Original Research Article

Effects of Dolutegravir (DTG) on Survival, Pupariation and Emergence in *Drosophila melanogaster*: The Rescue Role of *Brassica oleracea*

Abstract

The study aimed at determining the protective role of *Brassica oleracea* on dolutegravir-induced changes in Pupariation and Emergence of *Drosophila melanogaster*. Fruit flies aged 3-5 days old were exposed to different concentrations (0.5 to 4 mg/ 5 g diet) of dolutegravir and *Brassica oleracea* extract (7.5–1000 mg/5 g diet) for 7 days to determine the lethal concentration (LC_{50}). Flies were then exposed to the extract (50, 100, 200, and 400 mg/5 g diet) and controls (diet alone and vitamin C) to assess their effects on pupariation and emergence. A 14-day assay was also performed to evaluate the effect of the extract and toxicant (dolutegravir) on fly survival. The result showed a dose-dependent significant decrease ($P < 0.05$) and a dose-dependent significant increase ($P < 0.05$) in survival for flies exposed to dolutegravir and the extract respectively, when compared to the control group. Results showed a delay in pupariation and decrease in mean pupariation in flies exposed to dolutegravir alone; an improvement in the same parameters was observed in flies pre-treated with the extract before exposure to dolutegravir. Flies pre-treated with 200 and 400 mg extract per 5 g diet showed emergence that was comparable to flies in the control groups. A significant decrease ($P < 0.05$) was observed in the groups exposed to 50 and 100 mg extract per 5 g diet, suggesting no protection at these doses. This study concludes that *Brassica oleracea* leaf extract, at certain concentrations, is able to protect against dolutegravir-induced changes in pupariation and emergence in *Drosophila melanogaster*.

Keywords: Pupariation, Emergence, Drosophila, Dolutegravir, Brassica oleracea

1.0 INTRODUCTION

Drosophila melanogaster is a species of fly (the taxonomic order Diptera) in the family Drosophilidae. The species is often referred to as the fruit fly, though its common name is more accurately the vinegar fly. Starting with Charles W. Woodworth's proposal of the use of this species as a model organism, *D. melanogaster* continues to be widely used for biological research in genetics, physiology, microbial pathogenesis, and life history evolution[1].The life cycle of *Drosophila* is short and completes in about three weeks. Embryonic development, which follows fertilization and the formation of the zygote, occurs within the egg membrane. The egg produces larva, which eats, grows, and at length becomes a pupa. The pupa, in turn, develops into an imago or adult. The duration of these stages varies with the temperature. *Drosophila* cultures ought to be kept at room temperature where the temperature does not range below 20 ^{o}C or above 25^{o}C. They are bred on fermenting medium, which contains corn, dextrose, sugar, and yeast extract. Their breeding ratio is 1:3 (male: female). The male and the female are differentiated (under the microscope) by their size, markings on their abdomen and presence of sex combs following anesthetization[2]. Sexual dimorphism is characteristic of *Drosophila spp*. Therefore, males can be easily differentiated from females having differences in size and color. The females are about 2.5mm long while the male is somewhat smaller than the female, with dorsal site of the male body being darker due to a distinct black patch at the abdomen[3].

In the laboratory, *Drosophila* larvae typically wander and pupate on the walls of the container because the bottom of the rearing vial or bottle is usually entirely covered by food[4]. The distance that larvae pupate from the surface of the food 'pupation height' is a polygenic trait that responds effectively to bidirectional selection[5,6] and is influenced by light, temperature, humidity, pH, density, and parasitism[7,8,9]. When laboratory culture

conditions are enriched with horizontal semi-natural arenas (soil, agarose, etc.) around the feeding medium, larvae prefer to wander and pupate in these arenas [6,10].

Dolutegravir (DTG) is a second-generation integrase strand transfer inhibitor (INSTI) used in the management of HIV1. The FDA approved DTG in August 2013 for infected patients above the age of 12years and weighing over 40kg. Since its approval 7years ago, several types of research have been carried out to investigate the safety over a long period of use and its efficacy as well as the development of resistance by the virus to the drug. Studies have shown that Dolutegravir used in the management of HIV has the potential to cause oxidative stress in cells, which leads to several complications such as hepatotoxicity and other major side effects[11]. The study of DTG effect on pupariation and emergence of Drosophila might be beneficial in terms of evaluating the risk to patients placed on the drug for the management of HIV[12].

Brassica vegetables are the most important genus of the Brassicaceae family and consist of thirty-seven different species. They contain low fat, high vitamin, mineral, and fiber as well as various phytochemicals. Hydrolytic products of glucosinolates prevent oxidative stress, induce detoxification enzymes, stimulate the immune system, reduce cancer risk, inhibit malign transformation and carcinogenic mutations in addition to reduce the proliferation of cancer cell[13].

Metamorphosis in drosophila may be divided into two stages; a 12 hours prepupal period marked by pupariation (the onset of the larval-pupal transition)and a subsequent pupal period lasting 84 hours. Pupariation is marked by a sudden release of ecdysteroid hormone secreted from the ring gland. The larval cuticle becomes the puparium or pupal case that surrounds the metamorphosing fly until it ecloses. Apolysis is the term for the retraction of the epidermis from the cuticle of the third instar larva. Once apolysis is complete, a characteristic gas bubble forms in the prepupa abdomen, at this stage the

developing pupa is able to float in water. Next, the eversion of the head takes place, approximately 12 hours from the start of pupation. The process itself is sudden, lasting about 10 minutes and orchestrated by contractions of abdominal muscles. Head eversion marks the beginning of the true pupal state. During pupariation, the marginal disc undergoes eversion to form the basic shape of the adult head, thorax, and abdomen. Wing, leg, and haltere discs fuse to form the thorax. The eye-antennal complex fuses to form a single head capsule and the head and thorax fuse with the abdomen[14]

1.1 Aim of The Study

The aim of the study is to determine the rescue role of *Brassica oleracea* in protecting against Dolutegravir-induced changes in pupariation and emergence of *Drosophila melanogaster*.

2.0 Materials and Methods

The HAART Dolutegravir tablets 50 mg per tablet was used in this study. A total of thirty (30) tablets of the fixed-dose formulation were weighed to determine the average weight per tablet. The tablets were pulverized using porcelain mortar and pestle. The appropriate quantities of powder that will contain the desired amount of active ingredient were calculated and weighed using analytical balance (Meltlar Model No. MT-200B),

2.1 Plant Collection, identification and Extraction

The plant was purchased from Farin gada vegetable market Jos North Local Government area, Jos, Plateau State and the identification and authentication carried out at Federal College of Forestry and a voucher number obtained.

2.2 Animal Model

D. melanogaster (Harwich strain) at 3-5days old was obtained from the Africa Centre of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Jos, Nigeria. The fly stock was maintained at a temperature of 23±1°C and 60% relative humidity under 12 h dark/light cycles. The flies were fed on standard Drosophila medium composed of cornmeal (1% w/v), brewer's yeast (2% w/v), agar, and methylparaben (0.08% w/v).

2.3 Determination of LC50

The determination of LC50 was carried out following the methods described by Mohammad & Singh, 2009 and Charpentier et al., 2014,[15,16] with slight modifications. Sixty (60) flies of age range 1- 3 days were anesthetized under ice, counted and exposed to series of graded concentrations of 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 for 7 days. Mortality reading was taken at 24 hrs intervals during this period. Data obtained were subjected to dose-response simulation using Graphpad prism 8.0 for LC50 determination. The same was done for B. oleracea extract at 50 mg, 100 mg, 150 mg and 200 mg per 5 g diet.

2.4 Fourteen-day Survival Assay

In this experiment, sixty (60) flies of both genders (1-3 days old) were exposed to each per 5 g food in five replicates for 14 days as described by Abolaji et al., 2014[17]. The number of live and dead flies was scored daily till the end of the experiment, and the survival rate was expressed as a percentage of live flies.

2.5Treatment for Reproductive Ability (Pupariation and Emergence)

Sixty (60) virgin flies (both sexes), age range 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[17]. The same was done

for the extract at 50, 100, 200, and 400 mg respectively. Negative and positive control groups were also treated.

After five (5) days of treatment, 5 male and 5 female flies were randomly selected in each treatment group and transferred into fresh vials with normal food for 24 hours, and the eggs laid in each vial during this period was kept for 21 days for the emergence of adult flies. The reproductive ability of the flies, after exposure to DTG and extract, was assessed by counting the number of pupae and later the number of flies that emerged daily. The mean number of flies that emerged gives a measure of reproductive ability

2.6 Statistical Analysis

The results were analyzed using GraphPad Prism 8 (GraphPad Software Inc., CA).

RESULTS

The percentage yield was calculated to be 43.745 % and the phytochemicals present in *B. oleracea* leaf extract included Alkaloids, Saponins, Tannins, Flavonoids and Carbohydrates.

3.1 LC_{50} Determination

The LC_{50} of Dolutegravir was 2.144 mg/5 g diet while that of the extract could not be determined even at doses up to 1000 mg/5 g diet.

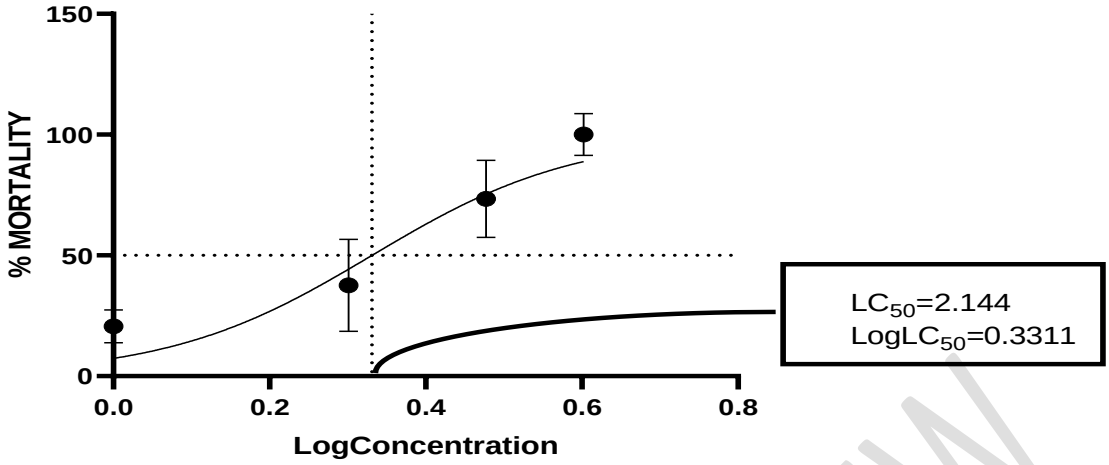

Fig 1: Percentage Mortality Vs Log Concentration of Dolutegravir in *Drosophila melanogaster*

3.2 Survival Assays

The result showed a dose-dependent significant decrease ($P < 0.05$) in the survival of the flies exposed to DTG while there was a dose-dependent significant increase in survival for flies exposed to different concentrations of the extract when compared to the control group

3.2.1 Fourteen-Day Survival Assay for DTG

There was a significant dose-dependent decrease in percentage survival in flies exposed to different doses of dolutegravir as compared to the control group that was on diet alone with survival dropping as low as 8.3% in flies exposed to 4 mg/5 g diet of DTG.

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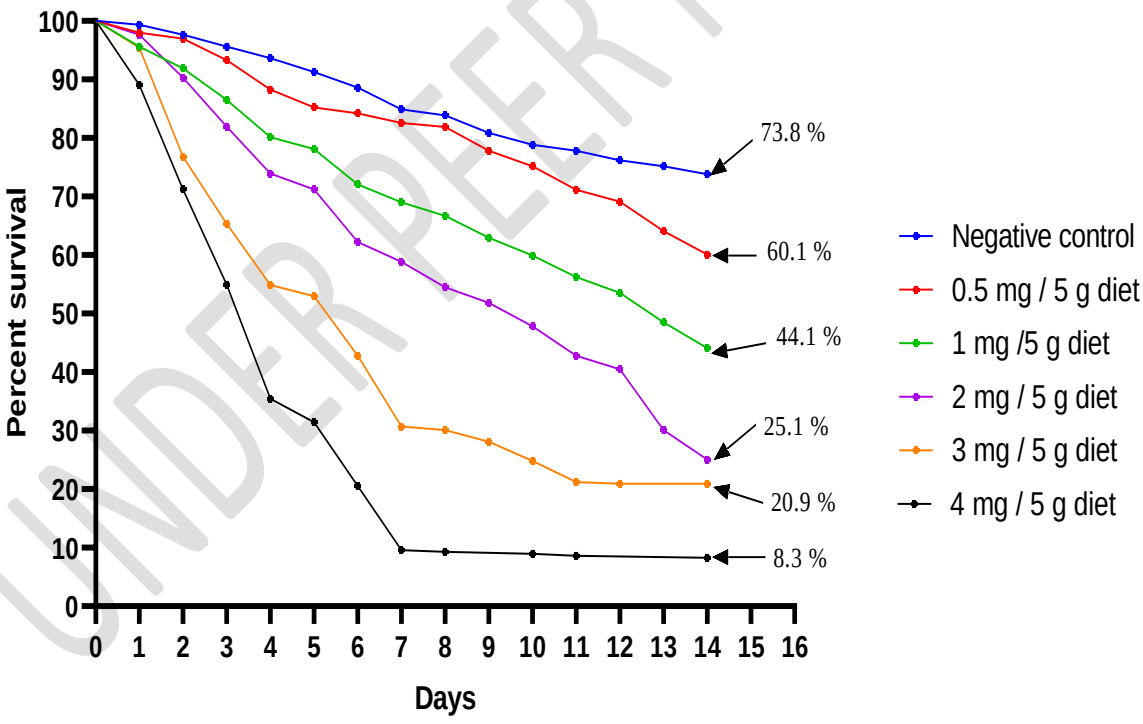

Fig 2: 14-Day Survival Assay for Dolutegravir Showing Percentage Survival Vs Days

3.2.2 Fourteen-Day Survival Assay for Extract

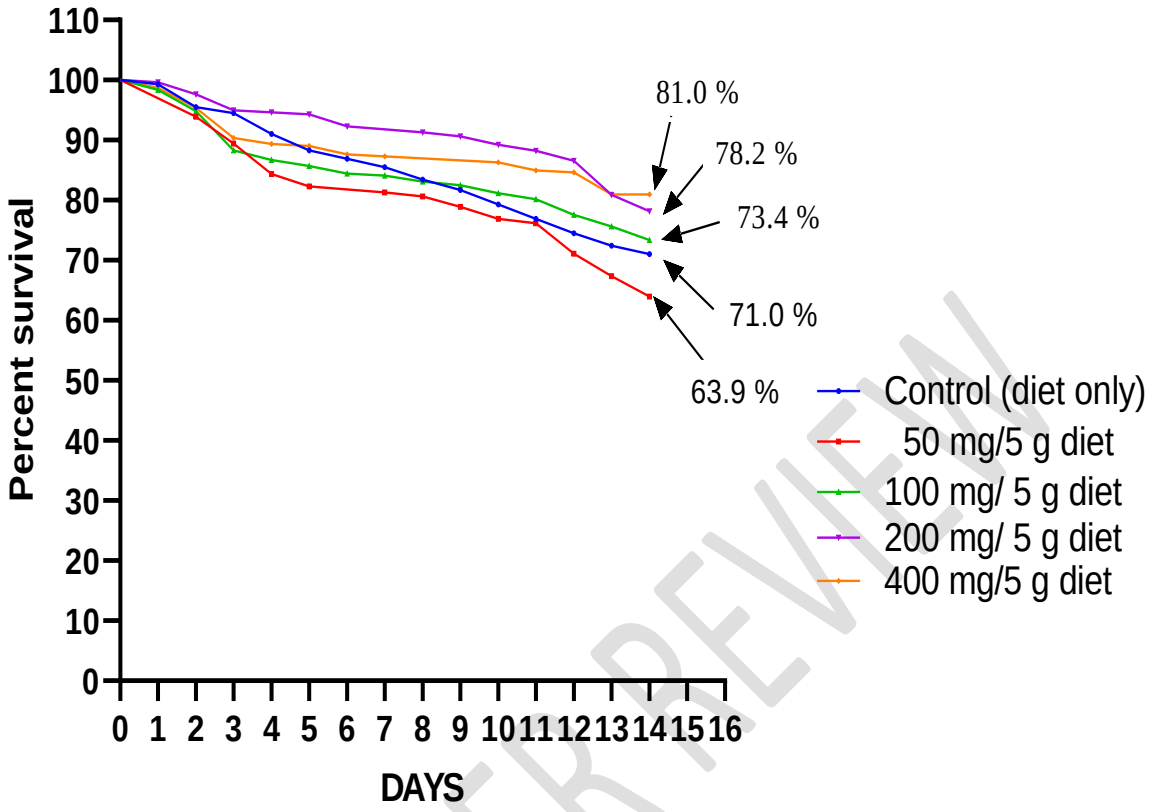

Fig 3: 14-day Survival Assay to different concentrations of the extract

3.3 Reproductive Ability

There was a delay in pupariation and decrease in mean pupariation in flies exposed to DTG alone while there was an improvement in same parameters in flies pre-treated with the extract before exposure to Dolutegravir. Flies pre-treated with up to 200 and 400 mg extract showed emergence that was comparable to flies in the negative and positive groups. A significant decrease was seen in the groups exposed to 50 and 100 mg extract suggesting that there was no protection at these doses.

3.3.1 Pupariation

There was a significant difference ($p<0.05$) in the groups treated with DTG only, 100 and 200 mg of the Extract.

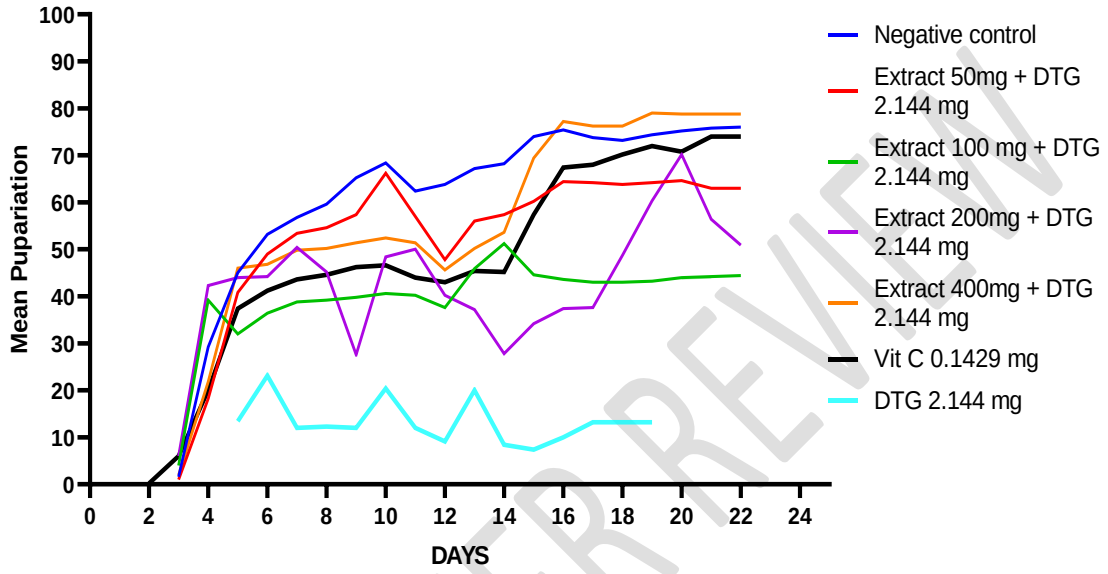

Fig 4: Mean Pupariation Vs Days in flies treated with the extract and DTG

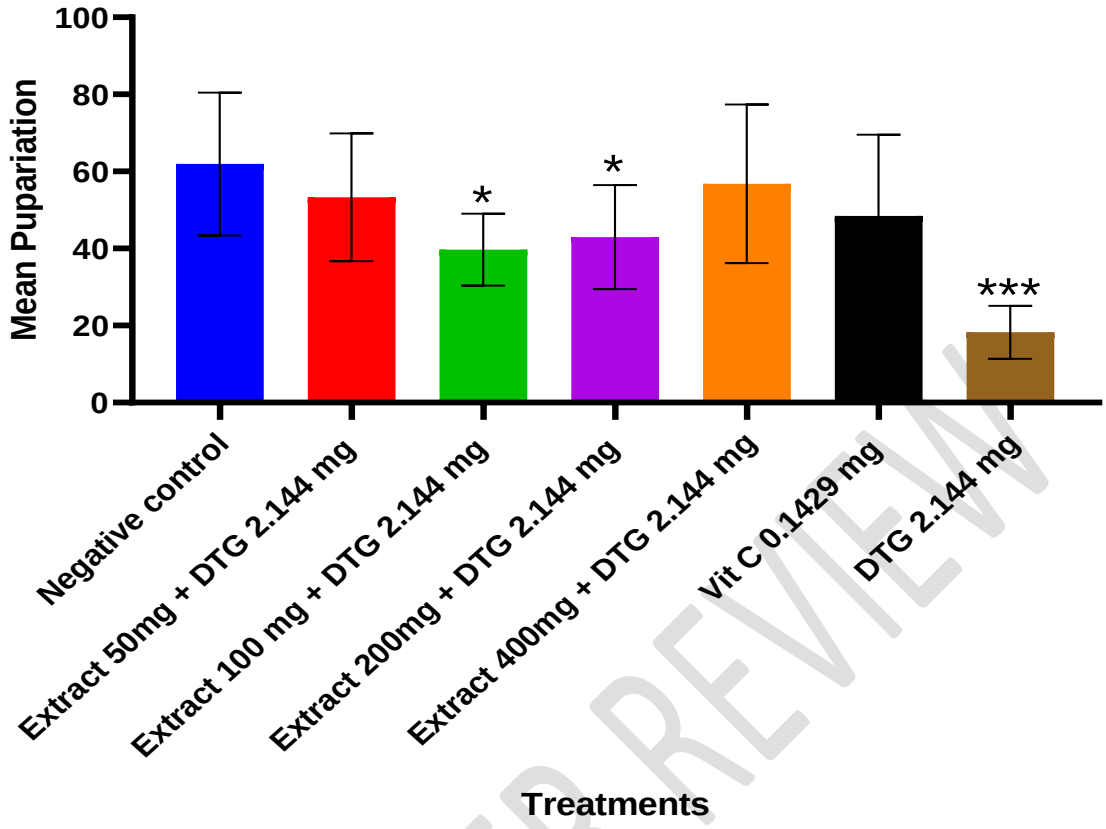

Fig 5: Effect of treatment on mean number of Pupae emerged

3.3.2 Emergence

Day-wise emergence pattern of the adult fly

Treatment of fruit flies with different concentrations of the extract and thereafter with dolutegravir (2.144 mg) showed considerable variations in the emergence of adult flies. Flies that received 200 and 400 mg per 5 g diet had mean emergence even higher than flies in thecontrol group and even in the group treated with vitamin C, a known antioxidant. The emergence time for flies in the control groups, and groups pretreated with the extract began on day 8 continued up to day 21 except for the group pretreated with the lowest dose of the extract (50 mg per 5 g diet) for which emergence stopped at

18 days however for groups treated with DTG only there was a delay in emergence (day 10) and it seized by day 17.

There was a significant difference in the groups treated with DTG only, 50 and 100 mg of the Extract

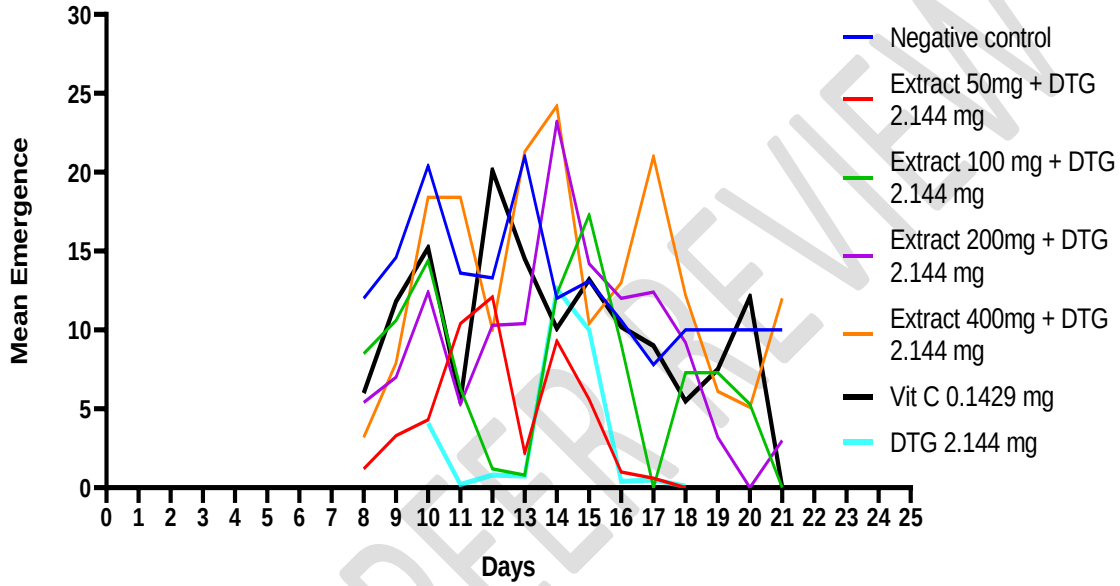

Fig 6: Effect of treatment on cumulative number of Pupae emerged

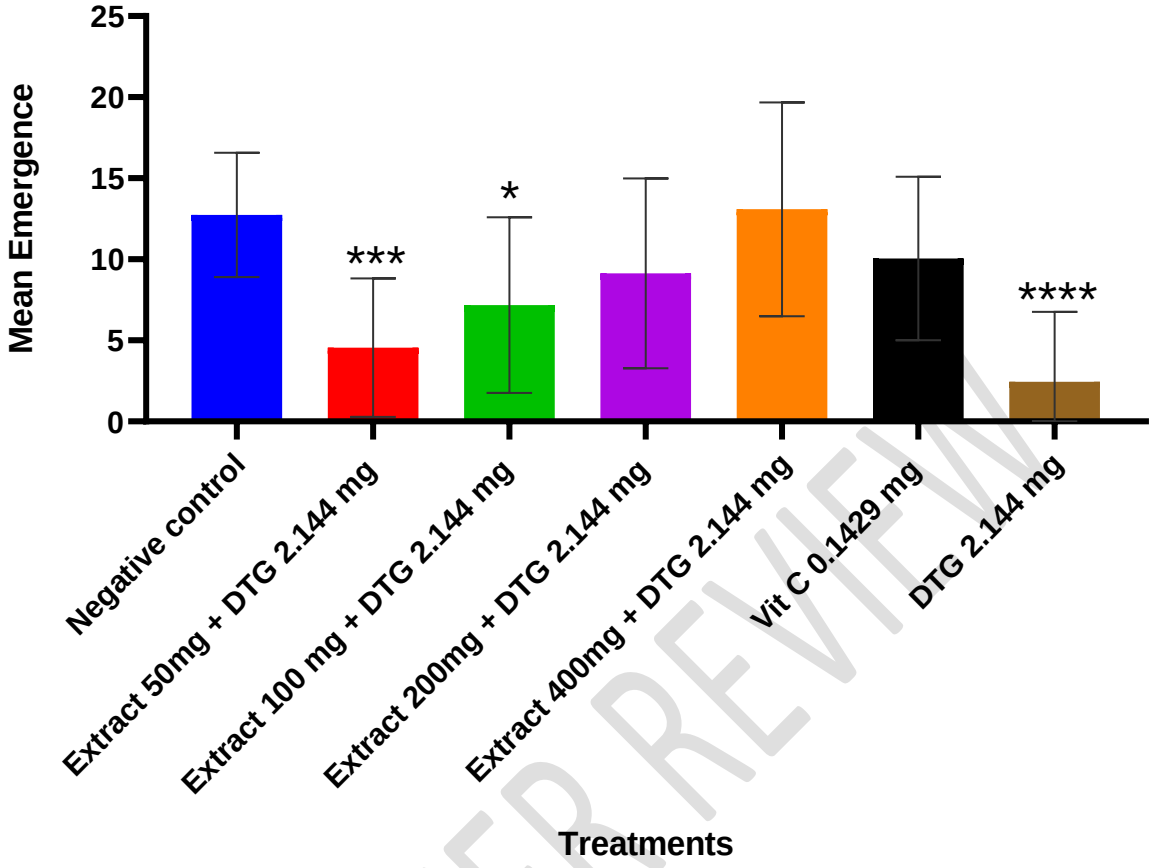

Fig 7: Effect of treatment on Mean Emergence

DISCUSSION

The phytochemicals present in *B. oleracea* leaf extract included Alkaloids, Saponins, Tannins, Flavonoids and Carbohydrates. A large number of natural compounds present in food materials have been reported to possess antioxidant properties[18]. Despite the prevalence of antioxidants such as vitamin C and E, the majority of the antioxidant activity of fruits, vegetables, spices, and herbs may be from compounds such as phenolic acids and flavonoids considered to be much greater than that of the essential vitamins[19,20.] The presence of such phytochemicals in the B. oleracea, therefore, suggests that it has antioxidant properties. Comparisons between the fully sequenced *Drosophila* and human genomes revealed that approximately 75 % of known human disease genes have a recognizable match in the genome of fruit flies consolidating its legitimacy as a model organism for medical research (Reiter *et al., 2001)* hence the choice of the fruit fly as a model.

Toxicity assay carried out showed significant toxicity in the flies with LC_{50} being 2.144mg per 5 g diet. As reported by Loomis & Hayes, 1996, in *Classification of LD_{50} based on dose range*, substances with LD_{50} below 5 mg/ kg are classified to be extremely toxic while substances with LD_{50} above 15,000 mg/kg are termed relatively harmless[21]. This indicates the drug DTG is moderately toxic (50-500 mg/kg) to drosophila at the doses used.

From the 14-day survival assay of flies treated with the extract, the higher percentage of survival of the flies treated with extract at 100, ,200 and 400 mg is indicative of the extracts ability to prolong life in drosophila and implies its antioxidant effects There decrease in survival of flies treated with DTG when compared to the negative control is indicative of toxicity of the drug on the fruit fly Drosophila melanogaster. With reasonably sized longevity experiments, differences as low as 1-2% are often highly statistically significant, but the overall impact of the intervention on health status may be minor. Therefore, both statistical and biological significance must be considered when interpreting the overall results of the experiment. Inference about the aging process from survival experiments can be augmented by measures of age-related declines in behavioral or physiological health measures, including climbing ability[22] and gastrointestinal wall integrity[23]. This, therefore, suggests significant differences in survival rate when exposed to different concentrations of the drug, the extract showed an ability to significantly improve survival and this can be attributable to the presence of alkaloids, flavonoids, and other phytochemicals that have been reported to have antioxidant activity.

The delay in pupariation (day 5) and decrease in mean pupariation seen with flies exposed to dolutegravir without pre-treatment with the extract and other groups is indicative of toxicity while the increase in pupariation in groups pre-treated with 100 and 200 mg of the Extract is indicative of the protective effects of the extract. In insects,

localized tissue injury often leads to global (organism-wide) delays in development and retarded metamorphosis. In Drosophila, for example, injuries to the larval imaginal discs can retard pupariation and prolong metamorphosis. Injuries induced by treatments such as radiation, mechanical damage, and induction of localized cell death can trigger similar delays. In most cases, the duration of the developmental delay appears to be correlated with the extent of damage, but the effect is also sensitive to the developmental stage of the treated animal. The proximate cause of the delays is likely disruption of the ecdysone signaling pathway, but the intermediate steps leading from tissue injury and/or regeneration to that disruption remain unknown[24]. In insects, various types of tissue damage can trigger a systemic injury response which results in prolonged larval and/or pupal stages[25].

The mean emergence in flies pretreated with the extract was higher than flies in the control group and also higher than the group treated with vitamin C, a known antioxidant. This is an evidence of the extract's ability to protect against DTG-induced toxicity and negative effects on fly emergence. The longer emergence time seen in flies pretreated with the extract and in the control groups as compared to groups exposed to only DTG also shows protection. In a previous study, evaluation of reproductive capacity in DTG-HAART exposed *D. melanogaster* showed significant ($P<0.001$) reduction in fly emergence at lower concentrations with 100 % emergence failure at higher experimental concentrations. The exposure of DTG-HAART naïve flies showed a significant ($P<0.001$) reduction in fly emergence without eclosion failure. This observation implies that DTG-HAART may have altered more overwhelmingly the reproductive capacity in the exposed adult flies than the developmental toxicity at the eclosion stage[26].

Conclusion

The extract of *B. oleracea* was found to possess antioxidant activity. The extract showed protective roles on dolutegravir-induced changes and pupariation on pupariation and emergence of Drosophila melanogaster. This study concludes that *B. oleracea* leaf extract, at certain concentrations, is able to protect against Dolutegravir-induced changes in pupariation and emergence in *D. melanogaster*.

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