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MODULATION OF DOLUTEGRAVIR-INDUCED GEOTACTIC DEFICITS IN *DROSOPHILA MELANOGASTER* BY HEXANE EXTRACT OF *BRASSICA OLERACEA*

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ABSTRACT

This study investigated the protective role of Brassica oleracea against dolutegravir-induced effects on geotactic behavior in Drosophila melanogaster. Conducted at the Africa Center of Excellence in Phytomedicine Research and Development, University of Jos, flies were exposed to varying concentrations of dolutegravir (0.5–4 mg/5 g diet) and B. oleracea extract (7.5–1000 mg/5 g diet) for toxicity (LC_{50} determination) and behavioral assessments. A five-day exposure to specific extract doses (50–400 mg/5 g diet) was followed by geotaxis assays and acetylcholinesterase (AChE) activity analysis. B. oleracea extract was non-toxic and improved AChE activity, negative geotaxis and climbing ability in flies exposed to dolutegravir. These results suggest that the hexane extract of B. oleracea has modulatory activity against dolutegravir-induced geotactic deficits in Drosophila melanogaster.

Keywords: *Brassica oleracea*, Diet, Dolutegravir

INTRODUCTION

Drosophila is a genus of small flies, belonging to the family Drosophilidae, whose members are often called “fruit flies”. *Drosophila melanogaster* remains a cornerstone in research and literature continues to highlight its significant contributions to understanding various biological processes, including development, aging, and disease mechanisms (Kumar, 2020). Movement disorders are neurological syndromes in which there is an excess of movement (hyperkinesia) or a paucity of voluntary and automatic movements (hypokinesia), unrelated to weakness or spasticity (Fahn, Greene, Ford, 2019). Dolutegravir is an antiretroviral medication classified as an integrase inhibitor, which is used in combination with other medications to manage HIV/AIDS (Sarode & Jindal, 2022).

Brassica oleracea (red cabbage) is a plant belonging to the family rubra, it is endemic to the Mediterranean region (Sandhya *et al*., 2023). This research assessed the toxic effects (LD_{50}) and geotactic ability of dolutegravir in *Drosophila melanogaster*, as well as the protective effect of *Brassica oleracea* extract.

EXPERIMENTAL DATA/PROCEDURE/ METHODS

Determination of LC_{50}

The Lethal Concentration (LC_{50}) was determined using the methods described by Mohammad & Singh, 2009 and Charpentier *et al*., 2014. Eight groups of flies in replicates of 5 each containing 60 flies each were set up. Group 1 was control and maintained in 5 g of diet for 7 days. While

group 2-8 were administered different concentrations of the extract (0.005 g, 0.01 g, 0.05 g, 0.1 g, 0.25 g, 0.5 g and 1 g respectively) in 5 g diet for 7 days. Daily records of mortality were taken for the period and the data analysed to determine the LC_{50} of the extract

Treatment for Movement Assays (Climbing Assays) and Acetylcholinesterase Activity

Sixty (60) flies, aged 1-3 days old, were exposed to dolutegravir (0.5 mg, 1.0 mg, 2.0 mg, 3.0 mg, 4.0 mg) and diet only (as control) per 5 g fly food respectively per 10 g fly food as described by Abolaji *et al*, 2014). Same was done for the extract at 50, 100, 200 and 400 mg respectively. Negative and positive control groups were also treated.

Movement Assays

The locomotor performance of flies exposed and unexposed to dolutegravir (DTG) respectively were investigated using the Climbing assay. Briefly, ten (10) treated flies were immobilized under mild ice anesthesia and placed separately in labelled vertical glass columns. A period of about 20 min was allowed for recovery after which the flies were gently tapped to the bottom of the column.

Climbing assays

Briefly, ten (10) treated flies were immobilized under mild ice anesthesia and placed separately in labelled vertical glass columns (length, 15 cm). A period of about 20 min was allowed for recovery after which the flies were gently tapped to the bottom of the column. The numbers of flies that climbed up to the 6 cm mark of the column in 6 seconds, as well as those that remain below this mark were recorded. Data was analyzed and expressed as the percentage of flies that climbed beyond the 6 cm mark in 6 s. The score of each group is an average of three trials for each group of treated and control flies.

Determination of Activity of AChE Enzyme

Treated flies were homogenised and the homogenates were centrifuged at 1.000 ×g for 5 min at 4°C, and an aliquot of the supernatant (S1) was used for measurement of AchE (Ellman *et al*., 1961.

Statistical Analysis

Data were analyzed by one-way Anova method, Dunnett's post-hoc test, with level of significance taken at 95% confidence level ($p<0.05$). GraphPad Prism 8 was also used. (GraphPad Software Inc., CA).

RESULTS

Acute Toxicity Assay for Dolutegravir: The concentration of the drug that killed 50% the sample population (LC_{50}) for the dolutegravir was determined as 2.144 mg/5 g diet.

Median lethal concentration (LC_{50}): The median lethal concentration (LC_{50}) of dolutegravir, formaldehyde and silymarin in *D. melanogaster* were established to be 3.0650 mg/5 g fly food, 7.500 µL/5 g fly food and 0.2743 mg/5 g diet respectively after 7 days. The LC_{50} of the crude extract of *B. oleracea* in *D. melanogaster* could not be determined after 7 days up to 1000 mg/ 5 g diet), implying it is not acutely toxic to the flies even at that concentration (Figure 1).

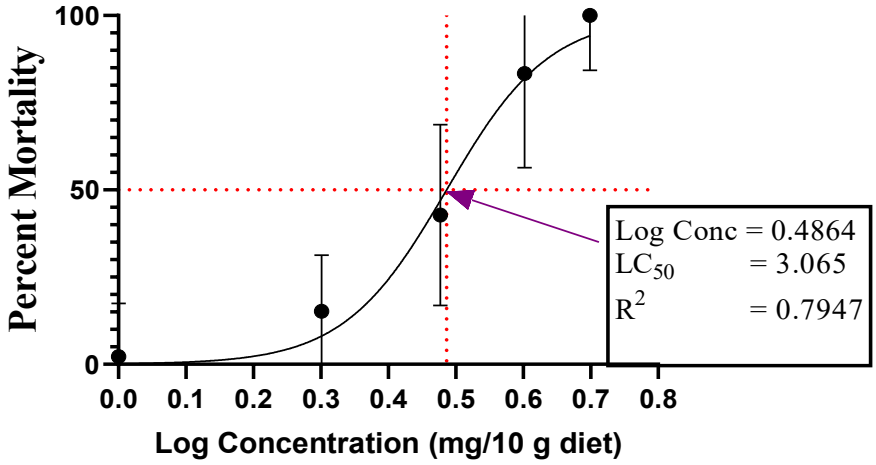

Figure 1 Median Lethal Concentration (LC_{50}) of Dolutegravir in *D. melanogaster*

Effects of Treatments on Geotactic Behavior

Effects of *B. oleracea* Extract Exposure on Climbing Ability in *D. melanogaster*

There was no significant difference when the different groups were compared to control and also no significant difference when compared to the DTG only group. But there was a significant difference in climbing ability between 50 mg and 200 mg/5 g diet extract (Figure 2).

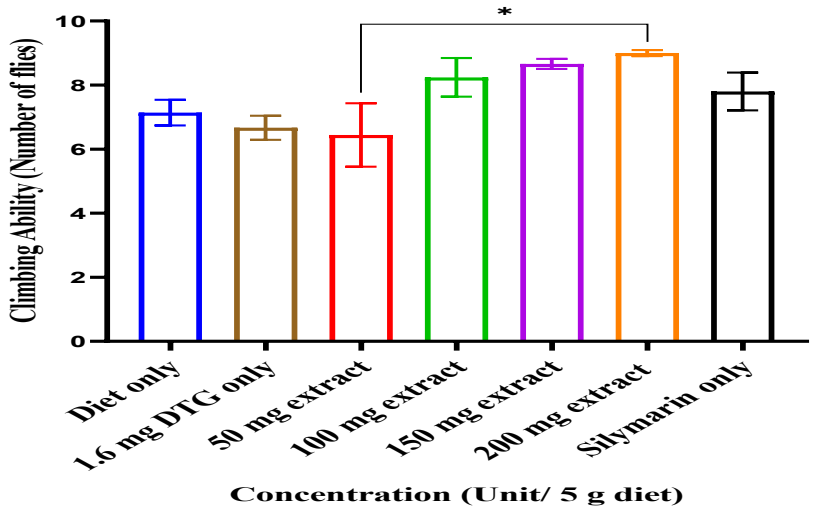

Figure 2 Effect of *B. oleracea* Extract Exposure on Geotactic Behavior (Climbing Ability) in *D. melanogaster* (One-Way ANOVA, Dunnett's Posthoc Test)

Key: * = Significance difference in climbing ability between 50 mg and 200 mg/5 g diet extract at $P < 0.05$; n=5

Effects of Treatments on Acetylcholinesterase Levels in *D. melanogaster*

Comparing the Diet-only group with all other groups, there was a significant increase in groups that were administered 50, 150 and 200 mg per 5 g diet of the extract, silymarin, a standard antioxidant, also showed a significant increase in acetylcholinesterase (AChE) activity. There was no significant difference when compared to the DTG-only group (Figure 3). Comparing the control group with other groups, there was a significant difference in the formaldehyde group and also the group pre-administered 50 mg of the extract before exposure to DTG. The group exposed to DTG only was compared to the other groups and a decrease in AChE activity was significant in groups pre-administered 150 and 200 mg of the extract before exposure to DTG. For Post administration of extract to check for amelioration, there was a significant difference in levels of AChE seen with administration of 200 mg extract after exposure to the toxicant. There was no significant difference in all the other groups when compared to DTG only group (Figure 3).

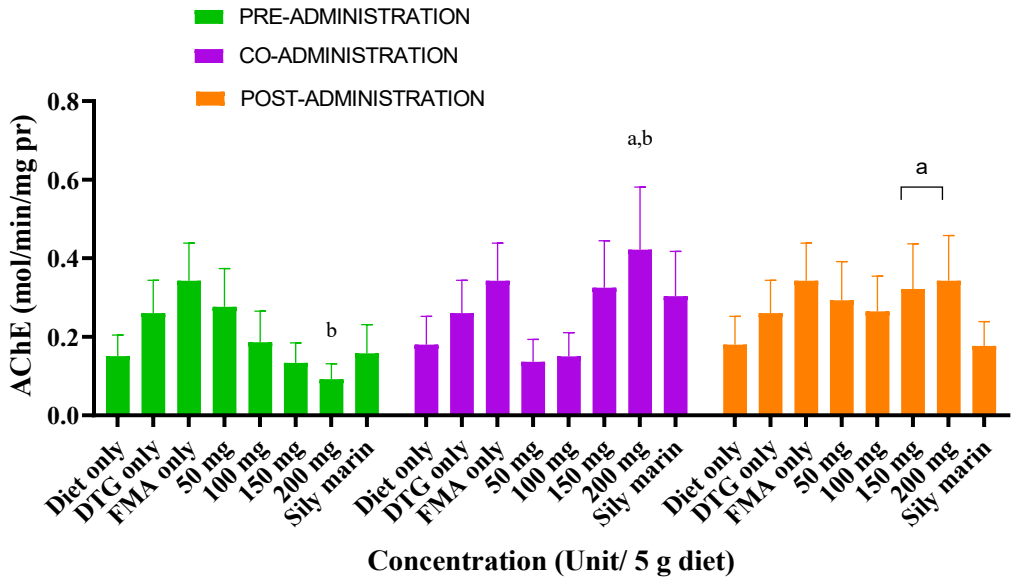

Figure 3 Effects of Pre-, Co- and Post-administration of *B. oleracea* Extract on Acetylcholinesterase (AChE) Levels in *D. melanogaster* (One-Way ANOVA, Dunnett's Posthoc Test)

Key: a = Significant difference compared to diet only at $P < 0.05$
b = Significant difference compared to DTG only at $P < 0.05$; Dolutegravir (DTG) = 1.6 mg; Silymarin =0.6 mg; Formaldehyde (FMA) =7.5 µL [All concentrations per 5 g diet]; ($P < 0.05$; n=5)

DISCUSSION

Acute Toxicity/ Median lethal concentration (LC_{50})

The crude extract of *B. oleracea* is not acutely toxic to *D. melanogaster*, even at that concentration. Concentrations higher than 1000 mg/5 g diet affected the diet, making it sticky and doses below 100 mg showed beneficial effects seen as increased percentage survival. According to Hodge and Sterner (2005), the extract can be considered to be practically non-toxic.

Effects of *B. oleracea* Extract on Geotactic Behavior

Effects of *B. oleracea* Extract Exposure on Climbing Ability in *D. melanogaster:* The significantly higher climbing ability with increase in concentration indicated the extract's ability to improve climbing ability and geotactic behavior. Climbing ability is used as a measure of assessment for sickness and health in drosophila with a decline in climbing ability indicative of poor health status of *D. melanogaster* (Linderman *et al*., 2012). The improvement in climbing ability seen in drosophila with exposure to B, oleracea extract is indicative of the plant's protective effects.

Effects of Treatments on Acetylcholinesterase Levels in *D. melanogaster*

The involvement of the cholinergic system in the development of neurodegenerative diseases, particularly among the elderly, has been well documented (Craig et al., 2011). However, its specific effects in Drosophila melanogaster remain unclear (Abolaji et al., 2020). In this study, the significantly increased ($P<0.05$) acetylcholinesterase (AChE) activity following dolutegravir (DTG) administration aligns with previous findings in D. melanogaster (Abolaji et al., 2020), where improved locomotion was observed due to AChE inhibition and the subsequent elevation of synaptic acetylcholine levels. Similar inhibitory effects on AChE were reported by Adewusi et al. (2011) using plant extracts. Reduced AChE activity has been linked to oxidative stress, supported by earlier studies showing that oxidative conditions suppress AChE levels, while antioxidant presence reverses this effect. Such reductions are likely due to enzyme inactivation by reactive oxygen species (ROS), which damage proteins (Santi et al., 2011).

The decline in climbing ability observed in fruit flies exposed to DTG is likely attributable to DTG-induced elevation of AChE activity, resulting in decreased acetylcholine (ACh) levels. AChE, primarily located at postsynaptic neuromuscular junctions, functions to hydrolyze ACh (McHardy et al., 2017). Therefore, increased AChE activity correlates with diminished ACh availability. This pattern is consistent with previous studies linking heightened AChE activity to oxidative stress (Melo et al., 2003). Xenobiotics such as dolutegravir have been shown to induce oxidative stress, contributing to movement disorders at the molecular level (Gazaryan & Ratan, 2010). Furthermore, movement disorders can also arise from hepatic or neurological damage associated with structural or functional liver impairment due to exposure to exogenous substances (Mulroy et al., 2021), with DTG being one such example. The generation of ROS by DTG not only underscores its neurotoxic potential but also implicates it in liver dysfunction, which may explain the impaired geotactic behavior observed in D. melanogaster in this study.

CONCLUSION

The hexane extract of *Brassica oleracea* exhibited modulatory effects against dolutegravir-induced geotactic deficits in *Drosophila melanogaster*.

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