LD_{50} OF *DROSOPHILA MELANOGASTER* EXPOSED TO DIFFERENT FISH SMOKING METHODS

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ABSTRACT
The aim of this study is to use the Drosophila model for investigations of toxicology study (LD50) of fish exposed to different smoking methods. This experiment was carried out at Animal products and Processing Laboratory, Department of Animal Production University of Jos, Jos Plateau state and the Drosophila Laboratory Africa Center of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria. The lethal dose (LD50) was determined using a standard protocol. Fish were smoked using different smoking methods such as gas, fire wool, charcoal, electric cooker and microwave. Sixty (60) flies (both sexes) of 1-3 day old were anesthetized under light ice, counted and exposed to 10 graded concentrations (10 mg, 50 mg, 100 mg, 200 mg, 300 mg, 400 mg 500 mg, 600 mg, 700 mg, 1000 mg) of the different smoked fish heat method and 1 ml distilled water (control) in 10 gm food for 7 days. The fish smoked with Gas had the highest LD50 (579.70mg) followed by raw fish 416.50mg, fish smoked with Firewood 416.50 mg, Charcoal 396.50mg, fish smoked with Microwave 391.50mg with least LD50 in fish smoked with Electric Cooker 360.20mg. In conclusion, from the result obtained, fish should be smoked with gas heat source because of the higher LD50.
keywords: LD_{50}, *Drosophila melanogaster*, fish, smoking methods and heat source

INTRODUCTION

The study of the lethal dose (LD50) provides valuable insights into the toxicological impact of various substances on organisms, offering a quantitative measure of their potential harm. In the context of this research, the focus is on Drosophila melanogaster, a model organism widely utilized in genetic and toxicological studies due to its short lifecycle and well-characterized genome (Lewis, 1960) . Specifically, this investigation delves into the LD50 of Drosophila melanogaster when exposed to different fish smoking methods, considering the presence of Polycyclic Aromatic Hydrocarbons (PAHs) as potential contributors to toxicity. Fish smoking, a common method of food preservation, involves the use of smoke generated during the combustion of wood or other materials. Processing such as drying and smoking of foods at high temperatures (grilling, roasting, frying) are major sources generating PAH (Guillen *et al.*, 1997; Phillips, 1999). Levels as high as 200 µg/kg food have been found for individual PAH in smoked fish and meat. In barbecued meat, 130 µg/kg has been reported whereas the average background values are usually in the range of 0.01-1 µg/kg in uncooked foods. The composition of this smoke varies depending on factors such as the wood type, combustion conditions, and smoking duration, leading to the formation of PAHs, known environmental pollutants with potential health implications (Kumar et al., 2019) . While fish smoking is generally regarded as a traditional and effective means of preservation, there is a dearth of research examining the potential toxicological effects of smoked fish consumption, particularly on non-target organisms.

Understanding the LD50 of Drosophila melanogaster following exposure to different fish smoking methods and considering the presence of PAHs is crucial for unraveling the potential hazards associated with the consumption of smoked fish. By employing Drosophila as a model organism, this research aims to provide insights into the lethality thresholds induced by distinct smoking methods and the associated PAH content, thereby contributing to our broader understanding of the potential risks posed by consuming smoked fish products. The goal of this study is to use the Drosophila model for investigations of toxicology study (LD_{50}).

MATERIALS AND METHODS

Experimental Site

This experiment was carried out at Animal products and Processing Laboratory, Department of animal Production University of Jos, Jos Plateau state.

Procedure

A total of fifteen *Oreochromis niloticus with* mean weight 658±25g were purchased. The live weights were taken using digital weighing balance. The fish were gutted, washed thoroughly with water to remove slime and blood; thereafter, the dressed weights were taken. The fish were transferred into a basket for proper draining of water prior to smoking. They were also covered with a muslin cloth to prevent dust and flies.

Smoking process

To ensure uniform smoking, charcoal (charcoal pot), gas heat source, and microwave, the fish was covered with a cardboard box to retain heat from the smoke and prevent dust and flies from contaminating it. The smoked products were measured for moisture loss by weighing them.

Drosophila Stock

The animal stock (Harwich strain of both sexes) used in this work was obtained from the Drosophila Laboratory Africa Center of Excellence in Phytomedicine Research and Development (ACEPRD), University of Jos, Nigeria. The flies were maintained and rare using corn meal food. The food contained, 100 gm of yellow-corn powder, 10 gm dry yeast (inactive and not hydrolyzed), 10 gm of agar-agar powder, 1 gm of methyl-4-hydrogen benzoate dissolve in 10 ml absolute ethanol and portable water adds to 1000 ml. All experiments were carried out using the same batch of corn powder, agar-agar powder, and Yeast. The fly's culture was maintained at 23 + 1°C, relative humidity ≈60% and 12 hours dark and light cycle (Abolaji, 2015).

Determination of 7 days LD_{50}

The lethal dose (LD_{50}) was determined using a protocol previously described by Iorjiim *et al*. (2020). Briefly, sixty (60) flies (both sexes) of 1-3 day old were anesthetized under light ice, counted and exposed to 10 graded concentrations (1 mg, 10 mg, 50 mg, 100 mg, 200 mg, 250 mg 300 mg, 350 mg, 400 mg, 450 mg) of the different smoked fish method and 1 ml distilled water (control) in 10 gm food for 7 days. Cumulative fly death was recorded every 24 hours for the duration of the treatment. The survival rate was subjected to dose-response simulation using GraphPad prism version 8. 0. 2 (623) for LD_{50} determination.

RESULTS

The lethal dose (LD_{50}), defined as the concentration of test material in a normal fly food (media) that cause 50% of fly death in 7 days. The concentration for use in the research was obtained from exposing the flies to ten staggered concentrations of the smoked fish for 7 days. The fish smoked with Gas had the highest LD_{50} (579.70mg) followed by raw fish 416.50mg, fish smoked with Firewood 416.50 mg, Charcoal 396.50mg, fish smoked with Microwave 391.50mg with least LD_{50} in fish smoked with Electric Cooker 360.20mg.

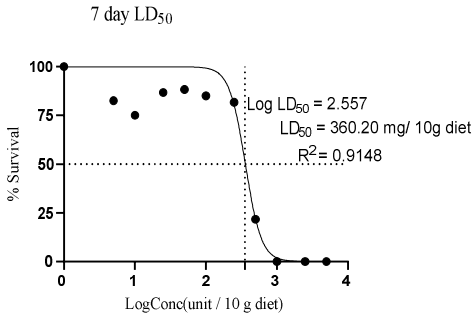

Figure 1. LD_{50} of D. melanogaster exposed to fish smoked with Electric Cooker

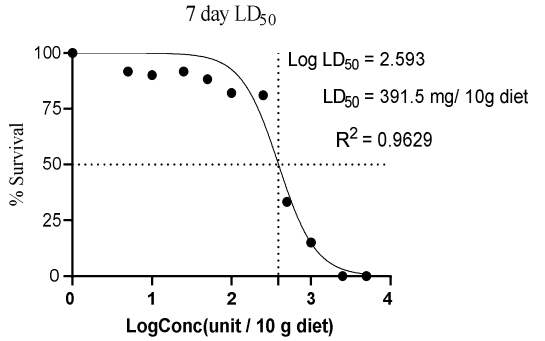

Figure 2. LD_{50} of D. melanogaster exposed to fish smoked with Microwave

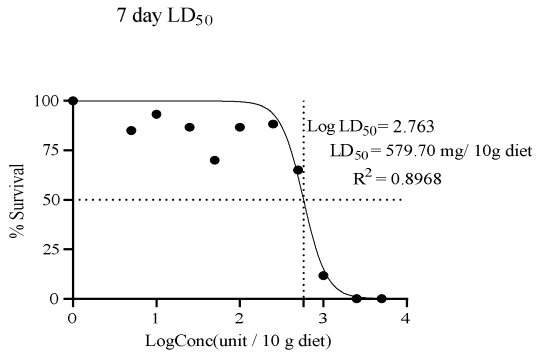

Figure 3. LD_{50} of D. melanogaster exposed to fish smoked with Gas

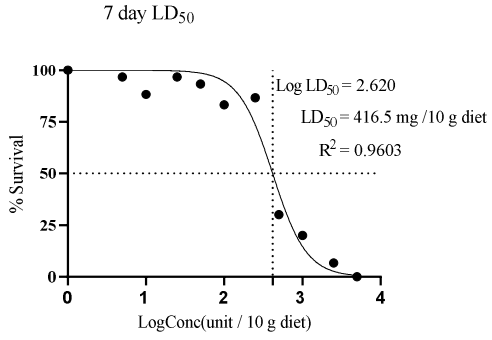

Figure 4. LD_{50} of *D. melanogaster* exposed to fish smoked with Firewood

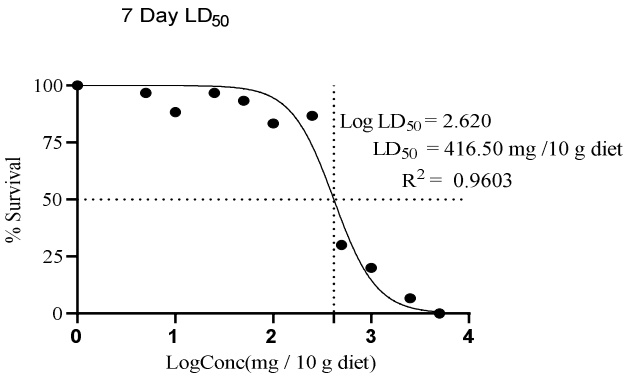

Figure 5. LD_{50} of D. melanogaster exposed to raw fish 416.50mg

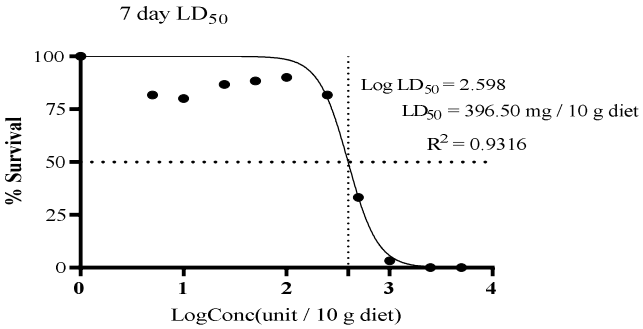

Figure 6. LD_{50} of D. melanogaster exposed to fish smoked with Charcoal 396.50mg

DISCUSSION

Processing procedures, such as smoking and drying, and cooking of food is commonly thought to be the major source of contamination by PAH. Depending on a number of parameters: time, fuel used, distance from the heat source and drainage of fat, type (grilling, frying, roasting), cooking results in the production in the food of a number of compounds including PAH. Although not precisely known, it is likely that there are several mechanisms of formation of PAH such as melted fat that undergoes pyrolysis when dripping onto the heat and pyrolysis of the meat due to the high temperature (Lijinsky and Shubik, 1965 a, b). A comparison of PAH levels in duck breast steaks, undergoing various processing and cooking treatments for 0.5 hour to 1.5 hours, showed that charcoal grilled samples without skin contained the highest amount of total PAH (320 μg/kg), followed by charcoal grilling with skin (300 μg/kg), smoking (210 μg/kg), roasting (130 μg/kg), steaming (8.6 μg/kg) and liquid smoke flavouring (0.3 μg/kg). For PAH that are classified as carcinogenic (IARC class 1 or 2 A and B), the trend was the same with the exception that smoked samples contained the highest amount (35 μg/kg). In addition, the highest amounts of total and carcinogenic PAH were observed after smoking of duck breast samples for 3 hours (53 μg/kg) (Chen and Lin, 1997).

In the result obtained for this study, it shows that gas heat source is the best heat source for smoking fish for better health due to its higher LD_{50} values obtained while the microwave and electric heat source are not good heat source for smoking fish due to their lower LD_{50} values which is due to the impact of higher wave exposure which cold lead to higher PAH

CONCLUSION

In conclusion, from the result obtained, fish should be smoked with gas heat source because of the higher LD_{50} which shows a lower level of toxicity.

REFERENCES

Abolaji A. O. (2015) "Ovotoxicants 4-vinylcyclohexene 1,2-monoepoxide and 4-vinylcyclohexene diepoxide disrupt redox status and modify different electrophilesensitive target enzymes and genes in Drosophila melanogaster," Redox Biol., vol. 5, no, pp. 328–339, 2015.

Chen, B.H., and Lin, Y.S., 1997. Formation of Polycyclic Aromatic Hydrocarbons during processing of duck meat. *J. Agric. Food Chem.*, 45, 1394-1403.

Guillen, M.D., Sopelana, P., and Partearroyo, M.A. (1997). Food as a source of polycyclic aromatic carcinogens. Rev. Environ. Health, 12, 133-146.

Lijinsky, W., and Shubik, P., 1965a. Benzo(a)pyrene and other polynuclear hydrocarbons in charcoal-broiled meat. Science, 145, 53-55.

Lijinsky, W., and Shubik, P., 1965b. Polynuclear hydrocarbon carcinogens in cooked meat and smoked foods. Ind. Med. Surg., 34, 152-154

Iorjiim W.M., Omale S., Etuh M.A, Bagu G.D., Ogwu S.O. and Gyang S.S. (2020). EFV b-HAART increases mortality, locomotor deficits and reduces reproductive capacity in Drosophila melanogaster. *J. Adv. Biol. Biotechnol*.;23(1):26–38

Lewis, E. B. (1960). A new standard food medium. Drosophila Information Service, 34, 117-118.]

Kumar, S., Khillare, P. S., and Agarwal, T. (2019). Source apportionment and health risk assessment of PM2.5-bound polycyclic aromatic hydrocarbons in ambient air of Lucknow, India. Environmental Science and Pollution Research, 26(8), 7823-7835.]

Nichols C.D., Becnel J. and Pandey U. B. (2012). Methods to assay Drosophila behavior. *J. Vis. Exp*. 61:1–5.

Phillips, D.H., Hewer, A., Seidel, A., Steinbrecher, T., Schrode, R., Oesch, F., and Glatt, H. (1991). Relationship between mutagenicity and DNA adduct formation in mammalian cells for fjord- and bay-region diol-epoxides of polycyclic aromatic hydrocarbons. *Chem. Biol. Interact*., 80,177-186.

Sonoshita M. and Cagan R.L. (2017). Modeling human cancers in Drosophila, 1stEd. Elsevier Inc.;121.