*West African Journal of Pharmacy (2024) 35 (1) 102 - 114*

# Antioxidant and attenuative effect of a mixture of *Helianthus annus* and *Taraxacum officinale* against dolutegravir-induced distortions in *Drosophila melanogaster*

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**ABSTRACT:**

**Background:** The investigation into the medicinal properties of plant extracts has become increasingly prominent in pharmacological research. This research area emphasizes the interplay between pharmaceutical drugs and medicinal plants, aiming to address and alleviate the side effects induced by conventional medications.

**Objective:** This study assessed the antioxidant and mitigatory effects of a *Helianthus annuus* and *Taraxacum officinale* herbal extract mixture on *Drosophila melanogaster* exposed to dolutegravir.

**Methods:** *Drosophila melanogaster*, aged three to five days, were exposed for seven days to different concentrations of dolutegravir and the herbal extract to determine the median lethal concentration. The antioxidant activity of the extract using stable 2,2-diphenyl-1-picrylhydrazyl was also evaluated. A separate cohort of fruit flies was divided into eight treatment groups, exposed to various concentrations of the plant extract (1-1000 mg/ 5g diet), a negative control, and silymarin for five days to assess the impact on catalase, glutathione-S-transferase, total thiol, total protein, and acetylcholinesterase. All groups, except the negative control, had dolutegravir incorporated into their diet, and one group was exposed to dolutegravir only.

**Results:** The results demonstrated a dose-dependent increase in catalase, particularly at 75-100 mg/5 g diet, compared to the control. Glutathione-S-transferase was significantly higher at the lowest extract and dolutegravir dose compared to groups exposed to dolutegravir alone. Protein levels were significantly lower compared to flies administered dolutegravir, indicating potential mitigative effects. The herbal mixture exhibited antioxidant activity surpassing that of silymarin.

**Conclusion:** In conclusion, the *Helianthus annuus-Taraxacum officinale* herbal mixture demonstrated ameliorative effects against dolutegravir-induced toxicity in *Drosophila melanogaster.*

**Keywords:** Antioxidant, Drosophila, Dolutegravir, *Helianthus Annus*, Mitigation, *Taraxacum officinale.*

# Effet antioxydant et atténuant d'un mélange de *Helianthus annus* et de *Taraxacum officinale* contre les distorsions induites par le dolutégravir chez *Drosophila melanogaster*

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**RÉSUMÉ**

**Objectif:** Le but de cette étude était d'évaluer les effets de l'extrait de thé *Helianthus annus* et *Taraxacum officinale* contre les altérations induites par le dolutégravir sur certains paramètres biochimiques et le taux de survie de *Drosophila melanogaster.*

**Méthodes:** Des différents groupes de *Drosophila melanogaster* ont reçu des concentrations variables (5 à 1 000 mg/5 g d'alimentation) de l'extrait de plante et du dolutégravir pendant 7 jours afin de déterminer la concentration de la dose létale. Un test de 28 jours a également été réalisé pour déterminer le taux de survie des mouches des fruits après l'administration conjointe de l'extrait végétal et du dolutégravir. Les drosophiles ont été divisées en six groupes de traitement, chacun recevant des concentrations différentes (allant de 25 à 1 000 mg/5 g d'alimentation) de l'extrait de plante, du dolutégravir et des contrôles pendant 5 jours. En outre, nous avons déterminé l'activité antioxydante de la silymarine et de l'extrait de *Helianthus annus/Taraxacum officinale* à l'aide de la méthode stable au 1,1-diphényl-2-picrylhydrazyl.

**Résultats:** Les groupes tests ont montré une augmentation dose-dépendante (75-100 mg/5 g d'alimentation) des taux de catalase, par rapport au groupe témoin ; tandis que la glutathion-S-Transférase était significativement ($p < 0,05$) plus élevée à la dose la plus faible d'extrait et de dolutégravir, par rapport aux mouches administrées avec du dolutégravir seul. Les niveaux de protéines observés étaient significativement ($p<0,05$) plus bas par rapport aux mouches ayant reçu du dolutégravir. Les résultats ont montré une diminution de la survie de Drosophila melanogaster exposée au dolutégravir et à l'extrait de manière dose-dépendante ($p < 0,05$) par rapport au groupe témoin. En outre, l'activité antioxydante de l'extrait de tisane a dépassé celle de la silymarine, un antioxydant bien connu.

**Conclusion:** L'extrait de thé du mélange de plantes *Helianthus annus-Taraxacum officinale* a démontré un effet protecteur contre la toxicité induite par le Dolutegravir chez la *Drosophila melanogaster.*

**Mots clés:** Antioxydant, Dolutégravir, *Drosophile, Helianthus Annus,* Protectrice et *Taraxacum officinale.*

## INTRODUCTION

In recent years, the field of pharmacological research has witnessed an unprecedented surge in the exploration of natural compounds and botanical extracts for their potential therapeutic applications. Among the various avenues of investigation, the interplay between pharmaceutical drugs and natural compounds has gained prominence, as researchers seek to unravel novel ways to mitigate drug-induced side effects.

Sunflower seeds, derived from *Helianthus annus*, offer a plethora of nutrients, minerals, and vitamins that are believed to bestow numerous health benefits, encompassing antioxidative, antibacterial, antidiabetic, antihypertensive, anti-inflammatory, and vascular healing properties.[1] *Taraxacum officinale* is regarded as a benign herb with choleretic, diuretic, anti-rheumatic, and anti-inflammatory properties that can mitigate various health conditions such as obesity, cancer, and cardiovascular disease.[2] Harnessing the potential synergy between these botanical extracts, the current study aimed to investigate their combined effects on Dolutegravir-induced alterations in *Drosophila melanogaster.*

Dolutegravir, a potent antiretroviral medication commonly used in the management of HIV infections, has demonstrated remarkable efficacy in suppressing viral replication. However, like many pharmaceutical agents, it is not exempt from the potential emergence of side effects. Oxidative stress, characterized by an imbalance between reactive oxygen species (ROS) production and the body's ability to neutralize them, has been implicated in various drug-induced toxicities.[3] In this context, the antioxidant properties of natural compounds have gained attention for their potential to mitigate oxidative stress-related damage.

*Drosophila melanogaster*, otherwise known as the fruit fly, with its well-characterized genetic background and relatively short lifespan, serves as an excellent model organism for studying drug-induced toxicity and assessing the therapeutic potential of various compounds. This exploration is not only confined to the realm of pharmaceutical research but also extends into the broader context of integrative medicine, emphasizing the potential of natural compounds to complement conventional drug therapies. The findings of this study may contribute valuable insights into the development of adjunctive therapies aimed at enhancing the safety and efficacy of pharmaceutical interventions.

### Materials

Beakers, vials, vial rack, brush, weighing balance, freezer/refrigerator, flask (for fly diet), stirring rod, Whatman filter paper, cotton wool, marker, tissue paper, distilled water, Silymarin (150 mg/capsule), Dolutegravir (50 mg/tablet).

## METHODS

### Sources of plant materials

Fresh whole plants of *Helianthus annus* and *Taraxacum officinale* were collected from Toro in Bauchi State, Nigeria and identified by Mr. Joseph J. Azila of the Federal College of Forestry, Jos, Nigeria. The seeds of *Helianthus annus* and leaves of *Taraxacum officinale* were separately dried and powdered using a mortar and pestle at the Technology Incubation Centre, National Board for Technology Incubation, Bukuru, Plateau state, Nigeria. The two powdered plant parts were then mixed together in a ratio of 1:1 (100 g each), the same proportion used in the commercially available product sold and consumed locally in Jos, Plateau state, Nigeria.

### Extraction and preparation

The process of extracting compounds from the plant material involved aqueous (water-based solution) maceration, with the extraction performed at 40$^{o}$C.[4] In this method, 200 g of the herbal tea was combined with 2 L of hot water in a container. The mixture was left to cool and placed in a refrigerator for three days. The mixture was taken out of the refrigerator three times a day within the three days and stirred to ensure complete extraction.[5] Afterwards, the sample was filtered using a Whatman No.1 filter paper and then a muslin cloth. The resulting filtrate was concentrated to dryness utilizing a water bath set at 30°C. The concentrated extract was then placed in a drying cabinet to remove any remaining moisture. The extract was weighed and it was carefully stored in a tightly sealed container. To maintain its quality, the extract was stored in a refrigerator until it required for use.

### *Drosophila melanogaster* stock and media

The Harwich strain of *Drosophila melanogaster* was cultured at the Africa Centre of Excellence in Phytomedicine Research and Development (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 natural night and day cycle.

**Free radical scavenging ability (DPPH Assay)**

The free radical scavenging activity of the herbal tea extract was determined with different solvents: Viz aqueous (100 %), methanol (80 % v/v), n-hexane (100 %) were investigated using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method.[6] Various concentrations of the extract and standards were prepared (500, 250, 125, 62.50, 31.25, 15.62, 7.8125, 3.91, 1.95, and 0.98 µg/mL). All the solutions were prepared with methanol as solvent. 2 mL of each prepared concentration was mixed with 4 mL of 50 µM DPPH solution in methanol and the experiment was done in triplicate. The mixture was incubated in the dark for 30 minutes, after which the absorbances were read at 515 nm against a blank using a UV-VIS spectrophotometer (Shimadzu, UV-1620PC, Japan).

**Acute toxicity determination ($LC_{50}$)**

**$LC_{50}$ of the mixture of *Helianthus annus* and *Taraxacum officinale* extract in *Drosophila melanogaster.***

The acute toxicity of the mixture of *Helianthus annus* and *Taraxacum officinale* was done based on a method previously described[7], with some modifications. Briefly, the fruit flies were divided into eight groups each receiving various concentrations of the extract (i.e., 1 mg, 5 mg, 10 mg, 50 mg, 100 mg, 250 mg, 500 mg and 1000 mg) in replicates of three vials per group, with each vial containing sixty (60), 1-3-day old flies of both sexes. The different concentrations of the extract were incorporated into 5 g each of fly diet and then transferred into sterilized empty vials. One group remained untreated, receiving only the diet as the negative control, while another group was exposed to silymarin, a standard antioxidant. The fruit flies were then transferred into the vials containing the mixture of diet and extract. Daily mortality was recorded for seven (7) days and the data obtained used to plot a graph of percentage mortality versus log concentration. The lethal concentration 50 ($LC_{50}$) was then extrapolated from the graph.

**$LC_{50}$ of dolutegravir in *Drosophila melanogaster***

The fruit flies were divided into eight groups in replicates of three vials per group with each vial containing 60 flies of both sexes, aged 3-5 days. Different concentrations of dolutegravir (5, 10, 25, 50, 100, 250, 500 and 1000 mg) were incorporated into 5 g of each of the fruit flies' diet and then transferred into sterilized empty vials. One group remained untreated, receiving only the diet as the negative control, while another group was exposed to silymarin, a standard antioxidant. The flies were then transferred into the vials containing the diet and test drug. Total mortality was recorded daily for seven days and the data obtained used to plot a graph of percentage mortality versus log concentration. The lethal concentration 50 ($LC_{50}$) was then extrapolated from the graph.

**Assessment of effect of co-administration of dolutegravir and plant extract**

Five (5) test groups of 60 flies each in 5 replicates were pre-exposed with four different concentrations of extract (25, 50, 75, and 100 mg respectively) per 5 g of fly diet for five days followed by dolutegravir (2.144 mg in 5 g of fly food) in each group for another 5 days. Flies in the positive control group were exposed to silymarin (125 mg in 5 g of fly food), while the negative control group was only fed the diet for a duration of 10 days in both treatments.

For the biochemical assays, flies that had been exposed to different concentrations of drugs (DTG and Silymarin) and extract as above were immobilized under ice before weighing, followed by homogenization in 0.1 M phosphate buffer solution (pH 7.4, ratio 1 mg: 10 µL). Briefly, homogenization involved placing the flies in vials and anaesthetizing them on ice before transferring to Eppendorf tubes for subsequent weighing. The fruit flies were then crushed inside the vials with a glass rod. A centrifuge (Eppendorf-Germany, model: AG, 5227 R) was set to revolve 4000 revolutions per min for 600 s 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), acetylcholinesterase (AChE), glutathione-S-transferase (GST) and catalase (CAT).

**Evaluation of biochemical parameters**

The method of Lowry and co[8] was adopted to determine total protein, while Ellman's method was adapted from Abolaji and colleagues[9] and used for total thiol assessment. Acetylcholinesterase (AChE) concentration was evaluated based on the method of Ellman,[10] while the catalase (CAT) level was determined according to standard methods.[11]

Protein quantification was performed using the Bradford method, as previously reported,[12] while the determination of the GST content of the sample was performed according to the method described by Moatamedi *et al*.[13]

Statistical Analysis: All data in this study were presented as Mean ± Standard Error of the Mean (SEM) and analyzed using One-Way ANOVA (analysis of variance) followed by

Tukey's post hoc Test to determine means with statistical differences. The decision rule of P<0.05 for significance was adopted for all means.

**RESULTS**

**Determination of antioxidant scavenging activity**

The 50 % DPPH radical scavenging activity ($LC_{50}$) of the extract and standard antioxidant, Silymarin are represented in Figures 1 and 2. $LC_{50}$ value of the extract was 113.4 µg/ml (Figure 1) and that of Silymarin was 121.4 µg/ml (Figure 2).

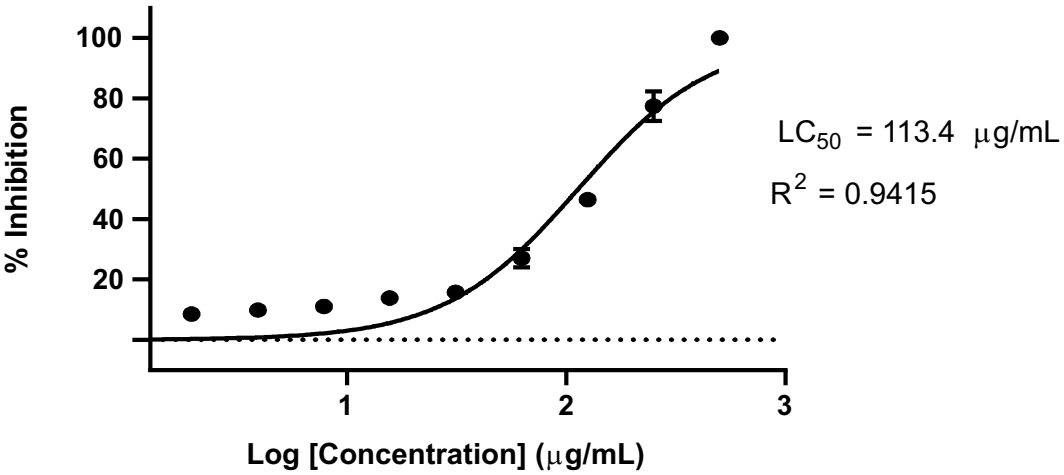


Fig. 1: Radical scavenging activity of the extract of *Helianthus annus* and *Taraxacum officinale.*

All experiments were performed in triplicate (n = 3). $LC_{50}$, Half maximum inhibitory concentration; $R^2$, Correlation coefficient.

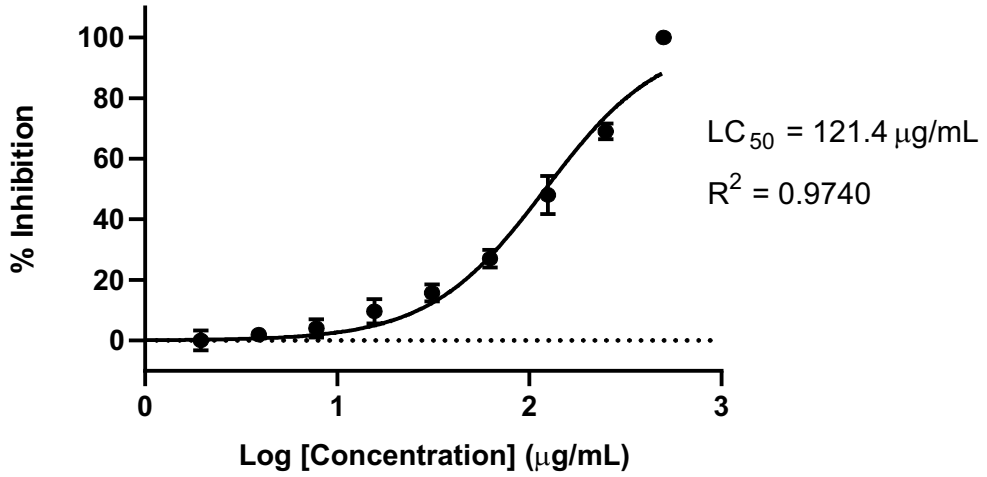


Fig. 2: Radical scavenging activity of Silymarin.

All experiments were performed in triplicate (n = 3). $LC_{50}$, Half maximum inhibitory concentration; $R^2$, Correlation coefficient.

**Determination of Acute Toxicity ($LC_{50}$)**

Lethal Concentration 50 ($LC_{50}$) of the toxicant, Dolutegravir, was determined to be 2.144 mg/5 g diet. The $LC_{50}$ of the extract, comprising *Helianthus annus* and *Taraxacum officinale* could not be determined even at the highest dose used (1000 mg/5 g diet). The fly diet became unstable and sticky above this dose making it difficult to proceed (Fig. 3 and Fig. 4).

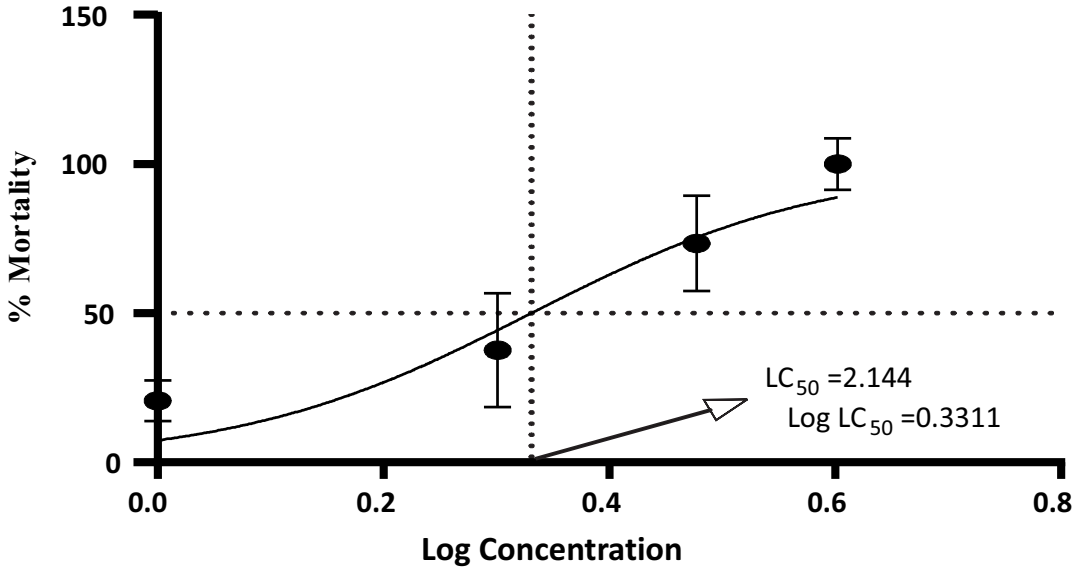


Fig. 3: Lethal Concentration 50 ($LC_{50}$) of Dolutegravir in *Drosophila melanogaster*

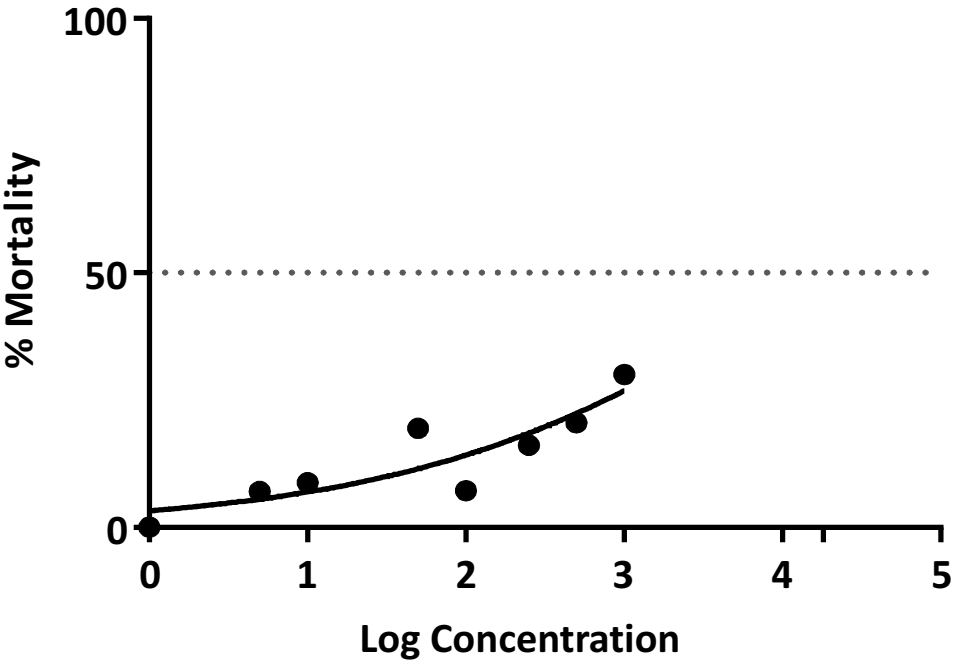


Fig 4: Lethal Concentration 50 ($LC_{50}$) ) of aqueous extract of *Helianthus annus* and *Taraxacum officinale* in *Drosophila melanogaster*

**Determination of Biochemical Parameters**

The catalase (CAT) level of *Drosophila melanogaster* exposed to the herbal tea extract and silymarin level after 5 days of induction of toxicity with dolutegravir is presented in Fig. 5. Catalase level in the DTG-only group was higher than the diet-only group. A dose-dependent elevation of CAT levels in all the extract-treated flies (25-100 mg/ 5 g diet) was observed, with a significant difference ($P<0.05$) compared to the control flies. The highest level was recorded in 100 mg/ 5 g diet treated flies, while the lowest was recorded at the lowest dose of extract at 25 mg/ 5 g diet (Fig. 5). CAT level was lower in the silymarin-treated group, compared to those fed on the diet only (negative control).

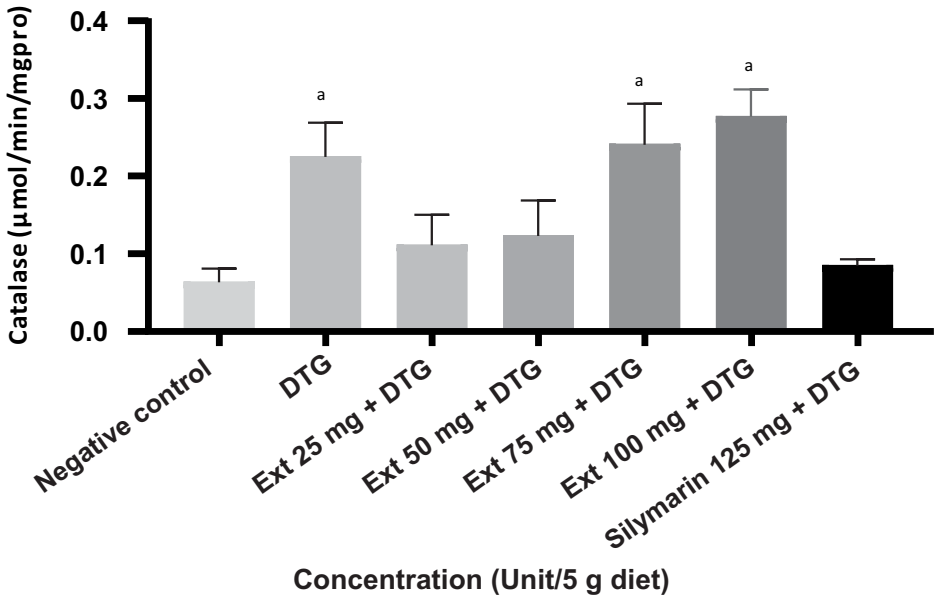


Fig. 5: Catalase level of *Drosophila melanogaster* exposed to the herbal tea extract after 5 days of induction with dolutegravir.

The values represent the mean ± SEM of five experiments. The results were considered statistically significant when *P<0.05; [a] versus negative control. Ext, Extract; DTG, Dolutegravir; DTG dose = 2.144 mg/ 5 g diet

The Glutathione-S-transferase (GST) level of *Drosophila melanogaster* exposed to the herbal tea extract after 5 days of induction with dolutegravir is presented in Fig. 6. Glutathione-S-transferase level in the DTG-only group was lower than the diet-only group (negative control). The highest activity was recorded at the lowest dose (25 mg/ 5 g diet treated flies). The level of GST decreased at the next highest dose (50 mg/5 g diet), and increased in a dose-dependent manner (75-100 mg/ 5 g diet) (Fig. 6).

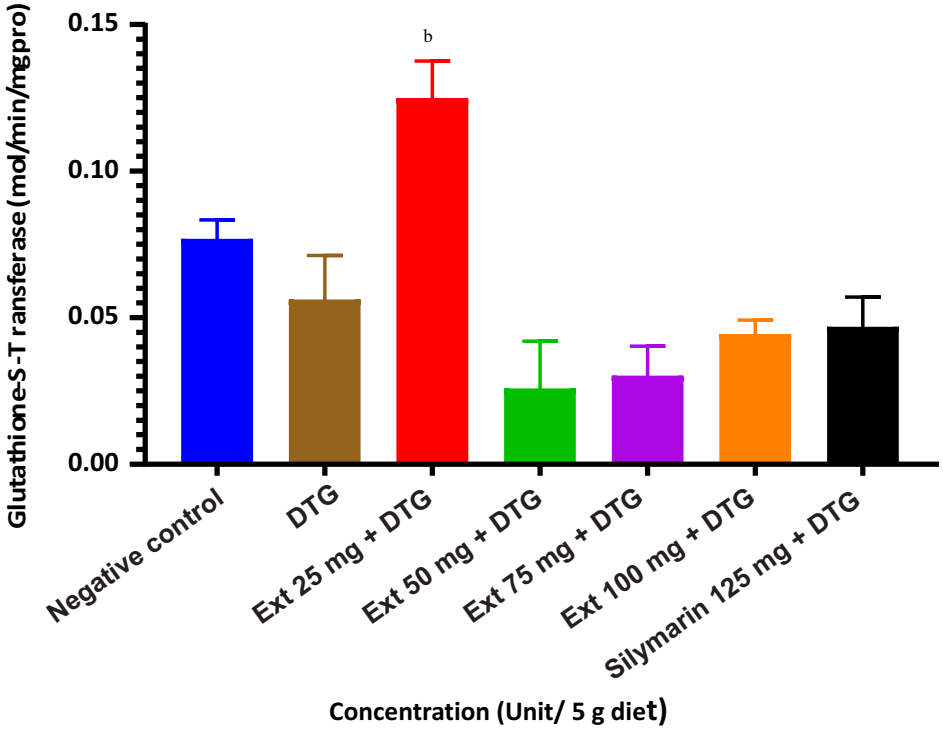


Fig. 6: Glutathione-S-transferase level of *Drosophila melanogaster* exposed to the herbal tea extract and Silymarin after 5 days of induction with dolutegravir.

The values represent the mean ± SEM of five experiments. The results were considered statistically significant when *P<0.05; [b] versus DTG. Ext, Extract; DTG, Dolutegravir; DTG dose = 2.144 mg/ 5 g diet.

Total Protein level of *Drosophila melanogaster* exposed to the herbal tea extract after 5 days of induction with dolutegravir is presented in Fig. 7. In the test groups, total protein was highest at the lowest dose (25 mg/ 5 g diet treated flies), compared to flies exposed to DTG only. The concentration of total protein increased in a dose dependent manner (50 -100 mg/ 5 g diet) (Fig. 7).

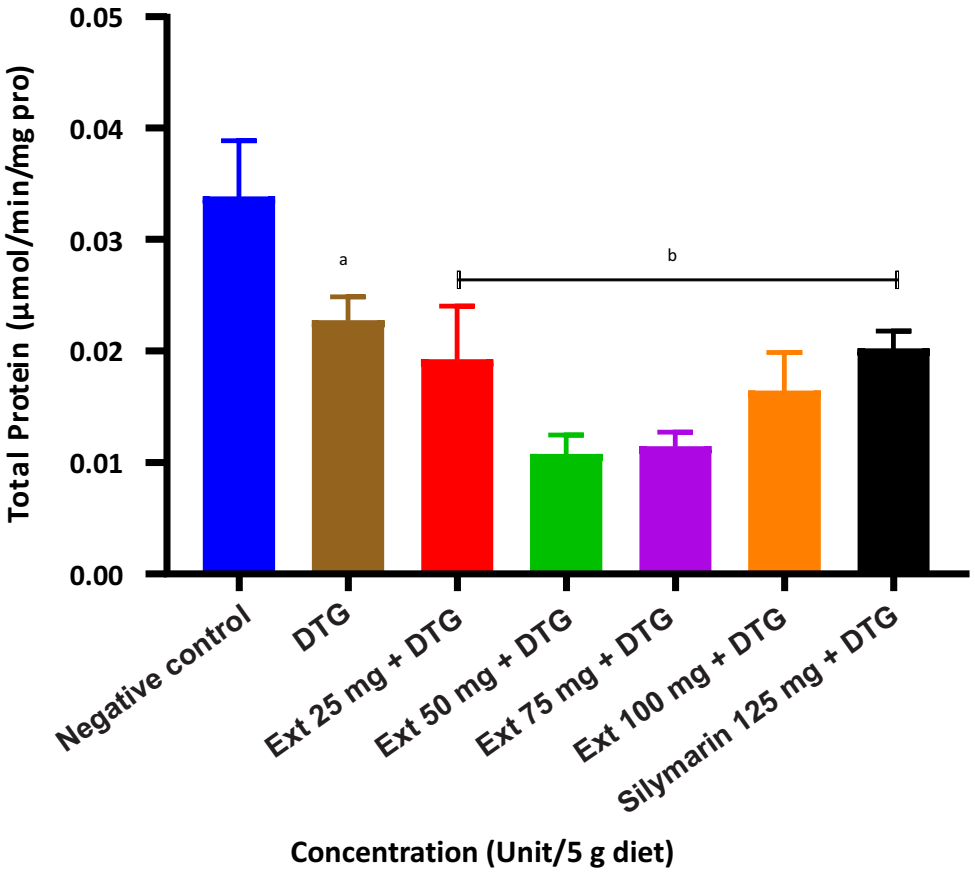


Fig. 7: Protein level of *Drosophila melanogaster* exposed to the herbal tea extract and Silymarin after 5 days of induction with dolutegravir.

The values represent the mean ± SEM of five experiments. The results were considered statistically significant when *P<0.05; [a] versus negative control; [b] versus DTG. Ext, Extract; DTG, Dolutegravir; DTG dose = 2.144 mg/ 5 g diet.

Total thiol level of *Drosophila melanogaster* exposed to the herbal tea extract after 5 days of induction with dolutegravir flies is presented in Fig. 8. In the test groups, total thiol was significantly lower (P<0.05) in the DTG-only group compared to the negative control. Flies exposed to extract and DTG at 50-100 mg/ 5 g diet showed significantly higher levels of total thiol (P<0.05) compared to flies exposed to DTG only (Fig. 8). Silymarin treatment resulted to a significantly higher level of total thiol (P<0.05) compared to DTG-only exposed flies.

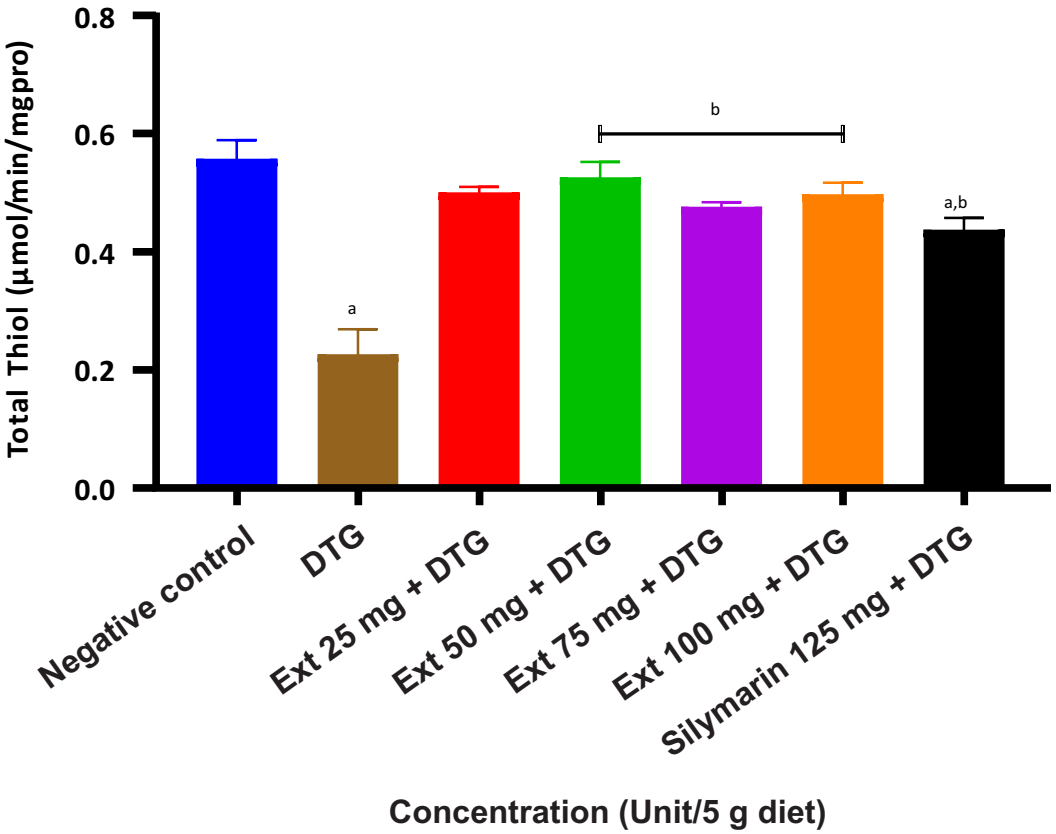


Fig. 8: Total thiol level of *Drosophila melanogaster* exposed to the herbal tea extract and Silymarin after 5 days of induction with dolutegravir.

The values represent the mean ± SEM of five experiments. The results were considered statistically significant when *P<0.05; [a] versus negative control; [b] versus DTG. Ext, Extract; DTG, Dolutegravir; DTG dose = 2.144 mg/ 5 g diet.

Acetylcholinesterase (AChE) level of *Drosophila melanogaster* exposed to the herbal tea extract after 5 days of induction of toxicity with dolutegravir is presented in Fig. 9. In the test groups, Acetylcholinesterase (AChE) was higher in the DTG-only group compared to the negative control. Flies exposed to extract and DTG at 50-75 mg/ 5 g diet showed a significant difference (P<0.05) compared to flies exposed to DTG only (Fig. 9). Silymarin caused a significant decrease in Acetylcholinesterase (AChE) (P<0.05) compared to DTG-only exposed flies.

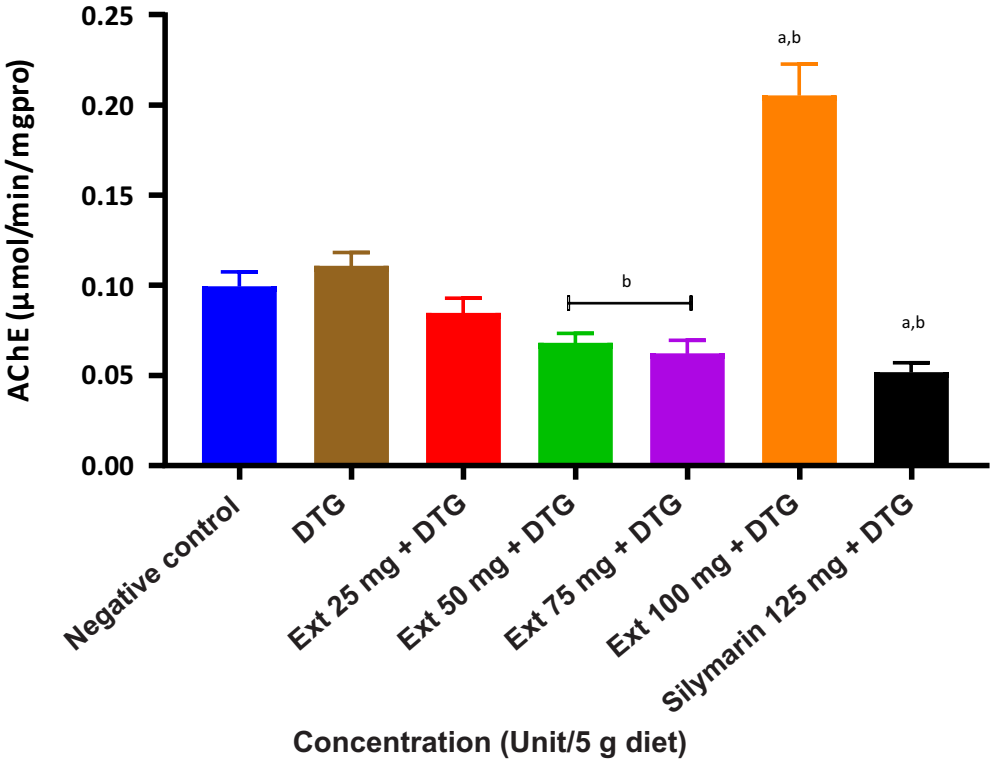


Fig. 9: Acetylcholinesterase (AChE) of *Drosophila melanogaster* exposed to the herbal tea extract and Silymarin after 5 days of induction with dolutegravir.

The values represent the mean ± SEM of five experiments. The results were considered statistically significant when *P<0.05; [a] versus negative control; [b] versus DTG. Ext, Extract; DTG, Dolutegravir; AChE, Acetylcholinesterase; DTG dose = 2.144 mg/ 5 g diet.

**DISCUSSION**

The antioxidative potential of both the herbal tea extract and silymarin, a well-explored antioxidant, was assessed using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay. In the current investigation, the herbal extract exhibited notably superior antioxidant activity in comparison to silymarin, as evidenced by its lower IC50 value. The higher the IC50 value, the lower the radical scavenging activity of antioxidant potential.[14] These results align with earlier research by various investigators,[15,16] which highlighted the antioxidant properties of sunflower and dandelion, respectively. This reaffirms the discernible radical scavenging effects observed in the present study.

The robust antioxidant effects observed in sunflower and dandelion extracts can be attributed to the presence of diverse phytochemicals, including flavonoids, vitamin E (recognized for its potent antioxidative properties), and essential minerals such as zinc and selenium.[17] Selenium serves as a crucial cofactor for several antioxidant enzymes, including glutathione peroxidases, which play a pivotal role in neutralizing detrimental free radicals.[18] Notably, dandelion leaves are enriched with flavonoids, alkaloids, vitamins, minerals, and beta-carotene, acting as a precursor to vitamin A and showcasing potent antioxidative capabilities.[19]

In this present study, survival of the fruit flies decreased with increase in concentration of dolutegravir (DTG), a similar finding previously reported.[7] Existing data suggests a connection between toxicities induced by dolutegravir and disruptions in mitochondrial function, leading to an excessive generation of reactive oxygen species (ROS) and subsequent onset of oxidative stress.[20] Likewise, a decrease in survival was observed in fruit flies exposed to varying concentrations of the extract. It is crucial to highlight that the impact of phytochemicals on Drosophila melanogaster is subject to variation based on factors such as concentration, duration of exposure, and

individual differences.[21] The absence of essential nutrients in certain extracts may result in diminished survival and reproduction rates of fruit flies. Simultaneously, some extracts might disrupt the hormonal regulation crucial for various physiological processes in fruit flies, leading to adverse effects on their survival and development. Furthermore, extracts have the potential to modulate the metabolic pathways of fruit flies, influencing energy production, growth, and development, thereby influencing overall survival. These aspects warrant thorough investigation in subsequent studies to accurately discern the specific effects of extracts on fruit flies.

It is noteworthy that certain phytochemicals may exhibit different effects when ingested as part of a mixture, compared to when they are isolated and administered in high doses. For instance, *Helianthus annus* is known to contain alkaloids, which, if ingested in substantial quantities, can be toxic to fruit flies.[22] The intricate interactions and varied effects of phytochemicals underscore the importance of comprehensive evaluations in elucidating their precise impacts on the physiology and survival of fruit flies.

The acute toxicity assessment was conducted for both the herbal extract and the drug, dolutegravir. In the classification of $LC_{50}$ , based on the dose range[23], the extract demonstrated a relatively non-toxic profile, with an observed $LC_{50}$ > 500 mg/5 g diet. This aligns with findings as previously reported,[24] suggesting a similar level of non-toxicity in their study. Furthermore, it is noteworthy that discontinuation of therapy in HIV-positive patients has been linked to the adverse effects associated with the use of dolutegravir.

Acetylcholinesterase (AChE) activity in fruit flies can serve as a biomarker for assessing the toxic effects of various chemicals. The observation, from our study, that levels of AChE decreased in a dose-dependent manner (25-75 mg/5 g diet) in the test groups correlates with a previous study.[25] It has been reported that significantly decreased acetylcholinesterase (AChE) correlates positively with increased oxidative stress[26], inferring that the extract (in this present study) was unable to protect against the toxic effects of DTG at these doses. The ability of the extract to cause a significant increase ($P<0.05$) in AChE at the highest dose (100 mg/5 g diet) compared to the positive control (DTG) indicates that the extract could mitigate oxidative stress in a dose-dependent manner. This scenario underlines the importance of conducting rigorous research and monitoring when considering combinations of medications and natural products to ensure both safety and efficacy.

The primary enzymatic defense system against oxidative stress involves catalase activity.[27] The efficacy of scavenging reactive oxygen species (ROS) through antioxidant enzymes, especially catalase (CAT) and superoxide dismutase (SOD), has been established to be directly linked to the vitality of sunflower seeds.[28] Disruptions or deficiencies in these enzymatic activities result in the accumulation of ROS, leading to diverse cellular injuries, including lipid peroxidation.[28]

In our study, where flies were exposed to dolutegravir, an unexpected increase in catalase activity was noted. This unusual rise could potentially be attributed to a compensatory upregulation in response to heightened oxidative stress induced by the toxicant[29,30]. In the present study, the extract, in the presence of the toxicant, induced a dose-dependent increase in catalase concentration, mirroring the findings of a prior study[31]. This observation underscores the extract's capability to mitigate dolutegravir-induced oxidative stress.

In fruit flies exposed to the toxicant, a decrease in Glutathione-s-transferase (GST) levels was observed, signifying oxidative stress. Glutathione-s-transferase is involved in detoxifying harmful compounds and is an essential part of the cellular defense against oxidative damage. However, in the context of this study, the extract demonstrated an attenuative effect, evident from the observed rise in levels of this antioxidant enzyme, specifically at the lowest dose. Similar results were observed in an earlier study[12], who demonstrated a slight increase in Glutathione-S-transferase (GST) activity in drosophila treated with a plant extract. The observation at the lowest dose may indicate that the plant extract's impact on GST levels is dose-dependent, meaning that even a minimal amount of the extract is sufficient to stimulate the increase in GST. The elevation of GST levels at the lowest dose compared to the higher doses may imply that the extract is capable of triggering this defense mechanism even at low concentrations.

Reactive Oxygen Species (ROS) are highly sensitive and possess a short half-life, making their measurement invivo for the determination of oxidative stress challenging.[32] Instead, biomarkers with longer half-lives, such as proteins, are utilized to assess the impact of reactive oxygen species. In the current investigation, exposure of fruit flies to dolutegravir in their diet resulted in a decrease in protein levels compared to flies that were

fed on the diet alone, similar to reports from a previous sudy.[20] The increase in total protein levels seen in response to an increasing dose of a plant extract can have several implications, reflecting the complex interactions between the extract and biological systems. Some plant extracts are known for their antioxidant properties, including *Helianthus annus* and *Taraxacum officinale*, which can protect cells from oxidative damage. Elevated total protein levels may suggest a response to oxidative stress, with cells producing proteins to counteract potential damage.

The thiol groups are important members of the antioxidant team and have been shown to destroy ROS and other free radicals by enzymatic and non-enzymatic mechanism.[33] Total thiol groups of proteins are mainly responsible for their antioxidant response, and they can serve as a sensitive indicator of oxidative stress.[34] Our findings showed an increase in total thiol level in the test groups when compared to flies exposed to DTG alone, which is similar to the study of Iorjiim *et al*.[6] Elevated total thiol levels indicate an improved antioxidant defense system as it can neutralize ROS and prevent them from causing cellular damage.[35] This further confirms the antioxidant properties of the two plants, helping to combat oxidative stress in the flies.

**CONCLUSION**

The herbal extract of *Helianthus annuus-Taraxacum officinale* herbal mixture demonstrated good antioxidant activity and was seen to alleviate Dolutegravir-induced toxicity in specific biomarkers in *Drosophila melanogaster*. This suggests potential health benefits of the herbal extract.

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