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Research Article

Preliminary mortality and eclosion: dose-determining factors of aqueous extract of *Hibiscus sabdariffa* in *Drosophila melanogaster*

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Drosophila melanogaster, *Hibiscus sabdariffa*, mortality, eclosion, concentration

ABSTRACT

Background: This study investigated the toxicity of *Hibiscus sabdariffa* extract using simple end point assays (mortality and eclosion assay) in *Drosophila melanogaster* (Harwich strain) flies. **Methods**: The study was carried out in two phases (phases I and II) and in an array of concentrations of 10, 20, 30, 40 and 50 mg/ml in phase I; 100, 250, 500, 750 and 1000mg/ml in phase II for the period of 168 hours. Thirty (30) 4-day old flies were used in each concentration and mortality was recorded every 24 hours for the period of the study. Eclosion was scored after emergence of the adult flies. **Results**: There was no significant difference (p<0.05) in mortality and eclosion between the (experimental) concentrations and the control in the first phase of this study, indicating low/zero toxicity to the parent and the emerged flies. However, higher concentrations of 250 through 1000mg/ml of the second phase showed significant (p<0.05) increased mortality and decreased eclosion scores, indicating high acute toxicity to *D. melanogaster*. **Conclusion**: The LC_{50} of *Hibiscus sabdariffa* after 168 hours was 427.1mg/10g diet in *Drosophila melanogaster* and much eclosion was recorded at doses between 10 - 100mg/10g diet as compared with doses between 250 - 1000mg/10g diet. Eclosion can be considered a factor in the determination of LC_{50} in *Drosophila melanogaster*.

INTRODUCTION

Hibiscus sabdariffa is widely cultivated throughout eastern, southern Asia and Africa (Lee *et al*., 1999). The flower, fruit, root, stem, and bark *of H. sabdariffa* all show pharmacological effects and have been widely used as medicinal treatment materials in Asia. The root bark of H. *sabdariffa* has been used as a traditional medicine with fungicide, antipyretic, and anthelmintic activities in the treatment of dysentery, eczema, tinea, and scabies in Asia (Shi *et al*., 2014). Recent studies have shown that the root bark of *of H. sabdariffa* shows anticancer (Shi *et al*., 2014), antioxidant, and human neutrophil elastase inhibitory activities. The antidepressant effects of some *Hibiscus* species have been investigated. The ethanol crude extract from the roots of H. *rosa-sinensis*, the ethanol extract of *H. sabdariffa* calyces, and the methanol extract from the flowers of H. *tiliaceus* showed significant antidepressant activity in animal models (Panesar *et al*., 2017).

Drosophila melanogaster and humans share many biological processes. For example, several neurobiological processes are shown to be similar in Drosophila and Homo sapiens, including using the same neurotransmitters and very similar mechanisms of neurotransmitter storage, release and recycling (O'Kane, 2011; Martin and Krantz, 2014). For decades, Drosophila has been used as a model organism to study human diseases, including neurological disorders (van Alphen and van Swinderen, 2013; Bellen et al., 2010). Taking Fragile X syndrome (FXS) as an example, the Drosophila FXS model has played an important role in elucidating the morphological and behavioral phenotypes associated with human FXS (Bhogal and Jongens, 2010). Drosophila has also been used to study complex psychiatric disorders, such as autism, schizophrenia, and attention deficit hyperactivity disorder (van Alphen and van Swinderen, 2013; Kaur *et*

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al., 2015).Evidence has shown that Drosophila and humans have similar depressive- like symptoms under stress (Shohat-Ophir *et al*., 2012; Yang *et al*., 2013).

METHODS

Animals

Drosophila melanogaster, (Harwich-S) was acquired from Africa Centre of Excellence in Phytomedicine Research and Development, Jos Droso-laboratory. The flies were placed in bottles (100mL) each containing *Drosophila* standard diet/food. To transfer the flies into the various bottles, standard yeast was sprinkled evenly on the top of the food in a single layer to attract the flies. Six hundred (600) flies were used for experimentation.

Equipment

Hand lens, stereo-microscope (florescent), forceps, Whatman's paper, paint brush, hand towel (2 pairs), cooking pot (1), glass wares, spectrometer, centrifuge (cold), Eppendiffs (50 pcs), Micropipettes (adjustable), culture vial, treatment vials, foam plug, weighing balance, cooking utensil, fly pi, egg chamber, weighing balance.

Chemicals

Corn flour 100g, baker's yeast 20g, agar agar 10g, methyl paraben, water 1700ml, aqueous extract of hibiscus sabdariffa, , 70% ethanol (1 litre), Phosphate buffered saline (PBS) to be supplied by Sigma-Aldrich.

Plant Extraction and Preparation

Dried extract of the flower of *Hibiscus sabdariffa* was bought from Samaru, and was freeze dried in the department of Pharmacognosy, A. B. U. Zaria. Dried flower of *Hibiscus sabdariffa* was extracted using, cold maceration method. Five hundred grams of powdered plant flower was soaked in 3 liters of distilled water, which was divided into different aliquots using conical flasks. The above was shaken for 24 hours **(**Olila *et al*., 2001). The infused fluid obtained was filtered with Whatman No. 1 filter paper and the filtrate, poured onto petridishes and dried in oven under low temperature **(**Olila *et al*., 2001**).** The process was repeated to obtain higher yield of crude extract. Another four hundred and eighty seven grams of powdered plant leaves was soaked in 4.87 liters of distilled water, which was divided into different aliquots using conical flasks. It was shaken for 24 hours **(**Olila *et al*., 2001).The infused fluid obtained was filtered with Whatman No. 1 filter paper and the filtrate was partitioned to obtain two different layers. The filtrate was poured onto petridishes and dried in oven under low temperature **(**Hsin-Ping, 2012**).**

The following concentrations of aqueous extracts of *hibiscus sabdariffa* was prepared as 10mg, 20mg, 30mg, 40mg, 50mg, 60mg, 100mg, 250mg, 500mg, 750mg, 1000mg (control) each with standard corn meal diet. Having it in mind that 1 fly weigh approximately 1mg (Bele *et al*., 2014).

For each experiment a total of 240 flies (20 flies per groups) starved for 14 to 16 hours in empty vials, and then fed on the different concentrations of the extracts for 30 minutes. The number of deaths were counted after1 hour of feeding (Bele *et al*., 2014). Three independent experiments were conducted each with three replicas; the last experiment was conducted with 120 flies (15 flies per groups) but 1fly per vial to ensure quality of the results obtained.

RESULTS

Lethal Concentration (LC_{50}) of Hibiscus sabdariffa in Drosophila melanogaster

There was no significant difference (p<0.05) in mortality and eclosion among the concentrations and control in the first phase of this study (Fig. 1). However, at higher concentrations (250mg/ml-1000mg/ml) of the second phase showed significance of (p<0.05) increased mortality and decreased eclosion scores, indicating high acute toxicity to *D. melanogaster* adults. Therefore, the safe concentrations of hibiscus sabdariffa extract in this study with insignificant differences in mortality and eclosion were 100 mg/g to 10 mg/g.

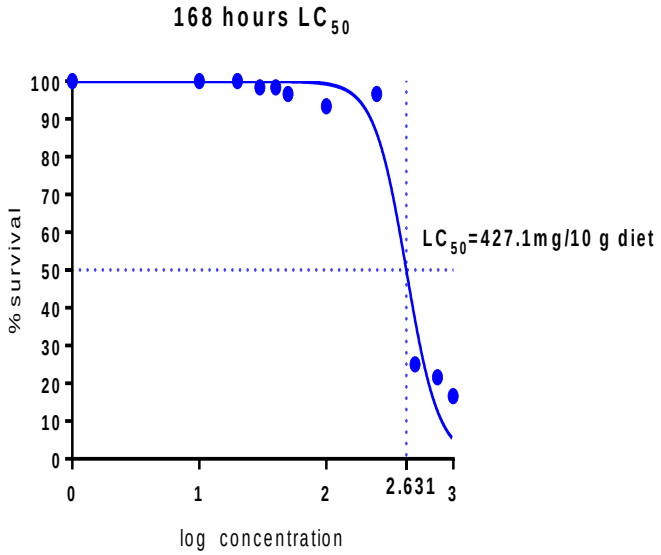

Fig. 1: LC_{50} of aqueous extract of *Hibiscus sabdariffa* using *drosophila melanogaster*

The flies were administered with aqueous extract of *H.s* which was incorporated into the flies diet ranging from 10mg,20mg, 30g,40mg, 50mg and 100mg/10g of diet for first phase and 250,500, 750 and1000mg/ for the second phase. The data was analyzed using probate analysis and the Lethal Concentration (LC_{50}) of *Hibiscus sabdariffa* in *Drosophila melanogaster* was found to be 427.1mg/10g diet. There is no significant difference (p<0.05) in mortality among the concentrations and control in the first phase of this study.

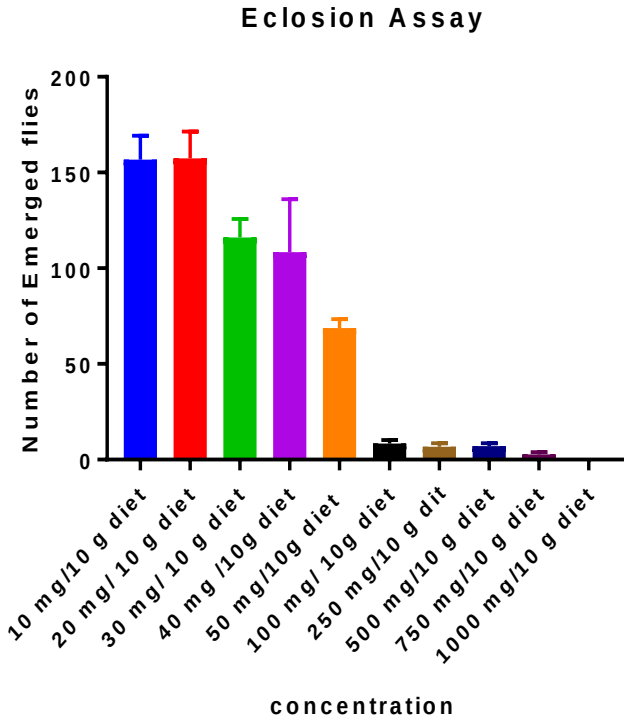

Fig. 2: Eclosion at different concentrations from 10-1000 mg

The flies were administered with with *H.s* which was incorporated into the flies diet ranging from 10mg,20mg, 30g,40mg, 50mg and 100mg/10g of diet for first phase and 250,500, 750 and1000mg/. The data were analyzed using probit analysis which shows that more flies eclosed from concentrations of the first phase as compared to the second, as less flies eclosed (Fig. 2). There is no significant difference (p<0.05) in eclosion among the concentrations and control in the first phase of this study compared with the second phase.

DISCUSSION

According to a study of Onyenekwe and coauthors, no deaths were observed in Albino mice after fourteen day's administration (i.p.) at doses of 1000–5000 mg/kg b.w./d., thus the calculated LD50 of aqueous extract of *H.s* was >5000 mg/kg body weight.

There is paucity of literature of acute toxicity with calculated LC_{50} for aqueous extract of *Hibiscus sabdariffa*using drosophila melanogaster. Hence this study has bridged that gap as the LC_{50} was calculated to be 427.19mg/10g of diet. Eclosion was also found to be very effective in determining the LC_{50}, this is because more flies eclosed in doses with lesser doses as compared with those of higher doses.

CONCLUSION

In conclusion, the results of this work reveal that the LC_{50} of hibiscus sabdariffa after 168 hours is 427.1 mg/10 g diet in drosophila melanogaster and there is much eclosion recorded at doses between 10-100 mg/10 g diet (indicating low/zero toxicity to the parent and the emerged flies) as compared with doses between 250-1000 mg/10 g diet, in which there is no eclosion recorded. It is then established that the safer doses of hibiscus sabdariffa using drosophila melanogaster model is between 10-100mg/ 10g of diet and eclosion can be considered a factor in the determination of lethal concentration in *Drosophila melanogaster*.

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