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Research Article
Dolutegravir Increased Mortality Rate, Climbing Deficits and Altered Biomarkers of Oxidative Injury in Harwich *Drosophila melanogaster*

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Abstract
Background and Objective: Dolutegravir (DTG) is safer compared to Efavirenz (EFV) as a component of antiretroviral combination therapy. However, recent studies suggested that DTG might be associated with oxidative stress toxicity in humans. This study intended to assess whether a non-target organism, *Drosophila melanogaster*, could replicate the DTG-induced oxidative stress and to determine the potential toxicity of a finished dolutegravir product because of possible environmental contamination. **Materials and Methods:** Flies (mixed sexes) were exposed to a series of ten DTG concentrations (0-50 mg per 10 g fly food) for a 7 days LD_{50} determination, followed by a 28 days survival assay, then a 5 days treatment to evaluate the effects of the test drug on negative geotaxis, fertility and some antioxidant biomarkers. Also, *in silico* molecular docking was performed to predict the possible molecular interactions of Drosophila acetylcholinesterase (AChE) and glutathione-S-transferase (GST) enzymes with the test drug. **Results:** The LD_{50} of DTG in *D. melanogaster* was 16.53 mg/10 g of food. The DTG showed a significant (p<0.05) reduction in 28 days survival and climbing ability as well as alterations in the levels of biochemical markers [total thiols (T-SH), AChE, GST, catalase (CAT), superoxide dismutase (SOD) and malondialdehyde (MDA)] of the exposed flies. The test drug interacted with similar amino acid residues with a significantly (p<0.05) higher molecular attraction for the active sites of GST but a lower (p>0.05) binding affinity for those of AChE compared to the respective natural substrates and standard inhibitors. **Conclusion:** Oral administration of DTG in *D. melanogaster* significantly increased mortality rate, climbing and antioxidant deficits through a competitive antagonism of GST and AChE activities. The DTG-induced oxidative stress was replicated in the flies. Thus, environmental contamination with dolutegravir sodium could adversely affect non-target organisms in an ecosystem.

Key words: Dolutegravir, *Drosophila melanogaster*, oxidative stress, acetylcholinesterase, glutathione-S-transferase, oocyte maturation, glucuronidation, malondialdehyde

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Available data suggest that Human Immunodeficiency Virus (HIV) infection is incurable. However, the progression of HIV-1 infection to Acquired Immunodeficiency Syndrome (AIDS) can be slowed with the effective utilization of antiretroviral drugs[1]. Despite the clinical relevance of antiretroviral drugs in humans, it is usually the nature of drugs to produce unintended impacts on non-target animals in an ecosystem[2]. The beneficial and adverse effects of DTG in humans have been investigated in clinical and toxicological studies[3-5]. However, the potential ecological effects of DTG on non-target species are scarcely known. In addition, it has been demonstrated that some drugs may produce toxic effects in some non-target animals at concentrations well below those used in safety and efficacy studies in humans[6]. Some consequences of pharmaceutical end products on a non-target organism such as the decreased testis and oocyte maturation in insects, inhibition of growth in aquatic plants and the emergence of antibiotic resistance in soil microorganisms have been reported[6].

The EFV-containing antiretroviral combinations have been the recommended treatment options for HIV-1 infection worldwide[7]. Recently, toxicity and efficacy concerns prompted the World Health Organization (WHO) to replace EFV (a Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI)) with a safer Integrase Strand Transfer Inhibitor (INSTI) dolutegravir[8]. Dolutegravir is now a mandatory component of combination antiretroviral therapy for all categories of HIV-1 patients[9]. After oral administration in humans, DTG is mainly metabolized in the liver by Uridine Diphosphate Glucuronosyltransferase 1A1 (UGT1A1) via the glucuronidation process[3]. Therefore, UGT1A1 inhibitors (e.g., atazanavir, diclofenac) could cause toxic plasma concentrations of DTG[10]. Common side effects associated with DTG include mild nausea, headache and diarrhea[3], however, recent shreds of evidence suggested that DTG-induced toxicities could be associated with mitochondrial dysfunction[5]. Mitochondrial toxicity induces excessive reactive oxygen species (ROS) production, leading to oxidative stress[11]. Oxidative stress is implicated in a variety of sicknesses[12,13] and is characterized by an imbalance between ROS production and antioxidants. The ROS plays a vital role in the propagation of specific molecular signals[14,15], which may be therapeutic at low concentrations[16]. However, excessive production of ROS results in oxidative injury to DNA, proteins and lipids[17]. Therefore, a study on the involvement of dolutegravir-induced toxicities in *D. melanogaster* is relevant as it could validate whether or not some of the DTG-induced toxicities in humans could be replicated in a non-target organism. In addition, the results of the current study might determine the potential toxicity of dolutegravir sodium, in non-target organisms in view of possible environmental contamination. The finished pharmaceutical product of DTG, dolutegravir sodium was used in this investigation to mimic the actual form it is used in therapeutics. Also, it is the finished DTG dosage form from the industries, distribution channels, clinics and homes that could be a most likely culprit in environmental pollution.

It is important to understand the off-target effects of DTG using an efficient model such as *Drosophila melanogaster* commonly known as the fruit fly. This model organism is widely accepted by scientists working to protect animal rights in research[18]. In addition, out of 929 genes implicated in various human diseases, 77% (n = 714) share genomic similarity with 548 of the Drosophila genome[19]. For example, all three forms of the antioxidant enzymes superoxide dismutase (CuZn-SOD, Mn-SOD and EC-SOD (extracellular SOD)) are found in different cells of vertebrates[20] and are well conserved in the fruit fly[21]. Exposure of *Drosophila melanogaster* to toxicants can occur through inhalation, injection or ingestion methods[18]. The administration method chosen for this investigation was to mix the test drug with a tasty substrate like sucrose or brewer's yeast-supplemented fly diet[22] to improve palatability[23]. This model organism has been successfully used to assess antiretroviral drug-induced genotoxicity[24] and antioxidant deficits[25]. In this study, the toxic potentials of DTG in terms of mortality rate, fertility, climbing performance (acetylcholinesterase activity and negative geotaxis) and some parameters of the antioxidant system on the Harwich strain of *Drosophila melanogaster* were investigated.

MATERIALS AND METHODS

Study area: This research work was carried out in January to July, 2021 at the Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Jos, Nigeria.

Reagents and antiretroviral drugs: The DTG 50 mg/tablet (Alubindo Pharma) were donated by General Hospital Gboko, Benue State, Nigeria. Ten tablets of DTG were weighed using an analytical balance (Mettler-MT200B) and the average weights were determined, followed by pulverization using porcelain mortar and pestle. The concentrations of active DTG were calculated relative to the excipients and weighed out. The weighed portions were separately dissolved with 1000 µL of 0.5% Dimethyl sulfoxide (DMSO) to produce a stock solution

of 50 mg mL^{-1}, which was further diluted to the desired concentrations with distilled water and thoroughly mixed with an appropriate amount of cold fly food. The structure of DTG contains two H-donor groups and many H-acceptor groups making it a moderately hydrophobic drug[26]. A solvent such as Dimethyl sulfoxide (DMSO), which possesses amphipathic properties due to a hydrophilic sulfoxide moiety and two hydrophobic methyl groups, has previously been shown to be a good solvent for DTG solubility (>45 mg mL^{-1})[26]. It has also been reported that DMSO is relatively non-toxic to fruit flies at concentrations below 0.5%[27]. The different concentrations of DTG used in the current study were chosen below 50% LD_{50} to avoid powder saturation in the 1000 µL DMSO.

The reagents used for the experiments were of analytical grade. Hydrogen peroxide (H_2O_2), GSH, 5,5'-dithiobis(2-nitro-benzoic acid) (DTNB) and 1-chloro-2,4-dinitrobenzene (CDNB) were from the Drosophila Laboratory, Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Jos, Nigeria. About 30% Trichloroacetic acid, 0.75%, Thiobarbituric acid, Tris-KCl buffer (0.15 M, pH 7.4), carbonate buffer (0.05M, pH 10.2), 0.1 M HCl, and 0.3 M Adrenaline were purchased from the Department of Biochemistry, National Veterinary Research Institute, Vom, Jos, Nigeria.

***Drosophila melanogaster*:** The Harwich strain of *Drosophila melanogaster* was cultured at the ACEPRD fly Laboratory University of Jos, Jos, Nigeria. Flies were fed with standard yellow corn meal medium mixed with brewer's yeast (1% w/v), agar (1% w/v) and methylparaben (0.08% w/v) and maintained under the prescribed temperature (23±1°C), relative humidity (60%) and 12 hrs dark/light conditions[22].

Determination of median lethal dose (LD_{50}) and 28 days survival assay: The median lethal dose (LD_{50}) was determined as previously described by Iorjiim *et al.*[25] using the feeding method of exposure. Sixty flies (1-3 days old) were fed ten varying concentrations of DTG (0, 5, 10, 15, 20, 25, 30, 35, 40, 45 and 50 mg) dissolved with 1000 µL of 0.5% DMSO per vial of 10 g fly food in three replicates for 7 days. Two different controls were run (with and without 0.5% DMSO), however, only the data from the DMSO control appear in the results as the two controls did not differ significantly in all parameters tested. The number of dead flies was recorded every 24 hrs throughout the experimental period. Percent survival versus logarithmic concentrations was plotted in a dose-response simulation using GraphPad Prism 8.0.2 (Fig. 1).

The technique of Abolaji *et al.*[22] was adopted for the 28 days survival assay to determine the concentrations of DTG and the number of days of exposure appropriate for the assessment of fertility, climbing ability and biochemical parameters. Five clusters of 60 flies per category in triplicates were treated with four different concentrations of DTG or control. The appropriate environmental conditions[22] were maintained throughout the experimental period of 28 days, while fly mortality values were recorded every 24 hrs. Percent mortality versus time (days) was plotted in a Kaplan-Mayer curve (Fig. 2a-b).

Fly treatment for negative geotaxis, reproductive ability and biochemical parameters: The duration of fly exposure to DTG was derived from the 28 days Kaplan-Mayer curve[21] (Fig. 2a-b). In this study, the 5th day was chosen as the treatment period because the flies showed early signs of toxicity (<30 % mortality) and thus early biochemical changes could also be detected during this period[21]. The negative geotaxis (climbing performance) is a behavioural test used to assess motor coordination competence in flies[21]. After a 5 days fly exposure to DTG by ingestion method, the climbing performance of the exposed or control flies was evaluated using the technique of Adedara *et al.*[28].

Fertility was assessed as described by Pam *et al.*[23]. Four groups of sixty virgin flies (both sexes) each in 3 replicates were exposed to the test drugs by ingestion for 5 days. Ten flies (5 males and females) from each treated or control group were mated in a plastic vial (15 by 1.5 cm) on a standard fly diet for 24 hrs. The matured flies were then removed from the vials and the presence of eggs in the vials was confirmed using a magnifying hand lens. The vials were then stored under the prescribed environmental conditions for 14 days[29]. The cumulative number of flies hatched during this period provided the measure of reproductive ability.

For the biochemical assays, flies that had been exposed to DTG as explained above were immobilized under ice before weighing, followed by homogenization in 0.1 M phosphate buffer (pH 7.4, ratio 1 mg:10 µL). A centrifuged (Eppendorf-Germany, model: AG, 5227 R) was set to revolve 4000 times per min for 600 sec at -4°C to spin the fly homogenate. The supernatant was micro-pipetted into labelled Eppendorf tubes for the determination of total protein (T-Pr), total thiols (T-SH) and malondialdehyde (MDA) levels and the activities of superoxide dismutase (SOD), acetylcholinesterase (AChE), glutathione-S-transferase (GST) and catalase (CAT).

Evaluation of biochemical parameters: Lowry *et al.*[30] techniques were used to determine total protein, while Ellman's method was adapted from Abolaji *et al.*[29] and used for total thiol assessment. Also, the composition of the reaction mixture, the incubation conditions and the wavelength for absorbance measurement were all as described by Abolaji *et al.*[29]. Total thiol content was calculated using (ε) = 9.6 mM^{-1} cm^{-1} (extinction coefficient) and adjusted per milligram protein content. Ellman *et al.*[31] technique was used to determine AChE activity. The composition of the test medium was distilled water (285 µL), 100 mM potassium phosphate buffer (180 µL, pH 7.4), 10 mM DTNB (60 µL), fly homogenate (15 µL) and 8 mM acetylthiocholine (60 µL) was added to start the reaction process. The absorbance of the reaction was measured in a spectrophotometer (Jenway, ≠7315) at 412 nm for 120 sec at 10 sec intervals. The method of Misra and Fridovich, adapted from Iorjiim *et al.*[25], was followed to determine SOD activity. Also, the reaction compositions and wavelength used to measure absorbance for SOD activity calculation were as defined by Iorjiim *et al.*[25]. The CAT activity was measured using Aebi's method described by Abolaji *et al.*[29]. A stock (solution A) was prepared with potassium phosphate buffer (100 mL, pH 7.0) and 19 mM H_2O_2 (194 mL). Fly homogenate (10 µL) was mixed with solution A (590 µL) and the rate at which H_2O_2 cleared away was checked at 240 nm at 25°C. A lipid peroxidation assay was performed as an indirect measure of *in vivo* reactive oxygen species (ROS) production. This was achieved by measuring the malondialdehyde content in the fly homogenate using the method of Varshney and Kale as previously described by Iorjiim *et al.*[25]. All experimental conditions were as published by Iorjiim *et al.*[25].

***In silico* modelling of molecular interactions:** The mechanism of the inhibitory activities and the nature of the biological interactions of DTG against Drosophila GST and AChE were evaluated using *in silico* molecular modelling.

Identification and collection of ligands and proteins: The corresponding chemical structure (SDF format) of DTG (ID: 54726191), glutathione (ID: 124886 ID),6-[(7-Nitro-2,1,3-benzoxadiazol-4-yl)sulfanyl]hexan-1-ol, (NBDHEX, ID: 9817686), acetylcholine(ID: 187) and galantamine (ID: 9651), were identified[32-34] and downloaded from the Pub-Chem database in 3D forms. Similarly, *Drosophila melanogaster* GST (PDB ID: 1 M0U) and AChE (PDB ID: 1Q0N) were identified[28,32] and downloaded from the protein data bank (RCSB PDB) in their crystallographic structure format.

Ligand and protein preparation: The techniques of ligand and protein preparation for docking analysis described by Johnson *et al.*[34] and Duru *et al.*[35] were adopted. According to these procedures, a file of the ligands was imported, minimized three times to achieve the optimal docking energy and converted to auto-dock ligand formats (PDBQT) via the Open-Babel plug-in using the PyRx software. Proteins were fetched by their IDs into the UCSF Chimera (version 1.14) and prepared for docking as defined by Johnson *et al.*[34] and stored in a PDB format.

Ligand-protein complex formation: Proteins prepared for docking were loaded into PyRx software, made as macromolecules and converted to PDBQT formats. Each protein was docked with its appropriate substrate, standard inhibitor and test drug. The docking grid box was maintained in the system's default dimensions and performed 9 conformations of ligand-protein interactions in PyRx's Vina wizard. Docked outputs were exported as ZIP files to PyMol2.3.3 workspace for visualization and analysis of ligand-protein complexes. The 2D and 3D models of the ligand-protein interactions were created and displayed with Discovery Studio 2020 software.

Statistical analysis: All data in this study were presented as Mean±Standard Error of the Mean (SEM) and analyzed using ANOVA (analysis of variance) followed by Turkey's *post hoc* Test to determine means with statistical differences. Survival curve analysis was performed using Log-rank (Mantel-cox) in GraphPad Prism version 8.0.2 for Windows. The decision rule of $p<0.05$ for significance was adopted for all other means, but $p<0.006$ was used for Kaplan-Mayer curves (Bonferroni-corrected threshold for 9 multiple comparisons).

RESULTS

Seven days LD_{50} determination: Exposure of *Drosophila melanogaster* to DTG for 7 days showed 100% mortality at concentrations 40, 45 and 50 mg, respectively with corresponding 95% survival in the unexposed group. The calculated LD_{50} = 16.53 mg/10 g fly food (Fig. 1).

Twenty eight days survival rate of DTG-exposed flies: *Drosophila melanogaster* exposed to DTG by the feeding method exhibited a significant ($p<0.05$) increase in mortality rate compared to the unexposed groups. The reduction in survival proportion was directly proportional to antiretroviral concentrations (Fig. 2a-b).

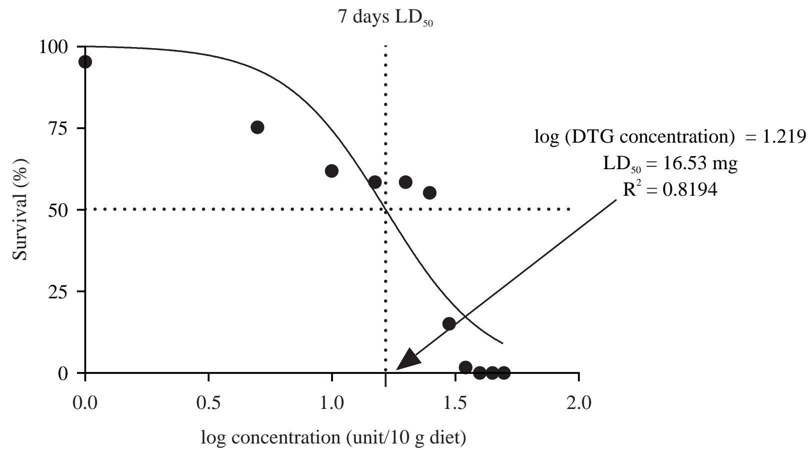

Fig. 1: Mean Lethal Dose (LD_{50}) of dolutegravir (DTG) in *Drosophila melanogaster*

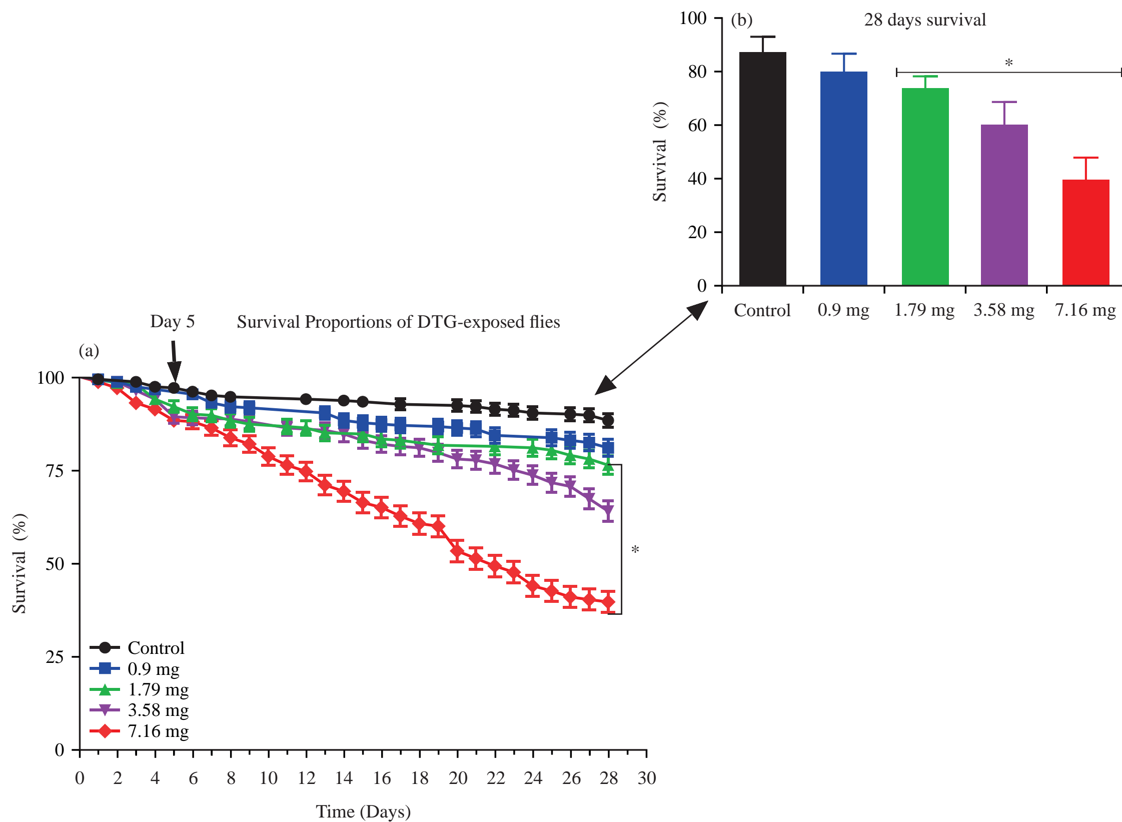

Fig. 2(a-b): DTG decreased the survival rate of *Drosophila melanogaster* dose-dependently after 28 days of exposure, (a) Survival (%) curve proportions analysis and (b) Survival (%) chart of 60 flies (both gender) after 28 days exposure of *D. melanogaster* to varying concentrations (x-axis) of DTG

Data were presented as Mean±SEM of three independent biological replicates and *Significantly ($p<0.05$) lower compared to control

Five days mortality, negative geotaxis and fly emergence: The result of 5 days fly exposure to DTG exhibited a non-significant ($p = 0.20$) increase in mortality rate. The DTG exposure, however, revealed a significant ($p = 0.01$) reduction in climbing performance at 7.16 mg/10 g fly food and a non-significant ($p = 0.07$) difference in fly emergence compared to the unexposed groups (Fig. 3a-c).

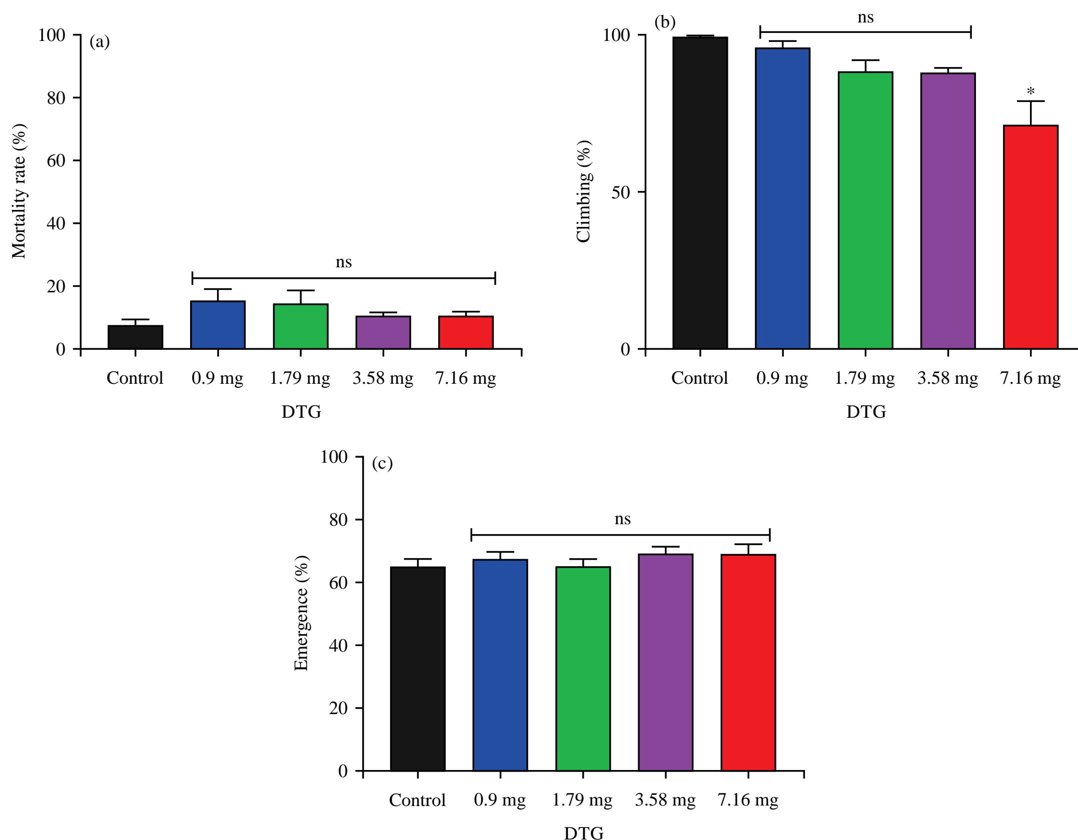

Fig. 3(a-c): Effects of DTG on (a) 5 days mortality rate, (b) Negative geotaxis (climbing ability) and (c) 14th day fly emergence rate in *D. melanogaster* after exposure for 5 days

Data were presented as Mean±SEM of three independent biological replicates, *Significantly ($p<0.05$) lower compared to the control group and ns: Non-significant ($p>0.05$) difference compared to unexposed flies

Changes in biochemical parameters of DTG-exposed flies: Following a 5 days exposure to DTG, the levels of T-SH significantly ($p<0.05$) reduced at all DTG experimental concentrations (0.9-7.16 mg/10 g fly food). There was also a significant ($p<0.05$) reduction in the activities of GST, SOD and CAT with a corresponding significant ($p<0.05$) increase in MDA level at 1.79-7.16 mg/10 g fly food (Fig. 4a-f).

***In silico* modeling of molecular interactions:** The result of *in silico* molecular interactions showed that the binding affinities of DTG for GST amino acids were significantly ($p<0.05$) higher than those of the inhibitor and substrate but significantly ($p<0.05$) lower for AChE compared to both the substrate and inhibitor (Fig. 5a-b).

2D and 3D analysis of ligand-protein interactions: The DTG occupied similar active sites as glutathione (substrate) and NBDHEX (inhibitor) at the GST binding pocket (Fig. 6a-c) through some amino acids such as, ARG A: 145, TYR A: 208, SER A:110 and GLN A:109. Similarly, Glutathione and NBDHEX interacted with another common amino acid TYR A:54 which is also seen in the DTG binding pocket. Concerning AChE (Fig. 7a-c), the test drug, galantamine (inhibitor) and acetylcholine (substrate) occupied the same amino acid residues such as TRP A: 83 and HIS A: 480. The DTG also interacted with another amino acid PHE A: 330 through a pi-alkyl bond. In addition, the AChE inhibitor (galantamine) and DTG interacted with other amino acid residues: TRY A: 71 and TRY A:370.

DISCUSSION

A dolutegravir serum concentration of 6.06 mg L^{-1} after oral exposure in humans resulted in severe neurological side effects such as fatigue, dizziness, restlessness and sleeplessness, which required treatment discontinuation[10]. *In vitro* studies using human culture cells suggested further that DTG toxicities might be associated with oxidative

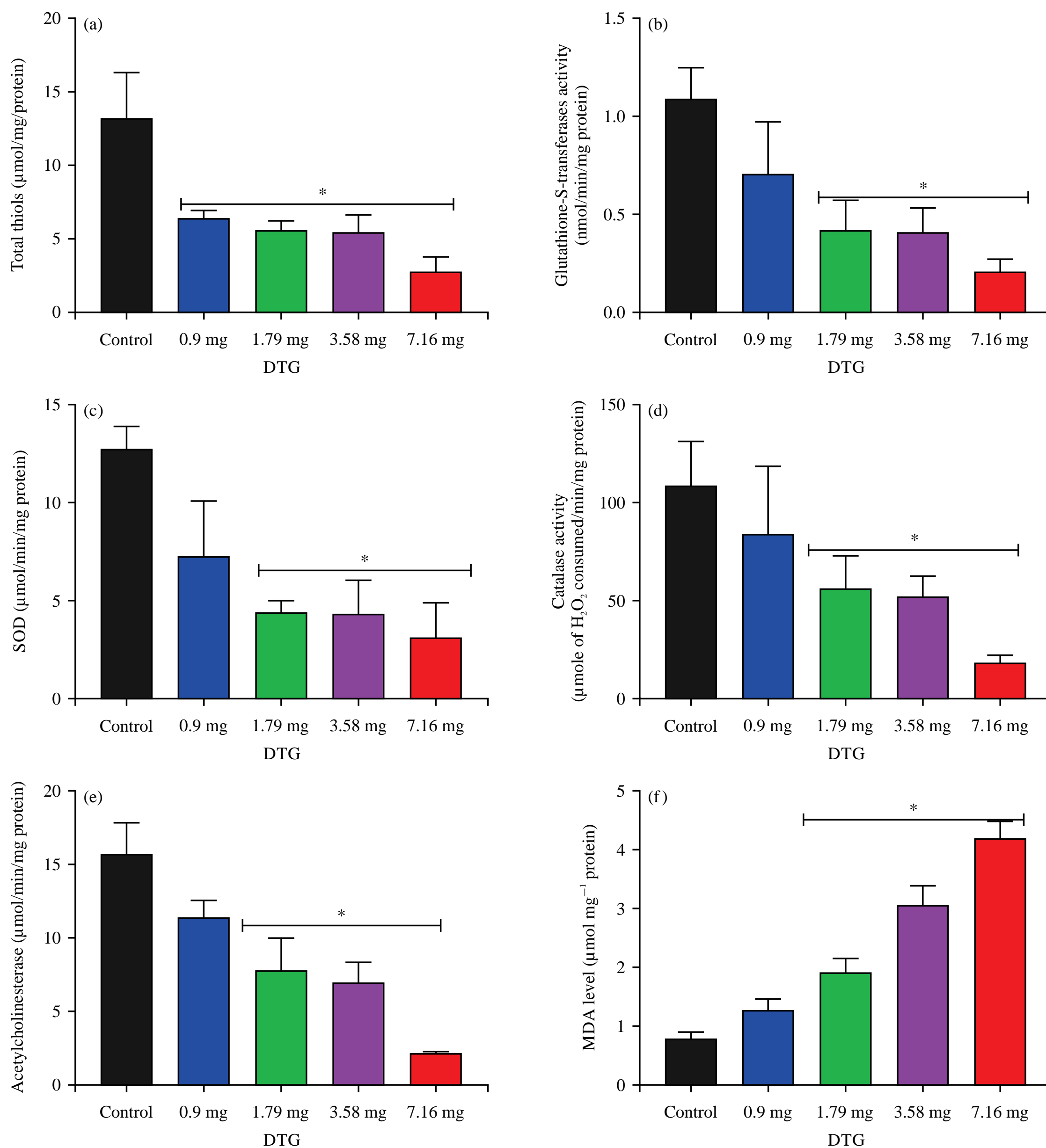

Fig. 4(a-f): Changes in biochemical parameters, (a) T-SH, (b) GST, (c) SOD, (d) CAT, (e) AChE and (f) MDA, after 5 days exposure of *D. melanogaster* to DTG

Data were presented as Mean±SEM of three independent biological replicates and *Significant ($p<0.05$) statistical difference compared to unexposed flies

stress[3,5,6] leading to neuronal inflammation[10]. The current study demonstrated that the Harwich strain of *Drosophila melanogaster* is prone to DTG-induced toxicities after oral exposure. The DTG significantly decreased 28 days survival and climbing ability and induced changes in the biochemical oxidative stress endpoints tested. The results of the lethal median dose (LD_{50}) (Fig. 1) together with the 28 days survival (Fig. 2a-b) in this study revealed that DTG was toxic to the flies. Previous studies have shown that the antiretroviral drug DTG disrupts mitochondrial functions in human microglial cell cultures[5] and induces oxidative stress toxicity, which has been observed *in vitro* as human erythrocyte death[36]. Therefore, the DTG-induced mitochondrial function deficits resulted in decreased cellular respiration, amplified inflammation signals and the production of free radicals[5,36]. For example, at a concentration of 4300 nM, DTG significantly changed the mitochondrial redox balance, ATP production and the metabolism of epithelial cells *in vitro* after 24 hrs exposure[5].

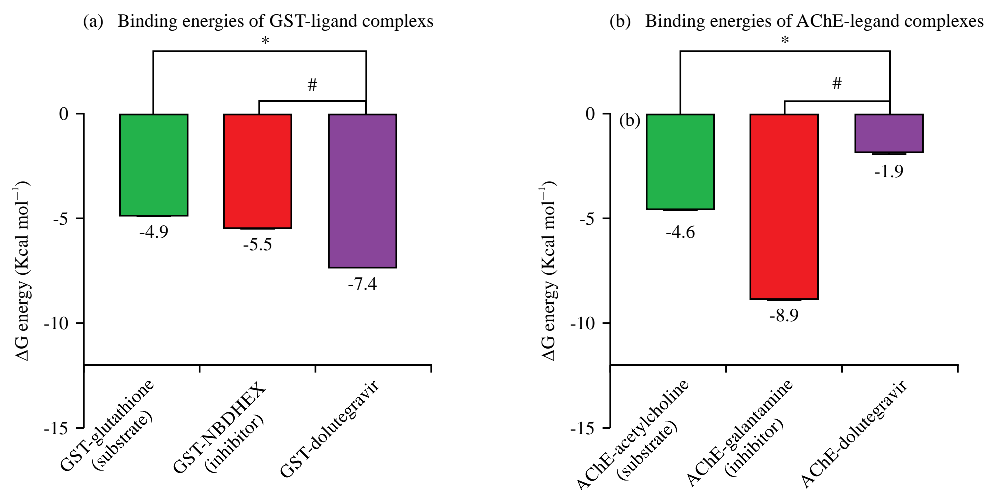

Fig. 5(a-b): Binding affinities of Drosophila enzyme-ligand complexes, (a) GST-DTG, GST-NBDHEX, GST-GSH and (b) AChE-DTG, AChE-galantamine, AChE-acetylcholine

*DTG-enzyme affinity is significantly (p<0.05) different compared to enzyme-substrate complex, #DTG-enzyme affinity is significantly (p<0.05) different compared to enzyme-inhibitor complex, GST: Glutathione-s-transferase, AChE: Acetylcholinesterase and NBDHEX: 6-[(7-Nitro-2,1,3-benzoxadiazol-4-yl) sulfanyl]hexan-1-ol

Thus, the significant (p<0.05) 28 days mortality increase among the exposed flies (Fig. 2a-b) in the current investigation could also be attributed to an antioxidant system failure orchestrated by DTG-induced toxicity. The DTG-treated groups showed non-significant (p>0.05) effects on mortality and fertility after 5 days exposure, but significantly reduced negative geotactic motion at the highest concentration (7.16 mg/10 g fly food) (Fig. 3a-c). This result could indicate that DTG exhibited delayed toxicity in *D. melanogaster* as a significant increase in mortality rate was detected in the 28 days survival assay.

Acetylcholine (ACh) is a parasympathetic neurotransmitter that mediates transient neurochemical control of muscle and gland cells and is rapidly metabolized by the AChE enzyme to choline and acetate[37]. Thus, a significant reduction in AChE activity favours neuronal ACh accumulation leading to sustained action potentials at the neuromuscular synapse resulting in muscle paralysis[37]. The role of the cholinergic system in the pathogenesis of neurodegenerative illnesses particularly in the elderly has been elucidated[38]. Although neurodegeneration due to cholinergic deficits has been replicated in vertebrate models, the exact outcome in *D. melanogaster* is yet to be unequivocally established[29]. However, both a decrease and increase in AChE could affect the flies adversely[29]. The significantly (p<0.05) decreased AChE activity (Fig. 4e) in this study is consistent with previous observations in *D. melanogaster*[29] and fish[39] where impaired locomotion was reported upon inhibition of AChE activity. Thus, the inhibition of AChE by DTG might have resulted in increased acetylcholine at the flies' synapses which was observed as a sluggish climbing ability in the DTG-exposed flies. The non-toxic effects of DTG on the reproductive function of exposed flies (Fig. 3c) despite a significant (p<0.05) AChE inhibition (Fig. 4e) is inconsistent with the result of Urra *et al.*[40], who reported improved fertility in rats after AChE inhibition with Huperzine-A. Huperzine-A is a plant antioxidant from *Huperzia serrata*[41], therefore, its fertility-enhancing activity could be driven by the antioxidant property of the compound as reported by Agarwal *et al.*[42] and not an AChE inhibitory effect. This result indicated that DTG induced no noticeable reproductive toxicity against fruit flies, unlike lamivudine and tenofovir disoproxil fumarate, which significantly (p<0.05) impaired both AChE activity and reproductive capacity after oral exposure in the study of Iorjiim *et al.*[43].

Further investigations were carried out to understand the contribution of DTG to the induction of oxidative stress in *D. melanogaster*. The DTG altered all tested antioxidant parameters (T-SH, GST, SOD and CAT) with a corresponding increase in MDA content (Fig. 4a-d and f), depicting a state of increased oxidative stress status in *D. melanogaster*. The MDA is a genotoxic product of ROS or enzyme-induced lipid peroxidation used as an indirect technique to determine the amount of circulating free radicals[44]. The increased MDA result in the current investigation agrees with that of Adaramoye *et al.*[45], who reported a significant increase in MDA levels after four weeks of exposure to nevirapine or tenofovir in Wistar rats[46]. Similarly, in the previous investigation[43], lamivudine or tenofovir disoproxil fumarate significantly impaired antioxidant capacity in fruit flies.

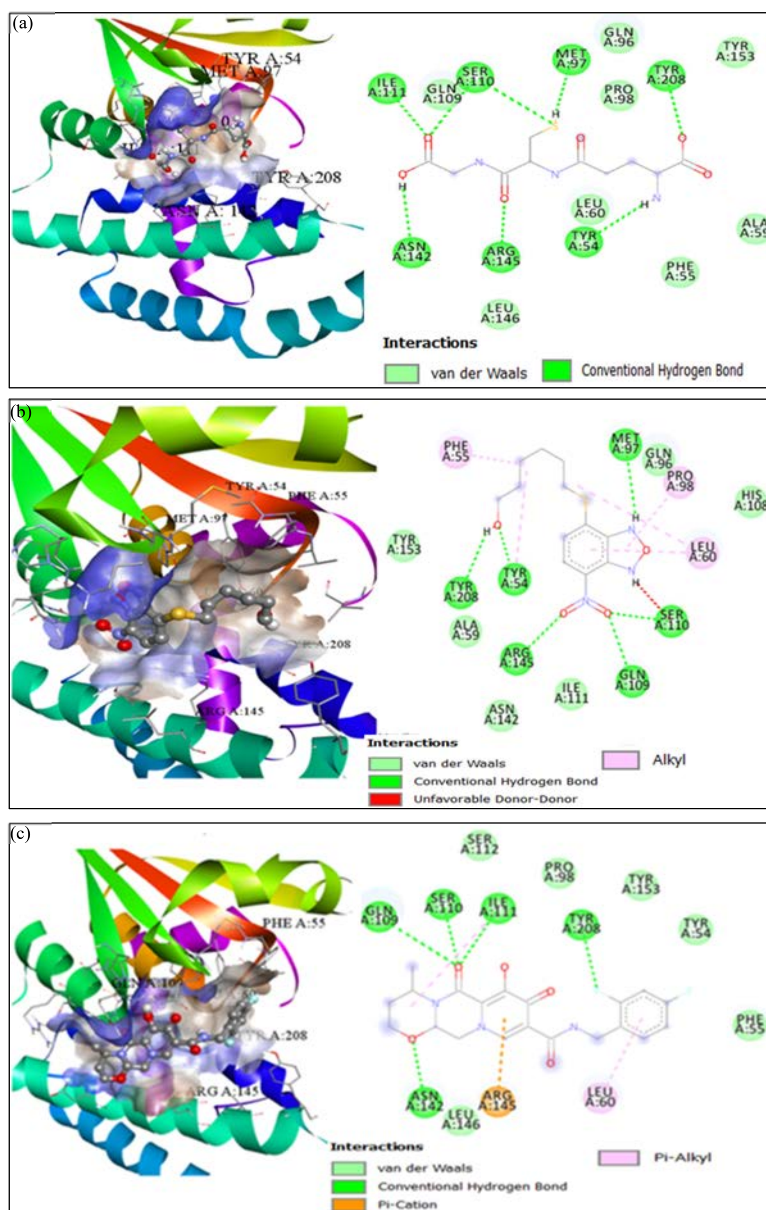

Fig. 6(a-c): Left (3D) and right (2D) presentation of the molecular interactions between the amino-acid of GST active-site gorge (grey surface) and (a) GSH (GST substrate), (b) NBDHEX (GST standard antagonist) and (c) Dolutegravir (DTG) (sticks)

The altered antioxidant biomarkers in the current study (T–SH, GST, SOD and CAT) (Fig. 4a-d) are an integral part of the Drosophila antioxidant system, which protects the organism against the harmful effects of ROS[47]. Thiols, e.g., glutathione (GSH) and homocysteine (HcySH) donate their sulfhydryl group (-SH) to free radicals during conjugation reactions and are generally susceptible targets for free radical attack[47]. As observed in this study among the DTG-exposed groups, a significant reduction in T-SH concentrations leaves the flies unprotected against oxidative damage. Glutathione-S-transferases (GSTs) on the other hand play a catalytic role during GSH conjugation reaction with xenobiotics and lipid peroxidation[48]. Thus, concurrent inhibition of GST activity and a decreased T-SH content as observed in this study might have compromised the antioxidant capacity of the flies. The CAT and SOD, like GSTs, are the first enzymes that protect living cells against free radical damage[49]. The SOD first catalyzes the dismutation of two superoxide anions to hydrogen peroxide and oxygen. The CAT then breaks down the two hydrogen peroxide molecules into one oxygen molecule and two water

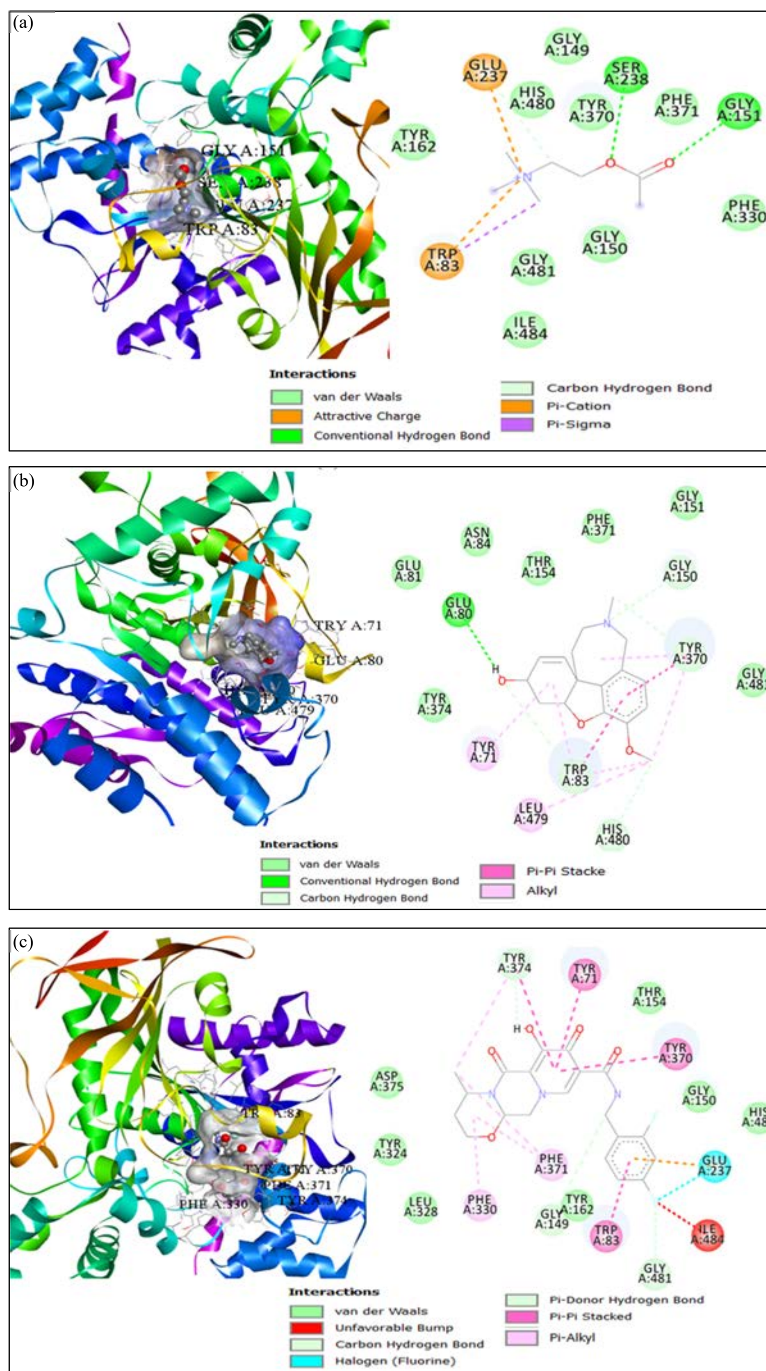

Fig. 7(a-c): Left (3D) and right (2D) presentation of the molecular interactions between the amino-acid of AChE active-site gorge (grey surface) and (a) ACh (substrate), (b) Galantamine (standard antagonist) and (c) Dolutegravir (DTG) (sticks)

molecules[49]. The significant decrease in the activities of CAT and SOD enzymes in the current study is consistent with previous assessments of some antiretroviral toxicity in non-target animal models. For example, Adaramoye *et al.*[45] reported a significant reduction in SOD and CAT activities in rat kidneys after tenofovir exposure[45]. Also, Oyeyipo *et al.*[50] found a significant reduction of these enzymes in rat's testicles exposed to EFV-containing HAART[50]. Similarly, lamivudine or tenofovir significantly decreased CAT and SOD activities in *D. melanogaster*[43].

The likely mechanism of DTG-induced toxicity in *Drosophila melanogaster* was investigated using molecular docking analysis of the test drug against the flies' GST and AChE. These enzymes were chosen because of their susceptibility after 5 days of exposure to DTG and the availability of their crystallographic (3D) structures in the protein data bank. The biochemical and behavioural effects of DTG-exposed flies were in agreement with the *in silico* analysis against AChE and GST. The DTG interacted with some key catalytic active amino acid residues such as TYR A: 208 and ARG A:145 on the GST binding site[51] similar to the substrate (glutathione) and standard inhibitor (NBDHEX) (Fig. 6a-c). The interaction of DTG with TYR A:208 was through an H-bond interaction, which could form a stable ligand-enzyme complex[34]. This observation in the current study agrees with the previous findings of Iorjiim *et al.*[43] and Johnson *et al.*[34] where *in silico* ligands and Drosophila GST interaction via the amino acid TYR A: 208 reduced the activity of the enzyme. Thus, the propensity of DTG to occupy the GST catalytic site with significantly ($p<0.05$) stronger affinity compared to glutathione and standard inhibitor (Fig. 5a-b) suggested a probable contribution of a competitive antagonism in the toxicity of this drug in *D. melanogaster*.

Again, it was observed that DTG interacted with known catalytic sites of AChE such as TRP A: 83, TYR 71, TYR 370[52] and PHE A:330[53] (Fig. 7a-c). An interaction between AChE with other antiretroviral drugs such as lamivudine and tenofovir disoproxil fumarate that revealed toxic potentials both *in silico* and *in vivo* has been reported by Iorjiim *et al.*[43]. However, in this study, the *in silico* result revealed a weak interaction between DTG and AChE (Fig. 5b and 7c), while the *in vivo* result suggested a cholinergic imbalance buttressed by significant ($p<0.05$) inhibition of AChE activity (Fig. 4e). The catalytic site of AChE is concealed close to the bottom of the deep and slim gorge[53], thus ligands could interfere with its catalytic activity via direct inhibition on its binding pocket or by stearic effect[54]. The DTG in our current study may have exerted a stearic hindrance effect together with the weak affinity on AChE catalytic site which manifested as a discrepancy between the *in silico* result and the *in vivo* effect on AChE. Overall, DTG has shown the propensity to compete with substrates for the active amino acid residues of *Drosophila melanogaster* GST and AChE. This result suggests a possible competitive antagonism of these enzymes as an off-target impact of dolutegravir that might partly account for its toxicity in the non-target organism *D. melanogaster*.

CONCLUSION

Dolutegravir-induced oxidative stress toxicity was replicated in a non-target specie *Drosophila melanogaster*, which significantly decreased the survival, climbing ability and cellular antioxidant defense capacity. *In silico* molecular interaction suggested the involvement of competitive antagonism of GST and AChE enzymes as likely mechanisms that partly accounts for DTG-induced toxicity in the flies. This study demonstrated that environmental contamination with the finished product of DTG could be hazardous to non-target species in an ecosystem.

SIGNIFICANCE STATEMENT

This study has shown that DTG-induced oxidative stress is reproducible in *D. melanogaster*. The study also demonstrated the toxic potentials of DTG against non-target organisms in the circumstance of environmental contamination with the finished pharmaceutical product. Thus, the fruit fly could be a good model for researchers in rapid toxicological assessment of antiretrovirals and other drugs in ecotoxicological studies.

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REFERENCES

1. Bassi, P., W. Gashau, K. Olaf, A. Dodoo, P. Okonkwo and P. Kanki, 2017. Prevalence of adverse drug reactions among HIV/AIDS patients on haart in University of Maiduguri Teaching Hospital (Umth), Nigeria: A four-year retrospective study. BMJ Global Health, Vol. 2. 10.1136/bmjgh-2016-000260.103.
2. Daughton, C.G., 2002. Cradle-to-cradle stewardship of drugs for minimizing their environmental disposition while promoting human health. I. Rationale for and avenues toward a green pharmacy. Environ. Health Perspect., 111: 757-774.
3. Kandel, C. and S. Walmsley, 2015. Dolutegravir-A review of the pharmacology, efficacy, and safety in the treatment of HIV. Drug Des. Dev. Ther., 9: 3547-3555.
4. Das, S., H. Taha and A. Das, 2015. Clinical effectiveness of dolutegravir in the treatment of HIV/AIDS. Infect. Drug Resist., 8: 339-352.

5. George, J.W., J.E. Mattingly, N.J. Roland, C.M. Small, B.G. Lamberty, H.S. Fox and K.L. Stauch, 2021. Physiologically relevant concentrations of dolutegravir, emtricitabine, and efavirenz induce distinct metabolic alterations in HeLa epithelial and BV2 microglial cells. Front. Immunol., Vol. 12. 10.3389/fimmu.2021.639378.
6. Boxall, A.B.A., 2004. The environmental side effects of medication: How are human and veterinary medicines in soils and water bodies affecting human and environmental health? EMBO Rep., 5: 1110-1116.
7. Rutherford, G.W. and H. Horvath, 2016. Dolutegravir plus two nucleoside reverse transcriptase inhibitors versus efavirenz plus two nucleoside reverse transcriptase inhibitors as initial antiretroviral therapy for people with HIV: A systematic review. PLoS ONE, Vol. 11. 10.1371/journal.pone.0162775.
8. WHO, 2018. Updated Recommendations on First-Line and Second-Line Antiretroviral Regimens and Post-Exposure Prophylaxis and Recommendations on Early Infant Diagnosis of HIV. World Health Organization. Switzerland, Pages: 79.
9. Urama, B., N. Anonyuo, A. Ibeme, B. Owoicho and C. Obi *et al*., 2019. Effects of stakeholder's management and engagement on the success of fixed dose combination (FDC) tenofovir/lamivudine/dolutegravir (TLD) introduction and transition: Nigeria experience. Public Health Open Access, Vol. 3. 10.23880/phoa-16000142.
10. Parant, F., P. Miailhes, F. Brunel and M.C. Gagnieu, 2018. Dolutegravir-related neurological adverse events: A case report of successful management with therapeutic drug monitoring. Curr. Drug Saf., 13: 69-71.
11. Smith, R.L., R. de Boer, S. Brul, Y. Budovskaya and H. van der Spek, 2013. Premature and accelerated aging: HIV or HAART? Front. Genet., Vol. 3. 10.3389/fgene.2012.00328.
12. Saeidnia, S. and M. Abdollahi, 2013. Toxicological and pharmacological concerns on oxidative stress and related diseases. Toxicol. Appl. Pharmacol., 273: 442-455.
13. Bullon, P., H.N. Newman and M. Battino, 2014. Obesity, diabetes mellitus, atherosclerosis and chronic periodontitis: A shared pathology via oxidative stress and mitochondrial dysfunction? Periodontology, 64: 139-153.
14. Koskenkorva-Frank, T.S., G. Weiss, W.H. Koppenol and S. Burckhardt, 2013. The complex interplay of iron metabolism, reactive oxygen species, and reactive nitrogen species: Insights into the potential of various iron therapies to induce oxidative and nitrosative stress. Free Radical Biol. Med., 65: 1174-1194.
15. Phaniendra, A., D.B. Jestadi and L. Periyasamy, 2015. Free radicals: Properties, sources, targets, and their implication in various diseases. Indian J. Clin. Biochem., 30: 11-26.
16. Sagai, M. and V. Bocci, 2011. Mechanisms of action involved in ozone therapy: Is healing induced via a mild oxidative stress? Med. Gas Res., Vol. 1. 10.1186/2045-9912-1-29.
17. Circu, M.L. and T.Y. Aw, 2010. Reactive oxygen species, cellular redox systems, and apoptosis. Free Radical Biol. Med., 48: 749-762.
18. Rocha, J.B.T., 2013. Drosophila melanogaster as a promising model organism in toxicological studies. Arch. Basic Appl. Med., 1: 33-38.
19. Reiter, L.T., L. Potocki, S. Chien, M. Gribskov and E. Bier, 2001. A systematic analysis of human disease-associated gene sequences in *Drosophila melanogaster*. Genome Res., 11: 1114-1125.
20. Birben, E., U.M. Sahiner, C. Sackesen, S. Erzurum and O. Kalayci, 2012. Oxidative stress and antioxidant defense. World Allergy Organ. J., 5: 9-19.
21. Abolaji, A.O., J.P. Kamdem, T.H. Lugokenski, E.O. Farombi, D.O. Souza and É.L. da Silva Loreto, J.B.T. Rocha, 2015. Ovotoxicants 4-vinylcyclohexene 1,2-monoepoxide and 4-vinylcyclohexene diepoxide disrupt redox status and modify different electrophile sensitive target enzymes and genes in *Drosophila melanogaster*. Redox Biol., 5: 328-339.
22. Abolaji, A.O., J.P. Kamdem, T.H. Lugokenski, T.K. Nascimento and E.P. Waczuk *et al*., 2014. Involvement of oxidative stress in 4-vinylcyclohexene-induced toxicity in *Drosophila melanogaster*. Free Radical Biol. Med., 71: 99-108.
23. Pam, D., A.M. Etuh, O.I. Oyeniran and I.W. Mdekere, 2021. Toxicity of *Mangifera indica* aqueous stem bark extract evaluated in *Drosophila melanogaster* used as model organism. Ann. Pharm. Fr., 79: 539-546.
24. de Moraes Filho, A.V., C. de Jesus Silva Carvalho, C.J. Verçosa, M.W. Gonçalves and C. Rohde *et al*., 2017. *In vivo* genotoxicity evaluation of efavirenz (EFV) and tenofovir disoproxil fumarate (TDF) alone and in their clinical combinations in *Drosophila melanogaster*. Mutat. Res. Genet. Toxicol. Environ. Mutagen., 820: 31-38.
25. Iorjiim, W.M., S. Omale, G.D. Bagu, S.S. Gyang and E.T. Alemika, 2020. Reproductive and oxidative stress toxicity of dolutegravir-based combination antiretroviral therapy in *Drosophila melanogaster*. J. Adv. Med. Pharm. Sci., 22: 26-40.
26. Alves, M., A.A. de Marchi, K.M. Doretto, L.G. Robello and M.C. Pigatto *et al*., 2019. Searching for a long-acting injectable formulation for the antiretroviral dolutegravir. Br. J. Pharm., Vol. 4. 10.5920/bjpharm.568.
27. Nazir, A., I. Mukhopadhyay, D.K. Saxena and D.K. Chowdhuri, 2003. Evaluation of the no observed adverse effect level of solvent dimethyl sulfoxide in *Drosophila melanogaster*. Toxicol. Mech. Methods, 13: 147-152.
28. Adedara, I.A., A.O. Abolaji, J.B.T. Rocha and E.O. Farombi, 2016. Diphenyl diselenide protects against mortality, locomotor deficits and oxidative stress in *Drosophila melanogaster* model manganese-induced neurotoxicity. Neurochem. Res., 41: 1430-1438.
29. Abolaji, A.O., K.D. Fasae, C.E. Iwezor, M. Aschner and E.O. Farombi, 2020. Curcumin attenuates copper-induced oxidative stress and neurotoxicity in *Drosophila melanogaster*. Toxicol. Rep., 7: 261-268.
30. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.

31. Ellman, G.L., K.D. Courtney, V. Andres Jr. and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-91.
32. Isaac, R.E., U. Ekbote, D. Coates and A.D. Shirras, 1999. Insect angiotensin-converting enzyme: A processing enzyme with broad substrate specificity and a role in reproduction. Ann. N. Y. Acad. Sci., 897: 342-347.
33. Nagaoka, S., S. Kawasaki, H. Kawasaki and K. Kamei, 2017. The angiotensin converting enzyme (ACE) inhibitor, captopril disrupts the motility activation of sperm from the silkworm, *Bombyx mori*. J. Insect Physiol., 103: 18-28.
34. Johnson, T.O., A.O. Abolaji, S. Omale, I.Y. Longdet and R.J. Kutshik *et al*., 2021. Benzo[a]pyrene and benzo[a]pyrene-7,8-dihydrodiol-9,10-epoxide induced locomotor and reproductive senescence and altered biochemical parameters of oxidative damage in Canton-S *Drosophila melanogaster*. Toxicol. Rep., 8: 571-580.
35. Duru, C.E., I.A. Duru and A.E. Adegboyega, 2021. *In silico* identification of compounds from *Nigella sativa* seed oil as potential inhibitors of SARS-CoV-2 targets. Bull. Natl. Res. Centre, Vol. 45. 10.1186/s42269-021-00517-x.
36. Al Mamun, B.A., E. Signoretto, R. Bissinger and F. Lang, 2016. Enhanced eryptosis following exposure to dolutegravir. Cell. Physiol. Biochem., 39: 639-650.
37. Cruz, P.M.R., J. Cossins, D. Beeson and A. Vincent, 2020. The neuromuscular junction in health and disease: Molecular mechanisms governing synaptic formation and homeostasis. Front. Mol. Neurosci., Vol. 13. 10.3389/fnmol.2020.610964.
38. Craig, L.A., N.S. Hong and R.J. McDonald, 2011. Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. Neurosci. Biobehav. Rev., 35: 1397-1409.
39. Kavitha, P. and J.V. Rao, 2008. Toxic effects of chlorpyrifos on antioxidant enzymes and target enzyme acetylcholinesterase interaction in mosquito fish, *Gambusia affinis*. Environ. Toxicol. Pharmacol., 26: 192-198.
40. Urra, J., J. Blohberger, M. Tiszavari, A. Mayerhofer and H.E. Lara, 2016. *In vivo* blockade of acetylcholinesterase increases intraovarian acetylcholine and enhances follicular development and fertility in the rat. Sci. Rep., Vol. 6. 10.1038/srep30129.
41. Simunkova, M., S.H. Alwasel, I.M. Alhazza, K. Jomova, V. Kollar, M. Rusko and M. Valko, 2019. Management of oxidative stress and other pathologies in Alzheimer’s disease. Arch. Toxicol., 93: 2491-2513.
42. Agarwal, A., A. Aponte-Mellado, B.J. Premkumar, A. Shaman and S. Gupta, 2012. The effects of oxidative stress on female reproduction: A review. Reprod. Biol. Endocrinol., Vol. 10. 10.1186/1477-7827-10-49.
43. Iorjiim, W.M., S. Omale, M.A. Etuh, A. Ubani, E.T. Alemika and S.S. Gyang, 2022. Senescence and oxidative stress toxicities induced by lamivudine and tenofovir in *Drosophila melanogaster*. Ann. Pharm. Fr., 80: 864-875.
44. JareÑo, E.J., J. Romá, B. Romero, N. MarÍn and M. Muriach *et al*., 2002. Serum malondialdehyde correlates with therapeutic efficiency of high activity antiretroviral therapies (HAART) in HIV-1 infected children. Free Radical Res., 36: 341-344.
45. Adaramoye, O.A., O.M. Adewumi, O.A. Adesanoye, O.O. Faokunla and E.O. Farombi, 2012. Effect of tenofovir, an antiretroviral drug, on hepatic and renal functional indices of Wistar rats: Protective role of vitamin E. J. Basic Clin. Physiol. Pharmacol., 23: 69-75.
46. Adaramoye, O.A., O.A. Adesanoye, O.M. Adewumi and O. Akanni, 2012. Studies on the toxicological effect of nevirapine, an antiretroviral drug, on the liver, kidney and testis of male Wistar rats. Hum. Exp. Toxicol., 31: 676-685.
47. Prakash, M., M.S. Shetty, P. Tilak and N. Anwar, 2009. Total thiols: Biomedical importance and their alteration in various disorder. Online J. Health Allied Sci., Vol. 8.
48. Cummins, I., D.P. Dixon, S. Freitag-Pohl, M. Skipsey and R. Edwards, 2011. Multiple roles for plant glutathione transferases in xenobiotic detoxification. Drug Metab. Rev., 43: 266-280.
49. Ighodaro, O.M. and O.A. Akinloye, 2018. First line defence antioxidants-superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX): Their fundamental role in the entire antioxidant defence grid. Alexandria J. Med., 54: 287-293.
50. Oyeyipo, I.P., B.T. Skosana, F.P. Everson, H. Strijdom and S.S. du Plessis, 2018. Highly active antiretroviral therapy alters sperm parameters and testicular antioxidant status in diet-induced obese rats. Toxicol. Res., 34: 41-48.
51. Agianian, B., P.A. Tucker, A. Schouten, K. Leonard, B. Bullard and P. Gros, 2003. Structure of a *Drosophila* sigma class glutathione S-transferase reveals a novel active site topography suited for lipid peroxidation products. J. Mol. Biol., 326: 151-165.
52. Harel, M., G. Kryger, T.L. Rosenberry, W.D. Mallender and T. Lewis *et al*., 2000. Three-dimensional structures of *Drosophila melanogaster* acetylcholinesterase and of its complexes with two potent inhibitors. Protein Sci., 9: 1063-1072.
53. Silman, I. and J.L. Sussman, 2008. Acetylcholinesterase: How is structure related to function? Chem. Biol. Interact., 175: 3-10.
54. Colovic, M.B., D.Z. Krstic, T.D. Lazarevic-Pasti, A.M. Bondzic and V.M. Vasic, 2013. Acetylcholinesterase inhibitors: Pharmacology and toxicology. Curr. Neuropharmacol., 11: 315-335.