***Original Research Article***

***Moringa oleifera* L. Leaf Fractions Improve Survival and Ameliorate Antiretroviral Drug-induced Toxicities in the Canton-S *Drosophila melanogaster***

ABSTRACT

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| **Aim.** This study aimed to evaluate the mitigative role of *Moringa oleifera* leaf fractions against tenofovir/lamivudine/dolutegravir (TLD)-induced toxicities in the Canton-S *Drosophila melanogaster*. **Study design**: This is an experimental study.**Place and Duration of Study**: This study was conducted at the Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria, in July-September 2024.**Methodology.** *Moringa oleifera* L. leaf fractions were screened for their phytonutrient content. Young flies (<3 days old) were exposed to TLD (1 mg - 595 mg/10 g diet) to determine the median lethal concentration (LC50). Then, flies were exposed to *M. oleifera* fractions (20 mg /10 g diet) for a survival assay using 0.3% DMSO (dimethyl sulfoxide, the vehicle). For fly emergence, climbing ability, and biochemical tests, flies were co-treated with both TLD (10 mg/10 g diet) and *M. oleifera* leaf fractions (20 mg/ 10 g diet) for 7 days. Subsequently, antioxidant markers, survival rate, climbing ability, and fecundity parameters associated with antiretroviral toxicity in flies were evaluated. P < 0.05 was considered statistically significant. **Results:** The results showed that *M. oleifera* leaf fractions contain antioxidant phytonutrients, including flavonoids and phenols, and increased survival, climbing ability, and reproductive capacity compared to control flies. It also improved antioxidative markers in flies, evidenced by increased total thiol levels and glutathione S-transferase (GST) activity, along with a decrease in malondialdehyde (MDA) levels. Furthermore, *M. oleifera* leaf fractions mitigated TLD-induced climbing defects, reduced fly emergence, and alleviated both acetylcholinesterase activity deficits and increased malondialdehyde production (*P*<0.05). **Conclusion.** This study demonstrated the protective potential of *M. oleifera* leaf fractions against TLD-induced oxidative stress in the Canton-S strain of *D. melanogaster* through antioxidant mechanisms. |

Keywords: *Drosophila melanogaster*, oxidative stress, antioxidant, *Moringa oleifera,*

**1. INTRODUCTION**

 The highly active antiretroviral therapy (HAART) remains the hope of people living with human immunodeficiency virus (HIV) infections (Smith et al., 2013). Nevertheless, chronic administration of antiretroviral drugs is often associated with oxidative stress toxicities orchestrated by HIV infection or HAART drugs (Smith et al., 2013; Amegashie et al., 2025). We recently reported dolutegravir and efavirenz-induced functional deficits associated with oxidative stress in the Harwich strain of *Drosophila melanogaster* (Iorjiim et al., 2023a; Iorjiim et al., 2023b). Antiretroviral drug-induced oxidative stress remains a significant challenge in HIV management, especially in Sub-Saharan Africa, due to limited pharmacological interventions and resulting poor adherence to treatment (Amegashie et al., 2025; Pennap et al., 2013). Although HIV positive patients have resorted to self-supplementing with *M. oleifera* for its perceived antioxidant benefits (Gurumu et al., 2017; Anyanwu et al., 2019), there is a lack of empirical evidence supporting its efficacy and safety. Current strategies to address the menace of HAART drug-induced adverse effects are limited to switching drug regimens, which may not adequately address toxicity (Twimukye et al., 2021).

Notably, there is a gap in research using *D. melanogaster* to evaluate both the toxic effects of HAART and the therapeutic potential of *M. oleifera*. This highlights an urgent need for effective, affordable, plant-based adjunct therapies to mitigate drug-induced oxidative stress. *D. melanogaster* is a widely used model organism in toxicological research due to its genetic resemblance to vertebrates. For example, some key oxidative stress makers in humans, such as superoxide dismutase and catalase, are conserved in the fruit fly (Abolaji et al., 2013). Also, *D. melanogaster* is an important model organism in the screening of phytonutrients for medicinal properties (Ramirez-Moreno et al., 2022). Furthermore, the Canton-S strain of *D. melanogaster* demonstrates high susceptibility to environmental toxins and xenobiotics due to its lack of the cyp6 gene, which is implicated in the resistance of the fruit fly to xenobiotics, making it a good model organism in this study (Johnson et al., 2021). *M. oleifera,*  from the family Moringaceae, is known for its antioxidant, anti-inflammatory and highly nutritive properties (Fahey, 2005; Shah & Oza, 2022). The protective potentials of *M. oleifera* leaf extract in ameliorating HAART drug-induced oxidative stress toxicity have been reported (Ndlovu et al., 2020; Ndlovu et al., 2023). Therefore, in the current study, we evaluate the effects of *M. oleifera* leaf fractions on survival and TLD-induced oxidative stress toxicities using the Canton-S *D. melanogaster* as a model.

**2. EXPERIMENTAL DETAILS**

**2.1 Collection, Identification, and Extraction of Plant Material**

The leaves of *M. oleifera* were collected from Ahwa village, Gboko, Benue State of Nigeria. The plant was authenticated at the Federal College of Forestry, Jos, Plateau State of Nigeria, with a voucher number FHJ244. The fresh leaves were washed and dried in a shade (temperature range 25- 30 oC). The extraction was carried out with 80% Methanol as previously described (Iorjiim et al., 2020). The dried methanol extract was further partitioned using a published protocol (Gotep et al., 2021), with few modifications. Briefly, 50 g of the crude *M. oleifera* leaf methanol extract was suspended in 400 ml of distilled water in a 1000 ml beaker. After 24 h, the suspension was filtered with a Whitman (1mm) filter paper to obtain water-soluble and water-insoluble fractions. The water-soluble suspension was transferred to a 1000 ml separating funnel, shaken and allowed to settle. Thereafter, it was successively extracted starting from non-polar to polar organic solvents: n-hexane, chloroform, ethyl acetate, and n-butanol. First, n-hexane (200 ml) was added to the content of the separating funnel with gentle shaking to mix and allowed to separate into the hexane and aqueous layers. The n-hexane layer was collected, then another 200 ml of n-hexane was added, and the process was repeated three (3) consecutive times to get the n-hexane fraction. Other solvents were added to the aqueous suspension of *M. oleifera* methanol leaf extract in order of increasing polarity, and the procedure was repeated three times each to obtain ethyl acetate, chloroform, n-butanol and residual methanol fractions, respectively. These fractions were filtered using No. 1 Whatman filter paper, evaporated at room temperature to dryness and stored in a freezer till further use.

**2.2 Culture of Canton-S *Drosophila Melanogaster***

 The Canton-S strain of *D. melanogaster* was obtained from the Drosophila research laboratory of the Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria. Flies were reared and maintained on a standard fly diet and environmental conditions (Abolaji et al., 2020).

**2.3 Determination of Median Lethal Concentration (LC50)**

 The amount of the antiretroviral drug that could cause 50% mortality in flies in seven days was determined as described by Iorjiim and co-workers (Iorjiim et al., 2020), with slight modifications. Fifty (50) young flies (1- 2 day old) were made to sleep by placing them under ice. The flies were counted and put into plastic vials and fed with diet containing ten (10) varying concentrations of TLD (0- 595 mg) dissolved in 1000 µL 0.3% dimethyl sulfoxide (DMSO) or 1000 µL 0.3% DMSO (control) each per 10 g fly diet respectively for 7 days for five independent treatments in five replicates. Fly mortalities were counted and recorded daily during the experimental period. The total fly deaths per experimental concentration were subjected to a dose-response analysis in GraphPad Prism 8.0.2 to calculate the LC50.

 **2.4 Survival Assay**

 Fifty (50) flies (mixed sexes, 1-2 days old) were placed in a treatment group. Exactly 20 mg of *M. oleifera* leaf fractions (n-hexane, chloroform, butanol and ethyl acetate) were previously dissolved in 1000 µL 0.3% DMSO or 1000 µL DMSO, then mixed with TLD in fly diet. Flies were fed with the diet premixed with *M. oleifera* leaf fractions plus TLD 10 mg for 28 days. The dead flies were counted daily during the experimental period, and the fly survival rate was computed as a percentage of live flies.

**2.5 A 5-day Treatment for Fly Emergence, Climbing Ability and Biochemical Tests**

 Fifty (50) flies were fed with a diet mixed with 20 mg of *M. oleifera* leaf fractions plus TLD 10 mg or control (described in section 2.4), in five replicates for five days. After 5 days, ten (10) (five males and five females) were selected from each treatment group and used for the determination of climbing ability and fly emergence using published protocols (Adedara et al., 2022). Thereafter, the exposed flies (*M. oleifera* + TLD, or TLD alone) or controls were immobilised using ice, then weighed, and homogenised in 0.1 M phosphate-buffered saline (pH 7.0, fly: buffer = 1 mg:10 µL). With the aid of an Eppendorf centrifuge (Model No.: AG 5227 R, Germany, at 4 °C), the fly homogenates were centrifuged at a speed of 4000 rpm for 10 minutes. The supernatants were separated from the fly debris and used for the evaluation of Total thiol and Malondialdehyde contents, as well as the activity of glutathione S-transferase (GST).

**2.5.1 Climbing Activity (Negative geotaxis) and Reproductive Ability**

 The climbing ability of the experimental flies or controls was evaluated by the method published by Adedara et al. (Adedara et al., 2022), while the method of Iorjiim et al. (Iorjiim et al., 2020) was utilised for reproductive ability.

**2.5.3 Determination of Total Thiol and Glutathione S-transferase Activity**

 The Total thiol level was evaluated using the method described by Abolaji et al. (Abolaji et al., 2020). Briefly, exactly 510 µL 0.1M potassium phosphate buffer (pH 7.4) and 25 µL of fly homogenate were added to 30µL of 10 mM DTNB and incubated at room temperature for 30 min. Then, the absorbance of the reaction mixture was read at 412 nm wavelength in a spectrophotometer. The total thiol level was calculated (in mmol/mg protein) using 35µl GSH as standard.

**2.6.4 Glutathione-S-transferase (GST) activity**

 Glutathione S-transferase was evaluated using the method described by Omale et al. (Omale et al., 2020), using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. Exactly 600 µL of ‘solution A’ was prepared by mixing 20 µL of a buffer (0.25 M potassium phosphate, pH 7.0 plus 2.5 mM EDTA), 510 µL of 0.1 M glutathione at 25°C, 60 µL of the dilute fly homogenate (distilled water: sample = 1:5), and 10µL of 25 mM CDNB. The absorbance of the reaction mixture was read at 340 nm (for 2 min at 10 s intervals) using a spectrophotometer (Jenway, Model No.7315). The activity of GST was expressed in µmol/min/mg of protein utilising 9.6 mM1 cm-1 molar coefficient.

**2.6.7 Malondialdehyde (MDA) content**

 Malondialdehyde (MDA) content was evaluated by the method of Varshney and Kale described by Iorjiim and co-workers (Iorjiim et al., 2020).

**2.7 STATISTICAL ANALYSIS**

Data values were analysed using GraphPad Prism version 8.0.2 for Windows and presented as Mean ± Standard Error of Mean.. The one-way ANOVA (analysis of variance) with Tukey’s post hoc test were utilised to show the means with significant differences at P< 0.05. Furthermore, survival curves were analysed using the Log-rank (Mantel-Cox) test. Bonferroni adjusted P-value/K was used, where K was 4 comparisons. Statistical significance was taken at *P* < 0.01.

**3.0 RESULTS**

**3.1. Median lethal concentration (LC50)**

 After a 7-day exposure of flies to TLD (Fig.1), the calculated median lethal concentration (LC50) in *D. melanogaster* was 94.33 mg/10 g diet. Also, 100% mortality was observed at 250 – 595 mg/10 g diet.



Fig. 1. Median Lethal Concentration (LC50 ) of TLD in Canton-S *D . melanogaster*

 TLD= Tenofovir /Lamivudine/Dolutegravir

**3.2 Phytochemical Screening of the Extracts of *Moringa oleifera* Leaf Fractions**

 The result of phytochemical screening (Table 1) showed the presence of antioxidant phytonutrients, including flavonoids and Phenols, in all the fractions of *Moringa oleifera* leaf.

Table 1

Phytochemical Constituents and Yield of *M. oleifera* Leaf Fractions

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Phytochemical constituents  |  | n-Hexane | Ethyl acetate | Butanol | Chloroform  |
| AlkaloidsSaponins Tannins FlavonoidsPhenols Yield (% w/w) |  | ---++11.14 | +-+++25 | +-+++45.00 | ++-++7.5 |

*Key: + = present, - = absent*

**3.3 Survival of *D. melanogaster* After Exposure to *Moringa oleifera* Fractions**

The result of survival rate following a 28-day oral exposure to *M. oleifera* leaf fractions in fruit fly is as shown (Fig. 2). The mortality rate of flies fed with ethyl acetate and chloroform fractions improved significantly (*P*<0.001) compared to the control flies. However, the survival rate of flies exposed to the n-hexane and aqueous fractions showed a significant reduction (*P*<0.001) in survival compared to the control group. The ethyl acetate fraction showed the highest survival rate relative to the other *Moringa oleifera* leaf fractions.



Fig. 2. *M. oleifera* Leaf Fractions Reduced Mortality Rate in

 Canton-S *D. melanogaster* After a Chronic (28 Days) Exposure

 \**P*<0.05 Significant difference compared to Aqueous extract

 MOL= *Moringa oleifera* leaf

**3.4 Effects of *Moringa oleifera* Leaf Fractions on Antiretroviral Drug-induced Mortality**

 The Canton-S *D. melanogaster* was exposed to fly diet containing antiretroviral drugs, or antiretroviral drugs + *M. oleifera* fractions or control (Fig. 3). The TLD-exposed flies showed a significant (*P* < .001) death rate compared to the control. All the *M. oleifera* leaf fractions significantly (*P* < .001) improved the mortality rate compared to the TLD group.



Fig. 3. *M. oleifera* Leaf Fractions Modified TLD-induced Mortality in

 Canton-S *D. melanogaster* Afteran Acute (5-day) Exposure

 \**P*<0.05 Significant difference compared to Control group

 #*P*<0.05 Significant difference compared to the TLD-exposed flies

 LD = Tenofovir/Lamivudine/Dolutegravir

**3.5 *Moringa oleifera* Leaf Fractions Modified Antiretroviral Drug-induced Climbing and Acetylcholinesterase Activity Deficits**

 After a 5-day exposure to TLD and *Moringa oleifera* leaf fractions (Fig. 4), the flies exposed to TLD significantly reduced climbing performance (*P* < .001) compared to the control group. All the *Moringa oleifera* leaf fractions (n-hexane, butanol, chloroform and ethyl acetate) significantly (*P* < .001) improved the HAART drug-induced decrease in climbing performance (Fig. 4A) and AChE activity (Fig. 4B) deficits in *D. melanogaster* compared to the antiretroviral-exposed flies. The ethyl acetate fraction showed the best protective activity against TLD-induced reduction in AChE activity compared to the other solvent fractions (Fig. 4 B). The AChE activity deficit was modified to a non-significant (*P* = .20) status compared to the unexposed flies.



Fig. 4. *M. oleifera* Leaf Fractions Modified TLD-induced Climbing Deficits and reduction of AChE activity in Canton-S *D. melanogaster* Aftera 5-day Exposure.

 (A) Acetylcholinesterase (AChE) activity, (B) Climbing ability in fruit fly

 \**P*<0.05 Significant difference compared to Crude Extract

 #*P*<0.05 Significant difference compared to the TLD-exposed flies

 TLD = Tenofovir, Lamivudine, Dolutegravir

**3.6 *M. oleifera* Leaf Fractions Ameliorated Antiretroviral Drug-Induced Reproductive Deficits in *D. melanogaster***

 The result of fly emergence after a 5-day exposure to TLD or *Moringa oleifera* leaf fractions plus TLD, is shown (Fig. 5). Fly exposure to antiretroviral drugs significantly (*P* < .001) reduces eclosion of young flies compared to the unexposed group. Conversely, all the fractions of *M. oleifera* leaf exhibited significant (*P*<0.05) ameliorative activity against reproductive deficits compared to the TLD-exposed flies. The n-hexane and butanol fractions improved the eclosion rate to a non-significant level (P = .20) compared to the unexposed flies. However, the chloroform fraction, on the other hand, modified the TLD-induced reproductive defects, but the group performance was statistically lower (P < .001) compared to the unexposed flies.



Fig. 5. *M. oleifera* Leaf Fractions Modified TLD-induced Fly Emergence in

 Canton-S *D. melanogaster* Aftera 5-day Exposure

 \**P*<0.05 Significant difference compared to Crude Extract

 #*P*<0.05 Significant difference compared to the TLD-exposed flies

 TLD = Tenofovir, Lamivudine, Dolutegravir

**3.7 *M. oleifera* Leaf Fractions Modified Antiretroviral Drug-induced Total Thiol Reduction in *D. melanogaster***

 It was observed from the result shown (Fig. 6) that TLD reduced total thiol levels significantly (*P* < .001) compared to control flies. Interestingly, co-administration of TLD and four *M. oleifera* leaf fractions significantly (*P* < .001) modified the total thiol deficits compared to the antiretroviral-exposed group. The total thiol content in flies supplemented with the ethyl acetate fraction showed the best protective effects against total thiol deficits compared to both TLD (P < .001) and control (P = .05) flies.

Fig. 6. *M. oleifera* Leaf Fractions Modified TLD-induced Total thiol deficits in Canton-S *D. melanogaster* Aftera 5-day Exposure

 \**P*<0.05 Significant difference compared to Crude Extract

 #*P*<0.05 Significant difference compared to the TLD-exposed flies

 TLD = Tenofovir/Lamivudine/Dolutegravir

**3.8 *Moringa oleifera* Leaf Fractions Modified Antiretroviral Drug-induced Glutathione S-transferase Activity Deficits in *D. melanogaster***

 It was observed from the result (Fig. 6) that TLD induced glutathione S-transferase (GST) activity deficits significantly (*P* < .001) compared to control flies. However, co-administration of TLD and *Moringa oleifera* leaf fractions significantly (*P* < .001) modified the reduction in GST activity compared to the antiretroviral-exposed flies. The GST activity in flies supplemented with the ethyl acetate and chloroform fractions showed a significant increase in GST activity compared to both the TLD (P < .001) and control (P = .5) flies.

Fig. 7. *M. oleifera* Leaf Fractions Modified TLD-induced Fly Emergence in Canton-S

 *D. melanogaster* Aftera 5-day Exposure

 \**P*<0.05 Significant difference compared to Crude Extract

 #*P*<0.05 Significant difference compared to the TLD-exposed flies

 TLD = Tenofovir/Lamivudine/Dolutegravir

**3.8 *M. oleifera* Leaf Fractions Modified Antiretroviral Drug-induced Glutathione**

 **S-transferase Activity Deficits in *D. melanogaster***

 The result of MDA generation is as shown (Fig. 8). TLD exposure showed a significant (P = .01) increase in the MDA generation compared to the control flies. However, Co-administration of *Moringa oleifera* leaf fractions and TLD showed significant reductions in MDA generation at groups treated with n-hexane (*P* = .04), butanol (P = .05), and chloroform (P = .04) fractions compared to the TLD-exposed group. Interestingly, the ethyl acetate fraction of *M. oleifera* leaf showed the best rescue effects against TLD-induced MDA generation in *D. melanogaster,* which was significant (*P* = .002) compared to the TLD-exposed flies.

Fig. 8. *M. oleifera* Leaf Fractions Modified TLD-induced Malondialdehyde

 Generation in Canton-S *D. melanogaster* Aftera 5-day Exposure

 \**P*<0.05 Significant difference compared to Crude Extract

 #*P*<0.05 Significant difference compared to the TLD-exposed flies

 TLD = Tenofovir/Lamivudine/Dolutegravir

**4. DISCUSSION**

 *Moringa oleifera* leaf has been suggested as a potential remedy for antiretroviral drug-induced oxidative stress toxicities due to its high antioxidant properties (Ndoluv et al., 2020; Ndoluv et al., 2022; Ndoluv et al., 2023). Undeniably, antioxidant compounds like N-acetylcysteine and vitamin E, which scavenge free radicals, are linked with increased lifespan in *D. melanogaster* (Suckow & Suckow, 2006). We have previously utilised *D. melanogaster* as an alternative model for studying oxidative stress toxicities associated with antiretrovirals Iorjiim et al., 2023). This study aimed to evaluate the protective role of *M. oleifera* leaf fractions against antiretroviral drug-induced toxicity in the Canton-S strain of *D. melanogaster*. In this study, phytochemical screening of *M. oleifera* leaf fractions revealed the presence of flavonoids and phenolic compoundsS (Table 1). Several studies have reported the presence of phenolics (Castillo-Lopez et al., 2017; Luiza et al., 2019), and flavonoids (Chhikara et al., 2021; Rani et al., 2018) in *M. oleifera*. Also, the current findings agreed with previous results wherein the presence of flavonoids and phenolic phytoconstituents in *M. oleifera* leaf extracts was associated with the plant’s antioxidant effect (Aremu et al., 2018; Kou et al., 2018; Chenynier et al., 2013).

 Furthermore, fruit flies were exposed to different concentrations of TLD to establish a concentration-response relationship. The TLD-exposed flies showed a reduced survival during the 7-day exposure period, buttressed by a relatively low LC50 (Fig. 1). This result suggests that TLD induced toxic effects, which were detrimental to the survival of the flies, and could increase oxidative insults. Several pieces of evidence have shown that consumption of antioxidant-rich diets extends the organismal life span (Abolaji et al., 2020; Iorjiim et al., 2020). Interestingly, supplementation of the fly diet with *M. oleifera* leaf in this study established a statistically significant survival extension capacity of the ethyl acetate fraction of the plant at a concentration of 20 mg/10 g diet, compared to the control (Fig. 2).

 Again, results of exposure to TLD in the current study (Fig. 4) showed a significant (*P* = .04) reduction in climbing performance (Fig. 4A), AChE activity (Fig. 4A) and fly emergence (Fig. 5). Reports have linked climbing deficits and reproductive reduction in oxidatively stressed flies. Also, climbing deficits in fruit flies have been associated with decreased AChE activity, with a concomitant increase in free radical generation (Pam et al, 2021; Etu et al., 2019; Kavithal & Venkateswara Rao, 2007). Furthermore, oxidative stress has been implicated in reduced spermatogenesis, oocyte maturation and fertilisation in humans (Perkins et al., 2016; Haghnazari et al., 2016). Therefore, the observed TLD-induced reduction in climbing, reproduction and AChE activities could be a result of oxidative stress induction. *M. oleifera* acts as antioxidant via activation of the nuclear factor erythroid 2-related factor 2 (Nrf2)/antioxidant response element (ARE) pathway, which in turn initiates the *in vivo* expression of antioxidant enzymes (Cheng et al., 2019; Soliman et al., 2020). Activation of Nrf2 signaling pathway protects *D. melanogaster* against the harmful effects of oxidative and xenobiotic stress, and abate their consequences, including aging-associated functional senescence (Fuse & Kobayashi, 2017; Sykiotis & Bohmann, 2008).. Furthermore, It has been demonstrated that Nrf2 signaling in the *D. melanogaster* is activated by *M. oleifera* leaves (Sailaja et al., 2021). Thus, the observed ameliorative activity of *M. oleifera* leaf fractions against TLD-induced poor climbing, fly emergence deficits, and decreased AChE activity with corresponding reduction in MDA generation could be attributed to its antioxidant mechanism via Nrf2 activation.

 Glutathione S-transferases are cysteine-rich phase II family of multi-functional enzymes (Abolaji et al., 2020). They perform very important detoxifying roles against xenobiotics by conjugating glutathione (GSH) with electrophiles (Abolaji et al., 2020; Lushchak, 2012). Furthermore, thiols are compounds that contain a carbon-bound sulfhydryl moiety, which is very essential in free radical scavenging (Abolaji et al., 2020). Also, total thiols reflect the redox status of –SH groups, which are vital for the activity of many proteins involved in the maintenance of cell redox and protection of cells against pro-oxidants (Prakash et al., 2009). Antiretrovirals have been associated with the oxidation of proteins containing thiols (Mondal et al., 2004), as well as MDA generation (Ogunlade et al., 2022; Ikekpeazu et al., 2019). Malondialdehyde (MDA) is a toxic byproduct of lipid peroxidation, which has been used as an indirect measure of free radical generation in living tissues (Niedernhofer et al., 2003). The observation that *M. oleifera* leaf fractions restored both antiretroviral drug-induced reduction in total thiol level and inhibition of GST activity in *D. melanogaster* showed that TLD might have oxidised thiol groups on GST, resulting in the modification of its structure, thus altering its activity. Notably, TLD can also oxidise GSH and indirectly interfere with GST’s activity by depleting its natural substrate (Ndlovu et al., 2023; Phaniendra et al., 2015; Jones et al., 2000). In this study, *M. oleifera* fractions rescued fruit flies from antiretroviral drug-induced reduction in thiols levels and GST activity, and concomitantly reduced the MDA generation in the TLD-exposed flies. The ameliorative effects of *M. oleifera* may be via scavenging free radicals and/or its effect on the activation of Nrf2, an antioxidant pathway, which induces antioxidant enzyme expression.

**5. CONCLUSION**

 This study showed that *M. oleifera* leaf fractions contain antioxidant phytonutrients, flavonoids, and phenols, and supplementation of fly food with the fractions for 1–28 days improves survival rates. Additionally, the study deduced that fractions of *Moringa oleifera* rescued fruit flies from climbing failure, reduced emergence, decreased acetyl cholinesterase activity, and mitigated antioxidant deficits with corresponding malondialdehyde generation caused by TLD exposure. The ameliorative effects of *M. oleifera* could be attributed to the potent antioxidant activities of the plant’s phytonutrients. It can also be concluded that *M. oleifera* leaf fractions demonstrated protective activity against antiretroviral drug-induced oxidative stress toxicities in *Drosophila melanogaster* via antioxidant actions.

Ethical approval

Not applicable

**DISCLAIMER (ARTIFICIAL INTELLIGENCE)**

Authors hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have no competing interest to declare.

AUTHORS CONTRIBUTION

Iorjiim WM designed the study, performed the statistical analysis, wrote the protocol and the first draft of the manuscript. Omale S and Alemika ET managed the analysis and literature searches of the study. All authors read and approved the final manuscript.

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