



Highly Active Antiretroviral Therapy Depletes Some Antioxidant Parameters and Increases Free Radical Generation in *Drosophila melanogaster*

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Authors' contributions

This work was carried out in collaboration among 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 and GDB managed the analyses of the study. Authors WMI and MAE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Objective: This study intended to evaluate the toxic effects of Efavirenz-based highly active antiretroviral therapy (EFV_b-HAART) on some antioxidant parameters, and free radical generation in *D. melanogaster*.

Materials and Methods: The study was carried out at the Centre of Excellence in phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria, in 2019. Sixty (60) *D. melanogaster* (both sexes) 1-4 days old were exposed by ingestion to graded concentrations of

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EFV_b-HAART (93.11 mg, 46.56 mg, 23.28 mg, 11.64 mg) or 1000 µL distilled water (control) each per 10 g fly food for five days. All concentrations were diluted with 1000 µL distilled water and incorporated in cold fly food in five replicates. Treated flies were anesthetized under ice, homogenized, centrifuged, and the supernatant used to assay for Total protein, Total thiol, Glutathione-S-transferase, Catalase, Superoxide dismutase, and Malondialdehyde levels. Statistical significance was accepted at $P < 0.05$.

Results: The result showed significantly ($P < 0.05$) increased total protein ($1.05 \pm 0.0 - 1.34 \pm 0.12$ Vs. 0.56 ± 0.14 mg/ml) and Malondialdehyde levels ($1.63 \pm 0.20 - 3.72 \pm 0.53$ Vs. 0.79 ± 0.10 units/mg protein) in all tested groups versus unexposed. Conversely, Total thiol content ($1.96 \pm 0.33 - 0.38 \pm 0.10$ Vs. 5.31 ± 0.31 units/mg protein) Glutathione-S-transferase ($2.20 \pm 0.30 - 1.01 \pm 0.27$ Vs. 4.31 ± 0.24 units/mg protein), Catalase ($171.70 \pm 50.13 - 104.34 \pm 9.56$ Vs. 368.00 ± 7.56 units/mg protein) and Superoxide dismutase ($3.18 \pm 0.29 - 1.44 \pm 23$ Vs. 5.34 ± 1.35 units/mg protein) activities all decreased significantly ($P < 0.05$) as concentrations increased in all test groups versus unexposed.

Conclusion: Overall, our results suggest that the mechanism of EFV_b-HAART toxicity involves sterile immune response observed as increased protein contents, oxidative stress evidenced by depleted oxidative stress-antioxidant parameters, and possible free radical generation shown by increased malondialdehyde levels. Human-based studies are required for deeper understanding of these EFV_b-HAART toxicities.

Keywords: *Glutathione-S-transferase; highly active antiretroviral therapy; superoxide dismutase; Malondialdehyde.*

1. INTRODUCTION

Highly active antiretroviral therapy (HAART) has hitherto reduced the burden of HIV-related illnesses and deaths among HIV patients compared to HIV positive but untreated [1]. Nevertheless, HAART induces oxidative stress [2,3,4] and life expectancy in HAART treated HIV patients is shorter than the uninfected population [3]. Recently, lorjiim et al. [5] reported that *D. melanogaster* exposed to EFV_b-HAART developed locomotor deficits, decreased lifespan as well as reproductive capacity, and hypothesized that these observations might partly be oxidative stress-related toxicities. Therefore, this study aims to evaluate the toxicological impact of EFV_b-HAART on total protein, some antioxidants parameters (total thiols, Glutathione-S-transferase (GST), Catalase (CAT), Superoxide dismutase (SOD) and Malondialdehyde (MDA) levels (as a measure of free radical generation) in *D. melanogaster*.

Drosophila melanogaster has a significant number of advantages as a laboratory model in biological processes and toxicology, such as a short life cycle and a simple as well as cheap breeding method. Furthermore, the knowledge of physiology and biology of this organism allows a more in-depth understanding of mechanisms of action of analyzed substances [6,7,8]. Taking into account the advantages mentioned above of *D. melanogaster*, it seems to be an excellent

model to study, compare, and integrate the toxic effects of xenobiotics.

The free radicals, reactive oxygen species (ROS) are products of healthy metabolism or xenobiotic exposure, which may be beneficial as signaling molecules at low concentrations [9] or harmful when excessively high in a biosystem causing oxidative stress [10]. Oxidative stress (OS) results when there is a disequilibrium between ROS production and the detoxification of the reactive intermediates by a biological system, thereby contributing to cellular damage [11]. Excessive accumulation of ROS can damage biomolecules, including lipids, proteins, and nucleic acids [12]. Antioxidant defense mechanisms can remedy this excessive accumulation of ROS [12].

Both *in vivo* and *in vitro* studies have reported antiretroviral drugs-induced ROS generation [1-3]. The polymerase gamma toxicity theory of the nucleoside reverse transcriptase inhibitors (NRTIs (e.g. Lamivudine and Tenofovir), is a common conceptual framework upon which some of these studies on antiretroviral toxicities revolves [1-3]. Briefly, NRTIs competes with natural nucleosides for binding site on polymerase gamma (pol γ), the sole polymerase in the mitochondrial responsible for mitochondria DNA synthesis and repair, thus resulting to DNA chain termination. Consequently, the mitochondrial functions becomes compromised leading to decrease in energy production,

increase in inflammatory signals as well as ROS generation and apoptosis [3].

Many natural organic molecules are essential members of the antioxidant system known to scavenge free radicals by enzymatic and non-enzymatic mechanisms [13]. Thiol (-SH) group of proteins (e.g., glutathione, Thioredoxin and albumin) are mainly essential for their antioxidant response against reactive oxygen and nitrogen species [14] and may serve as a sensitive indicator of oxidative stress [15]. The enzyme superoxide dismutases (SODs) catalyzes the conversion of two superoxide anions into oxygen and hydrogen peroxide [16]. All three (3) forms of SOD, namely, CuZn- SOD (which binds both copper and zinc), Mn-SOD (which binds manganese), and EC-SOD (extracellular SOD), are found in various cells in vertebrate [16] and are well conserved in *Drosophila melanogaster* [17]. Catalase is responsible for the conversion of hydrogen peroxide (a harmful by-product of metabolism) to harmless oxygen gas and water [18]. Glutathione peroxidase (GPxs) (e.g., glutathione peroxidase 1, glutathione peroxidase 4, and glutathione S-transferase) are essential for the detoxification of xenobiotics through conjugation with glutathione for excretion as well as the repair of oxidative damage caused to macromolecules by reactive oxygen and nitrogen species [19]. GPxs also cooperated with Catalase in the catabolism of hydrogen peroxides [18,14].

2. MATERIALS AND METHODS

This research work was carried out at the *Drosophila* fly laboratory of Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Jos-Nigeria, between January to August 2018.

2.1 Reagents and HAART Drug

All chemicals used were of analytical grade. Distilled water (CAS: 7732-18- 5), Randox protein kit, reduced glutathione (GSH), Hydrogen peroxide, 1-chloro-2,4-dinitrobenzene, (CDNB), 5,5'-dithiobis (2-nitro-benzoic acid) (DTNB) were sourced through ACEPRD, University of Jos, Jos-Nigeria. Trichloroacetic acid (TCA) 30%, thiobarbituric acid (TBA) 0.75%, 0.15 M Tris-KCl buffer (pH 7.4), 0.1M HCl, 0.05M carbonate buffer (pH 10.2) and 0.3M Adrenaline were sourced through the Biochemistry Division, National Veterinary Research Institute (NVRI), Vom, Jos Nigeria.

The -HAART [EFV 600 mg (CAS 154598-52-4) + TDF 300 mg (CAS 202138-50-9) + 3TC 300 mg (CAS 134678-17-4) mg per tablet, Batch number 3075041, NAFDAC number A4-5090, expiry date October 2020 and manufacture: Gilead Sciences Inc] used was donated by the General Hospital Gboko, Benue State Nigeria. Thirty (30) tablets of the fixed-dose formulation were first weighed and the average weight determined and then pulverized using porcelain mortar and pestle. The appropriate quantities of powder containing the desired amount of active ingredient were calculated and weighed out using an analytical balance (Mettler Model No. MT-200B). These proportions of the powdered drug were then freshly diluted with 1000 μ L distilled water and mixed with the cold fly food. In all experiments, 1000 μ L distilled water served as negative control.

2.2 Animal Model

The *Drosophila melanogaster* (Harwich strain) used was sourced at the Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD). The fly stock was raised and maintained at constant temperature and humidity (23 \pm 1 $^{\circ}$ C; 60% relative humidity, respectively) under 12 h dark/light cycles. The flies were fed on *Drosophila* medium composed of yellow cornmeal (100 g), brewer's yeast (20 g), agar (16 g) and the preservative methylparaben (1 g) [7].

2.3 *In vivo* Antioxidant and Free Radical Assay

In vivo antioxidant and free radicals assay were carried out by exposing (via ingestion) sixty (60) flies to graded concentrations (11.64 mg, 23.28 mg, 46.56 mg, or 93.11 mg) of EFV_b-HAART per 10 g fly food in five replicates for five days as described by Alexander et al. [20]. Distilled water served as control. At the end of the exposure period (5 days), the flies from each group of control or HAART-treated flies were anesthetized in ice, weighed and homogenized in 0.1 M phosphate buffer, pH 7.0 (1 mg: 10 μ L), and centrifuge using cold centrifuge (Eppendorf AG, 5227 R, Germany, temperature 4 $^{\circ}$ C) for 10 min at 4000 rpm. The supernatants obtained were used to determine Total protein, Total thiol content, the activities of Catalase (CAT), Glutathione-S-transferase (GST), Superoxide dismutase (SOD) and Malondialdehyde (MDA) concentration.

2.3.1 Total protein determination

The protein content of the whole fly homogenate was determined by the Biuret method (Randox total protein assay kit), as described in the manufacturer's instruction manual.

2.3.2 Total thiol determination

Total thiol content was determined using the method of Ellman described by Alexander et al. [20]. The reaction mixture contained 510 μL potassium phosphate buffer (0.1 M, PH 7.4), 25 μL of the sample as well as 30 μL of DTNB (10 mM). After incubation for 30 min at room temperature, the absorbance was measured at 412 nm and used to calculate the sample total thiol levels (in mmol/mg protein) using 35 μL of GSH as standard.

2.3.3 Glutathione-S-transferase (GST) activity

The activity of glutathione-S-transferase (GST; EC 2.5.1.18) was determined by the method of Habig and Jacoby described by Alexander et al. [20] using 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate. The assay reaction mixture contained 600 μL of solution A (20 μL of 0.25 M potassium phosphate buffer, pH 7.0 with of 2.5 mM EDTA and 510 μL of 0.1 M GSH at 25°C), 60 μL of the sample (1:5 dilution) and 30 μL of 25 mM CDNB. An increase in absorbance was measured at 340 nm for 2 min at 10 s interval using a spectrophotometer (Jenway model No.7315). The data were expressed in mmol/min/mg of protein utilizing the molar extinction coefficient (ϵ) of 9.6 mM⁻¹ cm⁻¹ of the coloured GS–DNB conjugate formed by GST.

2.3.4 Catalase (CAT) activity

The measurement of catalase (CAT; EC 1.11.1.6) activity was carried out according to the procedure described by Abolaji et al. [17]. The reaction mixture containing 100 μL of potassium phosphate buffer, pH 7.0, 194 μL of 300 mM H₂O₂ to form solution A. 10 μL of the sample was reacted with 590 μL of solution A and monitoring the clearance of H₂O₂ at 240 nm at 25°C. The decrease in H₂O₂ was observed for 2 min (10 s intervals), at 240 nm using a UV–visible spectrophotometer (Jenway model No. 7315) and expressed as mmol of H₂O₂ consumed/min/mg of protein.

2.3.5 Determination of Superoxide Dismutase (SOD) activity

The SOD activity was determined by the method of Misra and Fridovich [21]. Briefly, 200 μL of the

sample was diluted in 800 μL of distilled water to make a 1 in 5 dilutions. An aliquot of 200 μL of diluted sample was added to 2500 μL of 0.05 M carbonate buffer (pH 10.2) in the spectrophotometer to equilibrate, and the reaction initiated by the addition of 300 μL freshly prepared 0.3 M adrenaline. The reference cuvette contained 2500 μL buffer, 300 μL of the adrenaline and 200 μL of water. The absorbance was monitored every 30 seconds for 150 seconds at 480 nm.

2.3.6 Assessment of Malondialdehyde level

Lipid peroxidation was determined by measuring the level of Malondialdehyde produced during lipid peroxidation according to the method described by Varshney and Kale [22]. An aliquot of 400 μL of the sample was mixed with 1600 μL of tris-KCl buffer to which 500 μL of 30% TCA was added. Then 500 μL of 0.75% TBA was added and placed in a water bath for 45 minutes at 80°C. This was cooled in ice and centrifuged at 3000 g for 5 minutes. The clear supernatant was collected, and absorbance measured against a reference blank of distilled water at 532 nm. Lipid peroxidation expressed as MDA formed/mg protein or gram tissue was computed with a molar extinction coefficient of 1.56 x 10⁵ M⁻¹ Cm⁻¹.

2.4 Statistical Analysis

The data are shown as mean \pm SEM (standard error of mean). One-way analysis of variance (ANOVA) was used for statistical analysis with Tukey's posthoc test to identify differences between test groups versus controls using GraphPad Prism version 8.0.2 for windows (GraphPad Software, La Jolla California USA, www.graphpad.com). The results were assumed as statistically significant at $P < 0.05$.

3. RESULTS

3.1 Total Protein

Five (5) days exposure of flies to EFV_b- HAART showed significant ($P < 0.001$) elevation of total protein content in the test groups compared to the unexposed in a dose-dependent manner. The protein values increased within the range of 1.05 \pm 0.06 – 1.34 \pm 0.12 mg/ml from the lowest to the highest concentration versus unexposed group 0.56 \pm 0.14 mg/ml.

3.2 Total Thiol Content and GST Activity

The results of total thiol content (Fig. 1A) and glutathione-S-transferase activity (Fig. 1B) after 5-days treatment was both significantly ($P < 0.001$) reduced in a dose-dependent manner compared to the unexposed groups respectively. The total thiol content decreased within the range of $1.96 \pm 0.33 - 0.38 \pm 0.10$ units/mg protein from the lowest to the highest concentration versus unexposed group 5.31 ± 0.31 units/mg protein. Similarly, glutathione-S-transferase activity decreased within the range of $2.20 \pm 0.30 - 1.01 \pm 0.27$ units/mg protein from the lowest to the highest concentration versus unexposed group 4.31 ± 0.24 units/mg protein.

3.3 Superoxide Dismutase (SOD) and Catalase (CAT) Activities

The results of SOD (Fig. 2A) and Catalase (Fig. 2B) activities after five (5) days treatment with EFV_b-HAART were both significantly ($P < 0.05$) reduced compared to the unexposed groups along concentration gradient respectively. The SOD activity decreased within the range of $3.18 \pm 0.29 - 0.144 \pm 0.23$ units/mg protein from the lowest to the highest concentration versus unexposed group 5.34 ± 1.35 units/mg protein. Also, CAT activity decreased within the range of $171. \pm 50.13 - 104.30 \pm 9.56$ units/mg protein from the lowest to the highest concentration versus unexposed group 368.00 ± 7.566 units/mg protein.

3.4 Malondialdehyde Content

The concentration of MDA after 5-day treatment was significantly ($P < 0.05$) elevated in the EFV_b-HAART-treated flies compared to the unexposed group (Fig. 3A). This significant elevation ($P < 0.05$) of MDA concentration was in hundredfold along the concentration gradient, as shown in Fig. 3B. From the highest experimental concentration the percentage increase was $93.11 \text{ mg} = 470.40 \pm 67.44\%$, $46.56 \text{ mg} = 431.30 \pm 16.34\%$, $23.28 \text{ mg} = 431.30 \pm 16.34\%$ and $11.64 \text{ mg} = 205 \pm 26.10\%$. The MDA level increased within the range of $1.63 \pm 0.20 - 3.72 \pm 0.58$ units/mg protein from the lowest to the highest concentration versus unexposed group 0.79 ± 0.01 units/mg protein.

4. DISCUSSION

The reactions of free radicals as normal physiological processes in a living system becomes harmful when its level is over-

expressed or elevated beyond the system's antioxidant capacity [23]. These free radicals may be generated exogenously (e.g. smoking or drugs in-take) or endogenously (e.g. normal cellular respiration or disease conditions or both [23]). Precisely, particulate matter from cigarette smoke for example, comprises of carcinogens (e.g. benzo[*a*]pyrene) and stable ROS (NO₂) that are not easily degradable [15,39]. Thus, these ROS may chronically react with biomolecules causing severe tissue damage [15,39]. The electron transport chain complexes I and III are the primary sites for superoxide generation during normal oxidative phosphorylation, however, in diabetes mellitus, increased glycolysis favours pyruvate generation. This raises the mitochondrial membrane potential, causing mitochondrial functional deficits and hence increased ROS production at complex II [15].

The antioxidant system plays a vital role in keeping the free reactive oxygen and nitrogen species in check by scavenging them when they are much in the system [23]. We investigated the toxic impact of EFV_b-HAART against the antioxidant system and free radical generation in *D. melanogaster* for the first time.

In this study, EFV_b-HAART significantly ($P < 0.05$) increased total protein after five (5) days exposure. Our result agree with the earlier report [24] that HAART exposure increases total proteins in humans. In drosophila, ROS released from damaged cells act as immediate damage signals called Damage Associated Molecular Patterns (DAMPs), which triggers the recruitment of hemocytes that continually circulate in haemolymph [25]. This cellular response bears a resemblance to the mammalian interleukins and JAK/STAT signaling in inflammatory responses [25]. The significant increase in total protein in *D. melanogaster* in our study might, therefore, be a sterile (non-microbial mediated) inflammatory response. This observed inflammatory response in *D. melanogaster* in our study may, in turn, be a similitude of the likely initial adoptive response to EFV_b-HAART-induced free radicals causing cellular aberrations in humans. Thus, we were encouraged by this result to look at the total thiol content and other antioxidant enzyme activities to ascertain the oxidative status of the EFV_b-HAART exposed flies.

Due to our suspicion of a sterile inflammatory response in EFV_b-HAART exposed *D.*

melanogaster observed as significantly ($P<0.05$) increased total protein, we investigated the impact on total thiol contents and GST activity respectively. Protein thiols scavenge 50-70% of ROS generated; therefore, the serum level of protein thiols in a biosystem indicates its antioxidant status [26]. Thiol groups are very susceptible to oxidation and considered as one of the essential plasma sacrificial antioxidants[20]. When the cells are exposed to oxidative stress, thiol groups are the first antioxidants that are consumed, thereby reducing the content and activity of the thiol groups in the living system [20]. GST, on the other hand, catalyzed the transfer of sulfhydryl (-SH) group from Glutathione to xenobiotic and other electrophilic compounds[19,26]. The decreased total thiols (Fig. 1A) in the EFV_b-HAART exposed groups may imply a deleterious impact of HAART-induced ROS on the fly antioxidant system. The GST activity (Fig. 1B) in our study may also have decreased probably due to either decreased expression (which our study could not determine) or the diminished total thiol content (Fig. 1A) from which this enzyme catalyzes the transfer of sulfhydryl (-SH) moieties onto xenobiotics. Our finding here observed as significant ($P<0.05$) decrease in both total thiol content and GST activity is particularly worrisome because it portrays the picture of a severely weakened antioxidant capacity in EFV_b-HAART exposed *D. melanogaster*. Our result further correlates with, and gives credence to the earlier reports [27,2] that HAART drugs induce oxidative stress in the HIV positive population resulting in decreased protein thiols and GST activity respectively. Thus, we sought further to know the SOD and CAT status in *D. melanogaster* exposed to EFV_b-HAART.

Superoxide dismutase catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen [28], and prevents the superoxide anion from reacting with nitric oxide to form reactive peroxynitrite [28: 17]. They are two steps involved in scavenging ROS by superoxide dismutase and catalase. The first step is catalyzing the dismutation of two superoxide anions to hydrogen peroxide (H₂O₂) and water, while in the second step, CAT catalyzes the decomposition of H₂O₂ to water and oxygen [29]. Therefore a decrease in SOD and CAT activity may indicate the imbalance of antioxidant system to free radical ratio. Our results (Fig. 2A and 2B) revealed a significant ($P<0.05$) decrease in SOD and CAT activities in the EFV_b-HAART-treated flies compared to unexposed groups

respectively. These results suggest the overproduction of free radicals in the EFV_b-HAART-treated flies. Thus, excessive free radicals circulating the fly haemolymph may have overwhelmingly induced oxidative damage to these antioxidant enzymes or irreversibly suppressed their gene expression or both. Previous *in vitro* studies using human endothelial cells [30] and *in vivo* rat model [31] agree with our findings in *D. melanogaster* that exposure to HAART drugs may increase ROS production causing diminished SOD and CAT activities. Thus, we saw the need to investigate further to know the impact of EFV_b-HAART exposure against the free radical generation in *D. melanogaster* through the determination of MDA content.

Reactive oxygen species attacks all biological molecules; however, the most susceptible ones are polyunsaturated fatty acids [32]. Reactions with these cell membrane constituents result in lipid peroxidation (LPO) [32]. Increased LPO impairs membrane function by decreasing membrane fluidity as well as the activity of membrane-bound enzymes and receptors [33]. Malondialdehyde (MDA) production, measured as thiobarbituric acid reactive substance (TBARS) levels, serves as a marker of LPO [2]. Malondialdehyde is an endogenous genotoxic product of enzymatic and ROS-induced LPO whose adducts are known to exist in DNA isolated from oxidatively stressed humans [34] and *D. melanogaster* [35] respectively. Thus, MDA level is widely used as a marker of lipid peroxidation in states of elevated oxidative stress [34,36]. Our result (Fig. 3A) revealed a significant ($P<0.05$) increase in the MDA levels in the EFV_b-HAART-treated groups compared to the unexposed group. This result suggests increased production of free radical generation along the concentration gradient since there was a consistent hundredfold increase in MDA levels from the least experimental EFV_b-HAART concentration of 11.64 mg (205.80±26.10%) to the highest concentration 93.11 mg (470.40±67.44%) compared with the unexposed group (100±12.75%) (Fig. 3B). These findings agree with that of Adaramoye et al. [37] who reported a significantly increased serum malondialdehyde level in Wistar rats exposed to Tenofovir disoproxil fumarate (a component of the HAART in our study). Sundaram et al. [38] also reported that the role of oxidative stress in disease progression was more complicated in HIV-infected patients receiving HAART compared to those who remain untreated.

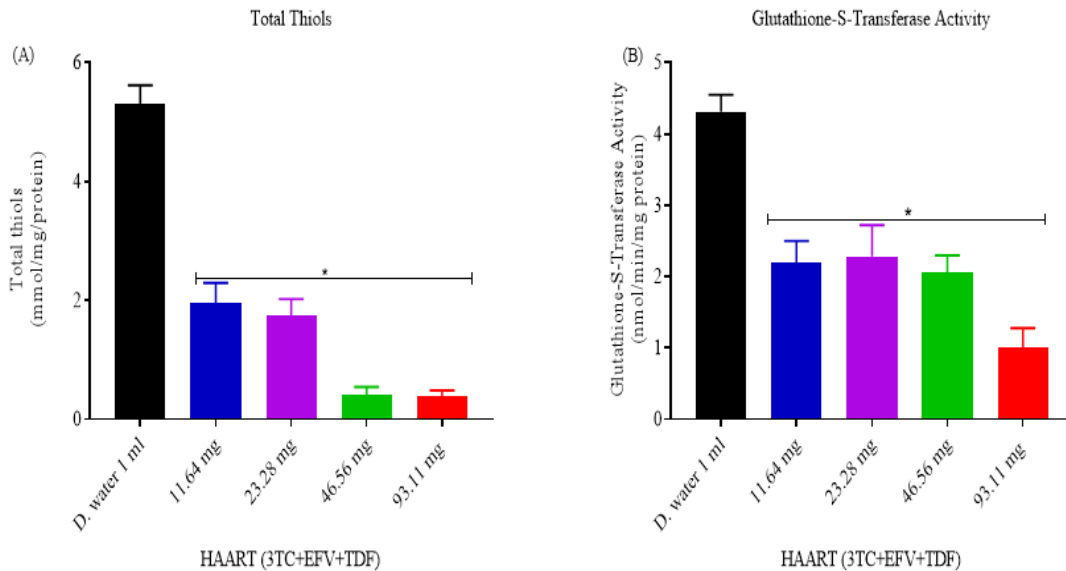


Fig. 1(A-B). (A) Total tiol content in whole fly homogenate of EFV_b- HAART exposed *D. melanogaster* after 5 days, (B) Glutathione-S-Transferase activity in whole fly homogenate of EFV-HAART exposed *D. melanogaster* after 5 days. Data presented as mean±SME of five independent biological replicated for each drug concentration (n=60). **P*<0.05 vs control. HAART = highly active antiretroviral therapy, 3TC = Lamivudine, EFV = Efavirenz, TDF = Tenofovir diisoproxyl fumarate

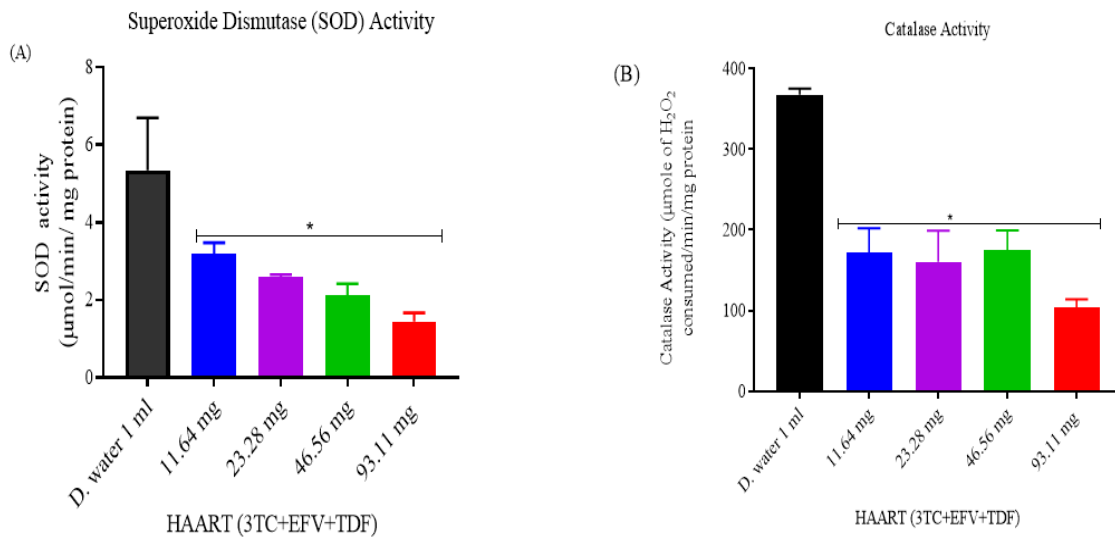


Fig. 2(A-B). (A) Superoxide dismutase activity in whole fly homogenate of EFV-HAART exposed *D. melanogaster* after 5 days. (B). Catalase activity in whole fly homogenate of EFV-HAART exposed *D. melanogaster* after 5 days. Data presented as mean±SME of five independent biological replicated for each drug concentration (n=60). **P*: 0.05 vs control. HAART = highly active antiretroviral therapy, 3TC = Lamivudine, EFV = Efavirenz, TDR = Tenofovir diisoproxyl fumarate

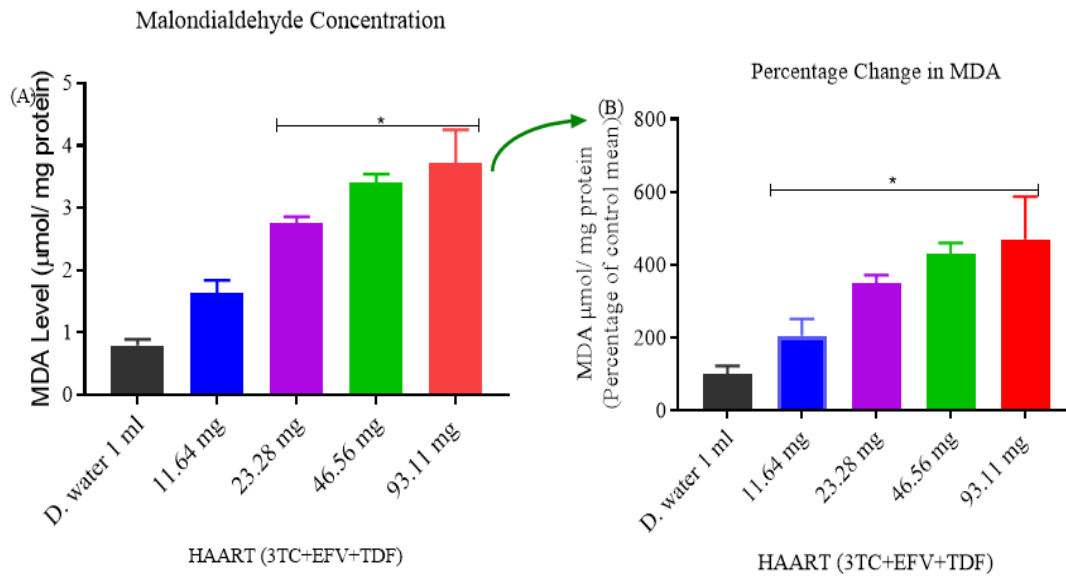


Fig. 3(A-B). (A) Malondialdehyde(MFS) concentration in whole fly homogenate of ERV-HAART exposed *D. melanogaster* after 5 days. (B) Malondialdehyde concentration (as percentage of control mean) in whole fly homogenate of ERV-HAART exposed *D. melanogaster* after 5 days. Data presented as mean±SME of five independent biological replicated for each drug concentration (n=60). *P<0.05 vs control. HARRT=highly active antiretroviral therapy, 3TC=Lamivudine, EFV=Efavirenz, TDF = Tenofovir diisoproxyl fumarate

It is important to mention here some of the limitations of our study. First, we could not confirm EFV-HAART toxicities using appropriate human based studies. Secondly, MDA is an unstable by-product of lipid peroxidation, thus the oxidative status in our study may have been underestimated.

5. CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

We showed in this study the toxic effects of EFV_b-HAART on the sterile inflammatory response, the antioxidant system and free radical generation in *D. melanogaster* for the first time. The inflammatory response, decreased antioxidant capacity and increased free radical generation in *D. melanogaster* evidenced by increased protein content, depleted antioxidant parameters or increased malondialdehyde level respectively portrays danger to the flies and these toxic events may do harmful to humans too.

5.2 Recommendations

We recommend further appropriate human-based studies for possible antioxidant supplementation.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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