human diseases such as diabetes mellitus, neurodegenerative disorders (e.g., Parkinson's disease, Alzheimer's disease), cardiovascular disorders (e.g., hypertension), and various cancers (prostate, breast, lung cancers) [31]. Therefore, search for effective and safe plant-based antioxidants for possible intervention against HAART drug-induced oxidative stress will contribute significantly to health and wellbeing among HIV positive population.

Research has proven that about 75% of the genes implicated in human diseases are well conserved in *Drosophila melanogaster* [32], making the model an excellent tool in toxicology and screening of nutraceuticals for medicinal activities [29]. Iojiim et al. [33] recently reported that *M. oleifera* leaf supplementation significantly increased the survival rate and augmented *in vivo* functions of the antioxidant system in *D. melanogaster.* Thus, the current study aimed at assessing the activity of *Moringa oleifera* leaf extract against longevity as well as its ameliorative potential on HAART drug-induced oxidative stress, locomotor deficits, and reproductive decline [15,16,17] using D. melanogaster model.

## 2. MATERIALS AND METHODS

### 2.1 Chemicals, Reagents, and HAART Drugs

All the chemicals or reagents used were of analytical grades. Vitamin C, Phosphate buffer saline (BPS), DPPH, reduced glutathione (GSH), Hydrogen peroxide, 1-chloro-2,4-dinitrobenzene, (CDNB), and 5,5′-dithiobis (2-nitro-benzoic acid) (DTNB) were sourced via the ACEPRD University of Jos, where the study was designed and performed. Trichloroacetic acid (TCA) 30%, Thiobarbituric acid (TBA) 0.75%, 0.15 M Tris-KCl buffer (pH 7.4), 0.1M HCl, 0.05 M carbonate buffer (pH 10.2) and 0.3 M Adrenaline provided by the Biochemistry Division, National Veterinary Research Institute (NVRI), Vom, Nigeria Dolutegravir-based HAART drug [TDF 300 mg + 3TC 300 mg + DTG 50 mg, TLD) mg per tablet (Batch No.3089392, Mylan laboratories Ltd). The Efavirenz-based HAART drug [TDF 300 mg + 3TC 300 mg + EFV 600 mg, TLE) mg per tablet (Gilead Sciences Inc, Batch No. 3075041, NAFDAC No. A4-5090,] were donated by General Hospital Gboko, Benue State Nigeria. Thirty (30) tablets of the HAART drugs were weighed and pulverized separately using porcelain mortar and pestle. The quantities of powder that will contain the desired concentrations were calculated and weighed out using an analytical balance (Meltlar Model No. MT-200B). Distilled water (1000 µL) assigned as a control in the current study, was also used to make a slurry of the powdered drugs or dissolution of plant extract before final incorporation into fly food.

### 2.2 Plant Collection, Authentication, and Preparation

The fresh leaves of *Moringa oleifera* were collected from Dogo Village of Benue State, Nigeria, during raining season. Taxonomic identification and authentication of MOL were made at the Federal College of Forestry, Jos, Nigeria, with voucher specimen number FHJ244. The leaves were washed with clean portable water, dried under shade, blended into a coarse powder using a wooden mortar and pestle. The *M. oleifera* leaves were extracted with methanol using the method described by Alexander et al. [34] with minor changes. 250 g of MOL was weighed and soaked (1:10 w/v) in an 80% (v/v) methanol co-solvent system for 72 hours with intermittent shaking. The macerate was filtered using Whatman No.1 filter paper, freeze-dried at -4°C. The lyophilized pellets were weighed to determine the percentage yield and stored in the refrigerator in an amber airtight container for future use.

### 2.3 *Drosophila melanogaster* Stock and Culture

Harwich strain of *Drosophila melanogaster*, originally from the National Species Stock Centre (Bowling Green, OH, USA), was sourced through the Drosophila research laboratory of the ACEPRD, University of Jos, Nigeria. The flies were fed and maintained under standard fly food and environmental conditions, respectively, as prescribed by Abolaji et al. [35].

### 2.4 Phytochemical Screening

The phytochemical screening of the methanol extract of MOL was carried out to identify the different classes of secondary metabolites using standard methods described by Nair et al. [36].

## 2.5 Experimental Design

Assessment of the activities of methanol extract of *Moringa oleifera* leaf on longevity, HAART drug-induced locomotor deficits, reproductive decline, and oxidative stress was carried out using short-range food regimens. Newly hatched flies (1-3 days old) were anesthetized under ice. Sixty (60) flies were counted into five groups in plastic vials (height, 11 cm, diameter, 2.5 cm) each in five replicates per group. Flies were first exposed to different concentrations of MOL (50 – 500 mg), or 25 mM ascorbic acid per 10 g fly food or control. Fly mortality was recorded daily for the entire life span. Secondly, flies were exposed to HAART drugs (46.56 mg /10 g fly food) with or without MOL (250 mg and 500 mg per 10 g fly food) for seven (7) days consecutively. The doses of HAART drugs, MOL extract, and the seven-day treatment period for this study were based on previous studies [16,17,33]. The animals were grouped as follows:

*The first design (Longevity assay)*

Group 1: Control lies fed with standard fly food
Group II: Flies fed with food containing MOL extract (50 mg per 10 g fly food
Group III: Flies fed with food containing MOL extract (200 mg per 10 g fly food)
Group IV: Flies fed with food containing MOL extract (350 mg per 10 g fly food)
Group V: Flies fed with food containing MOL extract (500 mg per 10 g fly food).
Group VI: Flies fed with food containing 25 mM ascorbic acid (AA)

*The Second design (Climbing assay, fecundity, and biochemical studies)*

Group I: Control flies fed with standard fly food.
Group II: Flies fed with food containing HAART drug (46.56 mg per 10 g fly food).
Group III: Flies fed with food containing HAART drug (46.56 mg per 10g fly food) and MOL (250 mg/10 g fly food.
Group IV: Flies fed with food containing HAART drug (46.56 mg per 10g fly food) and MOL (500 mg/10 g fly food.
Group V Flies fed with food containing MOL extract alone.

## 2.6 Longevity (Lifespan) Assay

Sixty (60) flies in five replicates were fed with food containing MOL extract (50 mg, 200 mg, 350 mg, and 500 mg) or standard fly food alone. Dead flies were counted, and the cumulative number of fly death recorded daily till the last fly in all the vails died [15].

## 2.7 Treatment for Climbing Assay, Reproductive Ability, and Oxidative Stress Biomarkers

Flies were treated for seven (7) days using the short-ranged food regimen described in the second experimental design above. After that, the treated flies were homogenized following standard procedures [17]. The whole fly homogenate was used to determine superoxide dismutase (SOD) activity, catalase (CAT) activity, and malondialdehyde (MDA) concentration, respectively.

### 2.7.1 Negative geotaxis (climbing ability)

The climbing ability of both treated and control flies was assayed using the negative geotaxis method described by Adedara et al. [37].

### 2.7.2 Reproductive ability

After seven (7)-day treatment, ten flies (5 males: 5 females) per each group (second experimental design) in five replicates were maintained in plastic vials for 24 hours, where they mated and laid eggs. After 24 hours, the adult flies were flipped while the eggs in their respective containers were monitored daily for fly emergence [17]. The cumulative number of flies that emerged up to the $14^{th}$ day of the experiment represents the reproductive capacity of the experimental animal.

### 2.7.3 Determination of superoxide dismutase (SOD) activity

The activity of SOD was determined using the method of Misra and Fridovic [17].

### 2.7.4 Determination of catalase activity

Catalase (EC 1.11.1.6) activity was determined as described by Abolaji, et al. [35] with slight modification. 100 µL of potassium phosphate buffer (pH 7.0) was added to 194 µL of 300 mM $H_2O_2$ called solution A. Exactly 10 µL of homogenate reacted with 590 µL of solution A and the $H_2O_2$ consumed was monitored at 240 nm at 25°C for 2 minutes (10-second intervals) using a UV-visible spectrophotometer (Jenway, model No. 7315). The result was expressed as mmol of $H_2O_2$ consumed/min/mg of protein.

#### 2.7.5 Determination of malondialdehyde (MDA) content

The malondialdehyde content as a measure of lipid peroxidation (LPO) was determined using the method of Varshney and Kale [17].

### 2.8 Statistical Analysis

Statistical data were expressed as mean±SEM (standard error of the mean) and analyzed using one-way analysis of variance (ANOVA) and Tukey's posthoc test to detect groups that were statistically different. The Log-rank (Mentel-cox) and Gehan-Breslow- Wilcoxon tests were used to analyze survival curves in Graphpad Prism version 8.0.2 for Windows (GraphPad Software, La Jolla California USA, www.graphpad.com). Results from ANOVA were considered statistically significant at $P<0.05$, while survival curves were considered statistically significant at $P<0.006$ (Bonferroni-corrected threshold for nine multiple curve comparisons).

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemical Constituents

The phytochemical result of *M. oleifera* leaf extract showed alkaloids, saponins, tannins flavonoids, cardiac glycosides, and carbohydrates, as shown in Table 1.

The methanolic extract of *Moringa oleifera* leaves in the present study showed phytochemicals (Table 1) similar to previous works that reported its therapeutic activities [22,38]. The antioxidant activity of a plant is an essential index of assessing its medicinal value, and plants with high flavonoid content have been shown to possess antioxidant and anti-inflammatory activities [39].

### 3.2 Longevity (Lifespan) Assay

The results of longevity studies (Fig. 1) showed that methanol extract of MOL significantly ($P<0.05$) increased the lifespan of *D. melanogaster* compare to the control groups. The highest concentration of MOL (500 mg / 10 g fly food) showed 92 days' survival representing 58.62% or 55.93% increase in lifespan compared to the 58 days or 59 days' survival in the negative control or positive control groups respectively. The median lifespan (ML) with corresponding hazard ratio (HR) for all MOL concentrations was: 500 mg (ML = 46 days, HR = 0.35±0.01), 350 mg (ML = 44 days, HR = 0.40±0.01), 200 mg (ML = 40 days, HR = 0.53±0.02), and 50 mg (ML = 39.5 days, HR = 0.62±0.01). The hazard ratios of both negative control (standard fly food + 1 ml distilled water: ML = 32 days, HR = 2.79±0.3), and positive control (Ascorbic acid: ML = 35 days, HR = 1.90±0.4) were higher than the MOL groups.

The result of longevity (Fig. 1) revealed that MOL supplementation significantly ($P<0.05$) increased the lifespan of *Drosophila melanogaster* by 34 days compared to either the negative control that died at day 58 or 33 days compared to the ascorbic acid supplemented group that died at day 59. The hazard ratios of MOL-exposed flies decreased consistently as the concentration of MOL extract increased. This showed that methanolic extract of MOL might have antiaging properties that conferred longevity on *D. melanogaster* as earlier reported by Aremu et al. [38] that methanolic extract of *M. oleifera* leaf improves the survivability rate of Wister rats infected with Trypanosoma brucei. The extension of lifespan by methanol extract of *M. oleifera* in *D. melanogaster* was the basis for its use in the current study to rescue fruit fly from HAART drug-induced toxicities.

### 3.3 Negative Geotaxis

The result of negative geotaxis (Fig 2 A-B) showed that both efavirenz-based HAART (TLE 46.56 mg) and dolutegravir-based HAART (TLD 46.56 mg) exposures significantly ($P<0.05$) reduced climbing activities compare to those fed with standard fly food, respectively. These HAART drug-induced climbing deficits were modified in all MOL extract supplemented groups. In the supplemented groups, (TLE 46.56 mg + MOL 500 mg), (TLD 46.56 mg + MOL 500 mg), and (TLD 46.56 mg + MOL 250 mg), climbing ability increased significantly ($P<0.05$) compared with the HAART drugs (TLE 46.56 mg or DH 46.56 mg) alone. Conversely, the treatment TLE 45.56 mg + MOL 250 mg insignificantly ($P>0.05$) increased climbing ability. Also, flies exposed to MOL extract 500 mg alone significantly ($P<0.05$) improved climbing activity in all our experimental designs compared to unexposed groups or HAART drugs alone.

The ameliorative effects of MOL extract against neuromuscular toxicity of Efavirenz-based or Dolutegravir-based HAARTs were evaluated using the negative geotaxis method. The methanol extract of MOL significantly ($P<0.001$) improved HAART drug-induced locomotor deficits (Fig. 2: A-B). Combination antiretroviral therapies are associated with premature aging,

including muscle weakness in humans [40]. Similarly, locomotor deficits in *D. melanogaster* exposed to HAART drugs have been reported [15,17]. Movement disorders are associated with decreasing acetylcholinesterase activity at the neuromuscular junction in *D. melanogaster* [41,42]. Therefore, the increased climbing ability in the current study might be due to increased acetylcholine activity at the neuromuscular junction occasioned by free radical scavenging effects of *M. oleifera* leaves.

### 3.4 Reproductive Ability

Treatment vials containing *D. melanogaster* eggs exposed to HAART drugs TLE 46.56 mg or TLD 46.56 mg or TLE 46.56 mg + MOL 250 mg showed 100% emergence failure (Fig 3 A-B). All treated vials containing TLE 46.56 mg + MOL 500 mg or TLD 46.56 mg + 500 mg MOL extract significantly ($P$<0.05) increased fly emergence compared with vials containing HAART drugs alone but significantly ($P$<0.05) lower than the untreated vials. Similarly, food supplementation with 500 mg MOL extracts alone significantly ($P$<0.05) increased fly emergence compared with the untreated vials or vials supplemented with HAART drugs + MOL extract 500 mg, respectively.

The reproductive ability of both treated and control groups was assessed using the fly emergence method. A significant ($P$<0.05) increase in fly emergence in MOL supplemented groups (TLE 46.56 mg + MOL 500 mg or TLD 46.56 MG + MOL 500 mg) compared to the HAART drugs alone was observed. *M. oleifera* 500 mg supplementation significantly (P<0.05) increased fly emergence compared with all treated and the untreated groups (Fig. 3 A-B). It was noted that supplementation with MOL extract 250 mg resulted in 100% emergence failure implying that the ameliorative effect of *M. oleifera* on reproductive capacity in *D. melanogaster* was dose-dependent. Reactive oxygen species (ROS) generation impedes spermatogenesis, reduced sperm quality, and promotes disturbance of oocyte intracellular $Ca^{2+}$ homeostasis, maturation, and fertilization in humans [43,44]. HAART toxicity with a significant free radical generation and reduction of fly emergence in *D. melanogaster* has been reported [17]. Meanwhile, other works [43,45] have suggested that antioxidant supplementation might ameliorate oxidative stress-induced reproductive deficits. The significant increase in fly emergence among the MOL supplemented groups in this study might be attributed to decreased free radical activity orchestrated by the antioxidant effects of *M. oleifera* leaves.

### 3.5 Superoxide Dismutase (SOD) Activity

The result of SOD activity (Fig 4. A-B) of both efavirenz-based HAART (TLE 46.56 mg) alone or Dolutegravir-based HAART (TLD 46.56 mg) alone showed significant ($P$<0.05) reduction in SOD activity compared with the unexposed groups respectively. Supplementation with MOL extract (TLE 46.56 mg + MOL 500 mg) or (TLD 46.56 mg + MOL 500 mg) significantly ($P$<0.05) reversed this observed decline in SOD activity compared with either of the HAART drugs alone. Conversely, the increase in SOD activity at MOL 250 mg supplementation was not significantly ($P$>0.05) different compared to TLE 46.56 mg or TLD 46.56 mg, respectively. *Moringa oleifera* leaf extract (500 mg) alone significantly increased SOD activity compared with the HAART treated groups, respectively.

### 3.6 Catalase (CAT) Activity

The results of CAT activity (Fig 5. A-B) of the Efavirenz-based HAART (TLE 46.56 mg) or Dolutegravir-based HAART (TLD 46.56 mg) alone showed significantly ($P$<0.05) decreased CAT activity compared to the unexposed groups respectively. Supplementation with MOL extract (TLE 46.56 mg + MOL 500 mg) or (TLD 46.56 mg + MOL 500 mg) significantly ($P$<0.05) ameliorated this deficit in CAT activity compared to either of the HAART drugs exposed groups. Conversely, the increase in SOD activity at MOL

**Table 1. Qualitative phytochemical constituents of methanol leaf extract of *M. oleifera* Lam**

| Phytochemical constituents | Test | Observation | Remarks |
|---|---|---|---|
| Alkaloids | Dragendorff's | Orange-red | + |
| Saponins | Frothing | Persistence foam | + |
| Tannins | Ferric chloride | Blue ink | + |
| Flavonoids | Alkaline reagent | Yellow | + |
| Cardiac glycosides | Salkowski's | Red-brown colour | + |
| Carbohydrate | Molisch's | Reddish violet colour | + |

*Key: Present (+)*

250 mg supplementation was not significantly (*P*>0.05) different from TLE 46.56 mg or TLD 46.56 mg alone. *Moringa oleifera* leaf extract (500 mg) alone significantly increased CAT activity compared with the HAART treated groups, respectively.

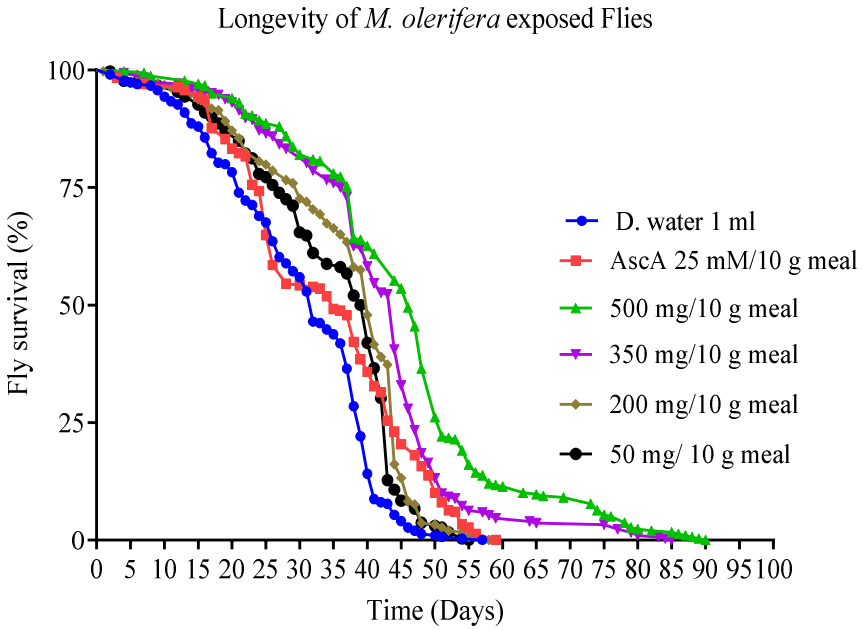


**Fig 1.** *Moringa oleifera* leaf extract extends life span of *D. melanogster* in a dose dependent manner. The highest concentration (500 mg) showed 92 days survival (medina survival of 32 days), hazard ratio (HR) = 0.23 against control 4.6. Data are presented as mean±SEM of five independent biological replicates carried out in two seperate experimens. *p<0.05 vs control.

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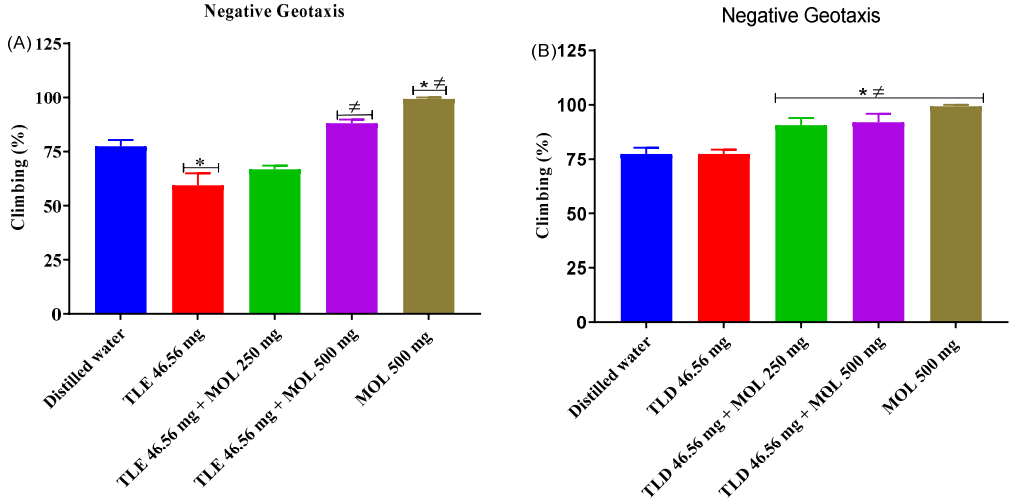


**Fig 2 (A-B)**. Methanol extract of *Moringa oleifera* leaf significantly (P< 0.05) modified HAART drug-induced climbing deficits in *D. melanogaster*. (A) Negative geotaxis of *D. melanogaster* after five day exposure to EFV-HAART alone or EFV-HAART + M. oleifera. (B) Negative geotaxis of *D. melanogaster* after five day exposure to DTG-HAART alone or DTG-HAART + M. oleifera.
Data are presented as mean±SEM of five independent biological replicates of each concentration. Each assay was caried out in two independent expriments. *P<0.05 vs control, ≠P<0.05 vs HAART drug alone. TLE= efavirenz -based HAART, TLD = dolutegravir-based HAART, MOL = *M. oleifera* leaf extract.

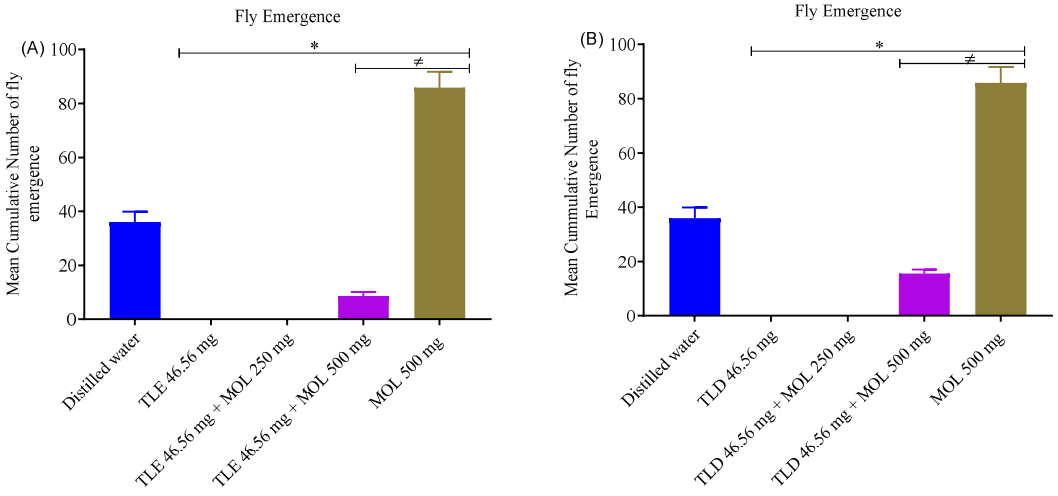


**Fig 3(A-B)**. Methanol extract of *Moringa oleifera* leaf extract recued HAART-drug induced reproductive deficites in *D. melanogaster*. (A). Fourteen day emergence of *D. melanogaster* after five day exposure to EFV-HAART alone or EFV-HAART + *M. oleifera* . (B) Fourteen (14) day emergence of *D. melanogaster* after five day exposure to DTG-HAART alone or DTG-HAART + *M. oleifera*. Data presented as mean±SME of five independent bological replicates for each drug concentration (n=60). * $P<0.05$ vs control, # $P>0.05$ vs HAART drug alone. TLE= efavirenz -based HAART, TLD = dolutegravir-based HAART, MOL = *M. oleifera* leaf extract.

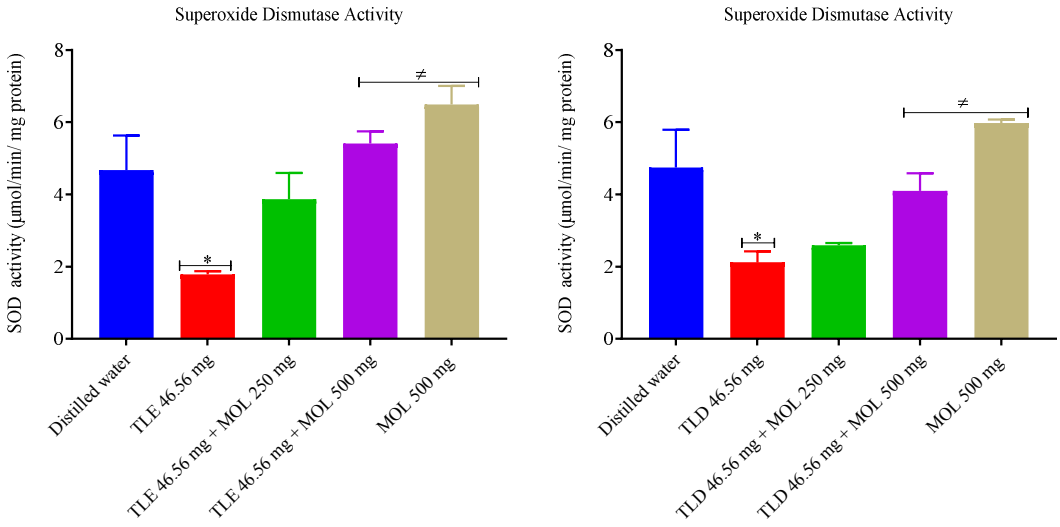


**Fig 4(A-B)**. Methanol extract of *Moringa oleifera* leaf modified HAART-drug induced SOD deficits in *D. melanogaster*. (A). SOD activity of whole fly hormoginate of *D. melanogaster* after five day exposure to EFV-HAART alone or EFV-HAART + *M. oleifera* . (B) SOD activity of whole fly hormoginate of *D. melanogaster* after five day exposure to EFV-HAART alone or EFV-HAART + *M. oleifera*. Data presented as mean ±SME of five independent bological replicates for each drug concentration (n=60). * $P<0.05$ vs control, # $P>0.05$ vs HAART drug alone. TLE= efavirenz -based HAART, TLD = dolutegravir-based HAART, MOL = *M. oleifera* leaf extract.

The ameliorative potential of MOL extract against HAART drug-induced decline in antioxidants SOD (Fig. 4 A-B) and CAT (Fig. 5 A-B) activities as presented, significantly ($P<0.05$) reduced the activities of SOD or CAT compared with the untreated groups respectively. Supplementation with methanol extract of MOL (TLE 46.56 mg + MOL 500 mg, or TLD 46.56 mg + MOL 500 mg) significantly ($P<0.05$) elevated these decrease in antioxidant enzyme activities compared to the

HAART-treated groups alone. The extract ameliorated the HAART drug-induced decline in antioxidant (SOD and CAT) activities in a dose-dependent manner. RONS are generated during normal cellular respiration or exposure to xenobiotics and may acts as signaling molecules at low concentrations but may be lethal at high concentrations [46]. Oxidative stress occurs if the balance between RONS production and antioxidant capacity is upset in a bio-system in favour of the former, thereby leading to cellular damage [47]. The initial response to overwhelming free radical generation requires the immediate increase in the enzymatic antioxidants (e.g., SOD and CAT) activities to curb further ROS build-up [48]. First, the dismutation of two superoxide anions to hydrogen peroxides ($H_2O_2$) and water is catalyzed by SOD. CAT in the second step rapidly converts $H_2O_2$ to water and oxygen to avoid hydroxyl radical formation through Fenton reaction [49], thus, restoring redox balance [50]. A surge in gene expression may initially occur due to the cells' effort to counter the moderate to severe effects of RONS [50]. Nevertheless, excessive oxidative stress leads to DNA modifications, such as degradation of DNA bases and proteins, resulting in decreased gene expression, which culminates in decreased antioxidant enzyme activities [50]. It has been hypothesized that the decreased antioxidant activities in HAART drug-exposed *D. melanogaster* might be due to a decreased in antioxidant enzyme gene expression or direct oxidative stress-induced damage on the antioxidant enzyme molecules or both [16,17]. The amelioration of the HAART drug-induced deficits in SOD and CAT activities by MOL extract supplementation might be due to increased gene expression of these enzymes or inhibition of free radical generation or both.

## 3.7 Malondialdehyde (MDA) Content

The results of MDA content (Fig. 6 A-B) showed a significant ($P$<0.05) increase in all the experimental animals fed with food containing HAART drugs (TLE 46.56 mg or TLD 46.56 mg) compared with the control groups. A non-significant ($P$>0.05) decrease in MDA content at efavirenz-based group (TLE 46.56 mg + MOL 250 mg or TLE 46.56 mg + MOL 500 mg) compared with TLE 46.56 mg group (Fig. 6A) was observed. Similarly, Supplementation with MOL extract at the dolutegravir-based HAART group (Fig. 6B, TLD 46.56 mg + MOL 250 mg) insignificantly ($P$ = 0.44) decreased MDA level when compared to the TLD 46.56 mg alone. However, a significant ($P$ = 0.02) decrease in MDA content at the TLD 46.56 mg + MOL 500 mg supplemented group (Fig. 6B) was observed compared to the TLD 46.56 mg group alone. Fly food supplemented with only MOL extract 500 mg significantly ($P$ = 0.001) decreased MDA content compared with either TLE 46.56 mg or TLD 46.56 mg, respectively.

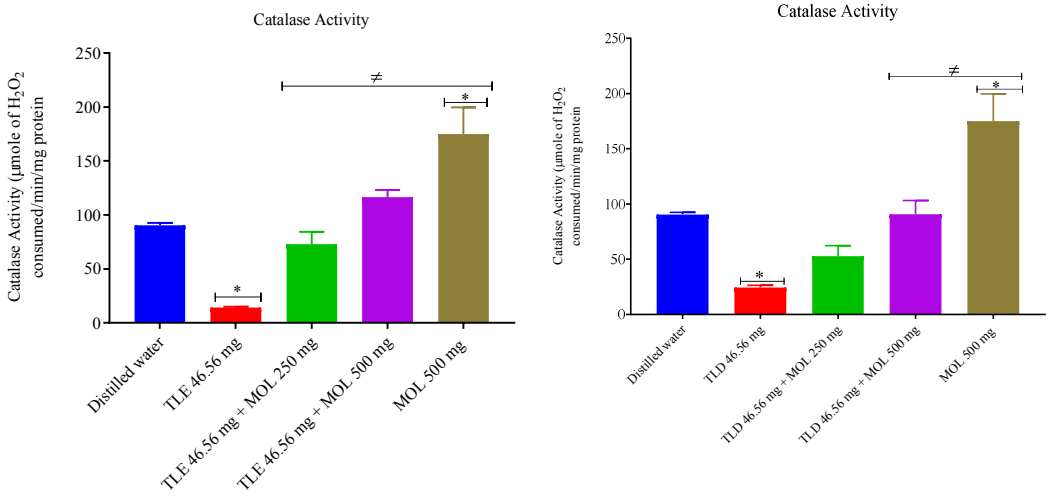


**Fig 5 (A-B)**. Methanol extract of *Moringa oleifera* leaf modified HAART-drug induced CAT deficits in *D. melanogaster*. (A). CAT activity of whole fly homoginate of *D. melanogaster* after five day exposure to EFV-HAART alone or EFV-HAART + *M. oleifera* . (B) CAT activity of whole fly homoginate of *D. melanogaster* after five day exposure to EFV-HAART alone or EFV-HAART + *M. oleifera*. Data presented as mean ±SME of five independent bological replicates for each drug concentration (n=60). * $P$<0.05 vs control, # $P$>0.05 vs HAART drug alone. TLE= efavirenz -based HAART, TLD = dolutegravir-based HAART, MOL = *M. oleifera* leaf extract.

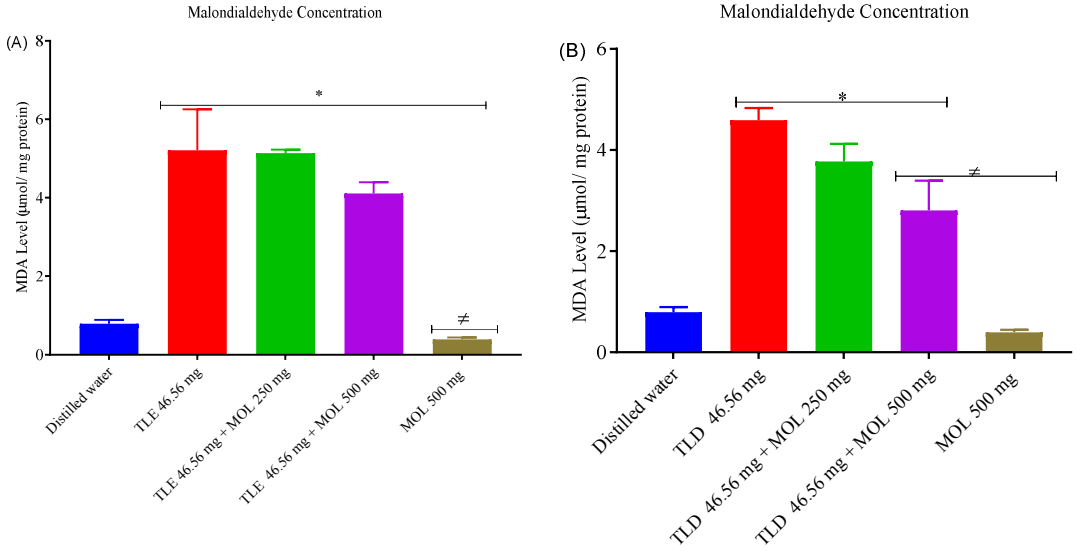


**Fig 6 (A-B)** . Methanol extract of *Moringa oleifera* leaf modified HAART-drug induced MDA increase in *D. melanogaster*. (A). MDA content in whole fly hormoginate of *D. melanogaster* after five day exposure to EFV-HAART alone or EFV-HAART + *M. oleifera* . (B) MDA content in whole fly hormoginate of *D. melanogaster* after five day exposure to DTG-HAART alone or DTG-HAART + *M. oleifera*. Data presented as mean ±SME of five independent bological replicates for each drug concentration (n=60). * $P<0.05$ vs control, # $P<0.05$ vs HAART drug alone. TLE= efavirenz -based HAART, TLD = dolutegravir-based HAART, MOL = *M. oleifera* leaf extract.

The effect of MOL extract on MDA concentration in the whole fly homogenate of HAART-exposed *D. melanogaster* was further investigated after the observed deficits in the activities of antioxidants enzyme (SOD and CAT) in this study. The supplementation with MOL extract in the efavirenz-based HAART group (Fig. 6A) showed an insignificant ($P<0.05$) reduction in the MDA content compared to the HAART-exposed non-supplemented group. However, in the dolutegravir-based HAART group, MOL supplementation (TLD 46.56 mg + MOL 500 mg) significantly ($P<0.05$) reduced the MDA level compared to the non-supplemented HAART (TLD 46.56 mg) group. Malondialdehyde (MDA) is a peroxyl radical-induced secondary product of hydroperoxides that was primarily changed from polyunsaturated fatty acids [51,50]. It serves as a measure of lipid peroxidation (LPO) during oxidative stress [50]. Oxidative stress-induced LPO decreases cell membrane function via loss of fluidity as well as alterations in membrane-bound enzymes and receptors [52]. Iorjiim et al. [33] and Luqman et al. [53] independently reported that MOL significantly ($P<0.05$) reduced the MDA level in *D. melanogaster* and mice models, respectively. Therefore, the results in the present study suggest that the ameliorative effects of MOL against HAART drug-induced toxicities might be attributed to inhibition of lipid peroxidation through scavenging of RONS. This study also implied that MOL extract rescued the fruit fly from TLD toxicities more satisfactorily compared to those of TLE combination. It was not ascertained, however, whether the observed decrease in MDA level in the present study was occasioned by decreased oxidative stress alone or enhanced MDA breakdown by aldehyde reductase or both.

## 4. CONCLUSION

The methanol extract of MOL promotes longevity in *D. melanogaster*. It also demonstrated ameliorative effects against HAART drug-induced toxicities, supported by improved locomotor deficits, reproductive decline, and deficits in oxidative stress biomarkers (SOD, CAT, and MDA) in HAART drug-exposed *D. melanogaster*. This study suggests that MOL extract extended lifespan and rescued *D. melanogaster* from HAART drug-induced toxicities via inhibition of lipid peroxidation and free radical scavenging actions.

## 5. RECOMMENDATIONS

More research should focus on HAART drug-induced toxicities and the ameliorative role of *M. oleifera* in antiretroviral therapy.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

It is not applicable.

## ACKNOWLEDGEMENTS

We appreciate Mr. Ogwu Sunshine Ochala and Ms Ugokwe, Chinalo of the ACEPRD, University of Jos, as well as Mr. Dan Shalong of the Biochemistry Division, National Veterinary Research Institute (NVRI), Vom, Jos, Plateau State of Nigeria, respectively for their technical supports.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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