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Survival Rate, Total Antioxidant Capacity and Malondialdehyde in *Drosophila melanogaster* Exposed to Bronze Borne Soil Samples in Benin City, South-South Nigeria

O.G. Igharo and E.O. Oloyede

[1]Department of Medical Laboratory Sciences, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.

*Corresponding author Email: osaretin.igharo@uniben.edu. Tel: +234 (0) 803 866 4898

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ABSTRACT: *Drosophila melanogaster* serves as an important research model organism in the fields of genetics and developmental biology. Recent studies have indicated the use of *Drosophila* in a number of other studies such as metabolic, nanotoxicological and immunological studies. Rearing of fly in the laboratory condition is an important task to work with this organism. To maintain the growth and development of the flies where the components of the food play important roles, this is done by mixing the compounds with fly food in proper concentration. *Drosophila* used in various toxicological or metabolic studies often involve oral intake of various nanoparticles, pathogens and molecules to study metabolism. This present study aimed at investigating the survival rate, total antioxidants capacity and malondialdehyde levels in *Drosophila melanogaster* exposed to bronze borne soil sample. A comparative cross-sectional study was matched in the parameters, the work was carried out on bronze borne soil sample collected from various locations; Igun foundry site, environmental and University of Benin to check the total antioxidant and malondialdehyde level, the methods employed was Enzyme Linked Immunosorbent Assay (ELISA) and Kinetic method (MDA/TBARS). Data were analysed using T test at 0.05 and were represented with the reference range. The result obtained showed that total antioxidant capacity was high in factory bronze soil sample compared to other soil samples which indicate high oxidative stress in *Drosophila* exposed to it. Total antioxidant capacity and malondialdehyde shows no significant variation and value. From the study it was observed that the level of total antioxidants capacity (marker for oxidative stress) was significantly higher and malondialdehyde (marker for lipid peroxidation) in *Drosophila melanogaster* was significantly lower when exposed to bronze borne soil sample. The survival rate of *Drosophila melanogaster* decreased with exposure to the concentration of bronze borne soil sample with high level of oxidative stress.

Keywords: Total Antioxidant Capacity, Malondialdehyde, Bronze borne soil sample, Drosophila melanogaster.

Introduction

Drosophila melanogaster is a species of fly in the family Drosophilidae. The species is often referred to as the fruit fly or lesser fruit fly commonly the "vinegar fly" or "pomace fly". It is one of the most intensively studied organisms in biology and serves as a model system or investigation of many developmental and cellular processes common to higher eukaryotes, including humans (Adams *et al.*, 2000).
Drosophila melanogaster has extensively been used as a robust model organism in genetics, developmental biology, aging, and other area of biomedical research, the genome contains both unannotated coding and non-coding genes, transcripts, exons and RNA editing sites, it is an attractive model for organism for studying the

mechanism of aging due to its relatively short lifespan, convenient husbandry and facile genetics. *Drosophila melanogaster* offers studies for Alzheimer's, Parkinsons and motor neuron diseases as well as models for trinucleotide repeat expansion diseases, including ataxias and Huntington's disease (Frank, 2010).

Bronze is an alloy and a golden hard, it has a reddish- brown colour with a brittle metal which compose typically modern bronze of 88% copper and 12% tin. It has high resistance to corrosion from saltwater and a melting point of 950^0C. Alpha bronze consists of the alpha solid solution of tin and copper. Alpha bronze alloys of 4-5% tin are used to make coins, springs, turbines and blades, some alloys contain hazardous metals such as Cadmium, Lead, Mercury, Arsenic etc. a typical characteristics of bronze is high ductility, it exhibits low friction against other metals, it display unusual property of expanding a small amount when solidifying from a liquid into solid (Helmensine and Anne, 2020).

In bronze casting environments, particulate matter is typically formed by metallic vapor condensation followed by oxidation reactions. Lead (Pb), mercury (Hg), cadmium (Cd) and Arsenic (Ar) serves as the main pollutants. Thus, foundry workers are exposed to different types of metals. Exposure to these metals results in pro-oxidant/antioxidant imbalance and can act as an intermediate in the formation of an oxidative stress state; levels of Malondialdehyde (MDA), the markers of lipid peroxidation increases and the activity of glutathione (GSH) enzyme decrease, the degree of oxidative stress could be affected also.

Total antioxidant capacity is the collaborative effect of all sample components i.e both (Synergistic or antagonistic effects) measured which depends on several factors, such as the rate of reaction between the reactive species and the concentration ratio between antioxidant and target, it acts by delaying or preventing the oxidation of some other chemicals, they are classified into enzymatic and non-enzymatic (Niki and Noguchi, 2000).

Malondialdehyde is an organic compound with the nominal formula $CH_2(CHO)_2$. It is a colourless liquid with highly reactive compound that occurs as the enol which occurs naturally and a marker for oxidative stress in health problems such as cancer, psychiatry, chronic obstructive pulmonary disease, asthma or cardiovascular diseases. Malondialdehyde increases with age and it is a stable end product of lipid peroxidation that can be used indirectly to measure the cumulative lipid peroxidation (Ho *et al.,* 2013).

Materials and methods

Experimental design: For the survival assay, flies of both gender of 1-3 days old were divided into four (4) groups with each group having 5 vials, each vial contained 35 flies with varied concentration of sand:

Group 1: F1-F5: Flies fed with sand from Igun factory in proportion of 100%-20% i.e (200 uL of sand extract + 0 uL of water) - (40 uL of sand + 160 uL of water)

Group 2: E1-E5 Flies fed with sand from Environmental Igun (100 % - 20 %)

Group 3: U1-U5 Flies fed with sand from Uniben as control (100 % - 20 %)

Group 4: N1-N4 Flies fed with normal meal without sand

The survival assay was carried out in three replicates of each concentration, the diet was changed every 7 days, during the period of the experiment. The survival rate was determined with all the concentration and both live and dead were recorded daily for 21 days. By the end of the experiment (21 days), the data obtained were accumulated and plotted as percentage of live and dead flies. The result was compared with the control.

Tissue homogenate preparation for biochemical assay: For the determination of biochemical assays, a second group experiment was carried out. In this experiment both gender flies were divided into five groups with each group having 4 vials each. Each vial contained 35 flies with vary concentration of sand:

Group 1: F1-F5: Flies fed with sand from Igun factory in proportion of 100%-20% i.e (200uL of sand extract + 0uL of water) - (40uL of sand + 160uL of water)

Group 2: E1-E5 Flies fed with sand from Environmental Igun (100%-20%)

Group 3: U1-U5 Flies fed with sand from Uniben as control (100%-20%)

Group 4: N1-N4 Flies fed with normal meal without sand

Total antioxidant capacity detection principle: A variety of antioxidants macromolecules, antioxidant molecules and enzymes in a system can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species. The total level reflects the total antioxidant capacity in the system. Many antioxidants in the body can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form a stable complex with the phenanthroline substances. The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520 nm.

Sample analysis: Analysis of total antioxidant capacity and malondialdehyde was carried out using enzyme linked immunosorbent assay (ELISA) and kinetic method (MDA/TBARS) respectively.

Principle of MDA/TBARS: MDA which is formed from the breakdown of polyunsaturated fatty acids serves as a convenient marker for the determination of the extent of lipid peroxidation. Assays is based on the reaction of

MDA with thiobarbituric acid (TBA), forming a MDA-2TBA adduct (Pink-red colored complex) that absorbs light at 535 nm.

Results

Survival rate and curve: Table 1 shows the survival rate of drosophila flies at different exposure to soil samples. It was deduced that all flies fed with factory soil had a significant and rapid death rate at the end of 21 days. The control flies fed with University of Benin soil sample and negative control had a rapid death rate at the beginning, however, have the highest level of survival after 21 days.
From the survival curve, the control sample has highest number of survived *Drosophila* at the end of survival study, while Drosophila with factory soil sample has the lowest survived rate compared to other groups (Environmental, Uniben and Negative Control).

Table 1: Survival curve of *Drosophila melanogaster* exposed to Bronze borne soil sample

Parameters (S.I Units)	Factory Sand	Environmental Sand	Uniben Sand	Negative control	F-value	P-value
TAC u/mL	$2.624^{B} \pm 0.73$	$3.386^{B} \pm 0.24$	$4.610^{A} \pm 0.51$	$3.565^{A} \pm 0.13$	2.93	0.049
MDA mmol/mL	0.016± 0.00	0.034 ± 0.00	0.014 ± 0.00	0.024 ± 0.00	3.00	0.064

Keys: Values are expressed as Mean ± SEM, **A** = High statistical value indicates no oxidative stress, **B** = Low statistical value indicates oxidative stress. **TAC** = Total Antioxidant Capacity, **MDA** = Malondialdehyde

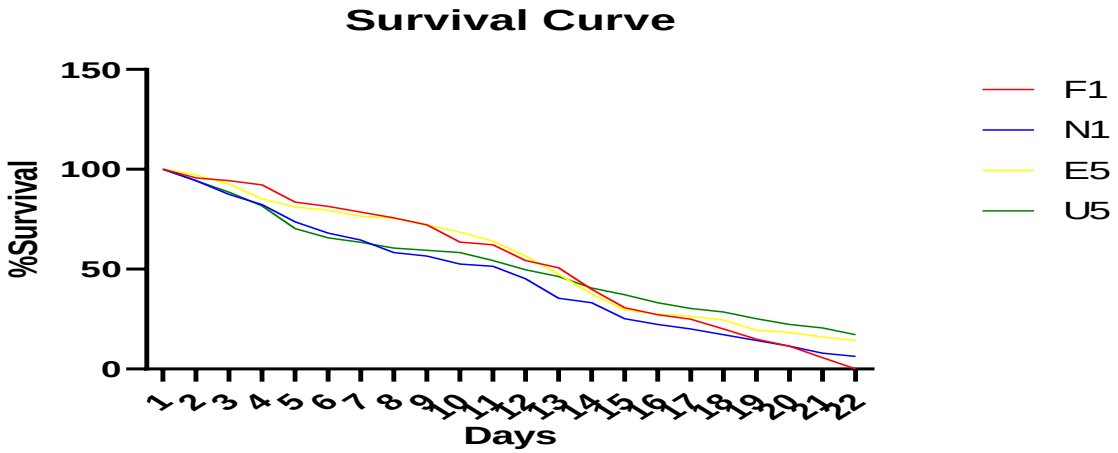

Figure 1: Survival curve of *Drosophila melanogaster* exposed to Bronze borne soil sample
Key: F = Factory Soil Sample, **N** = Negative Control, **E** = Environmental Soil Sample, U = University of Benin Soil Sample

Discussion

Drosophila melanogaster as a research model and choice of organism in genetics, developmental biology, toxicology and biomedical research has become a versatile, emerging and developmental model alternative to mammalian model. It has gained acceptance as a useful model for studying the cause of its similar gene (homologous) to humans which help in studying human diseases and gene despite its small body size.
Drosophila melanogaster is one of the alternative invertebrate models useful for genetics, cell biology, biochemistry and developmental biology (Abolaji *et al.,* 2013). It has been used to elucidate human diseases and recently adopted for toxicological studies (Wangler *et al.,* 2015). On the success achieved in its use in experimental studies, it met the standard of European Center for Validation and Alternative Method (ECVAM); reduction, refinement and replacement of the usage of laboratory animals.
Bronze use has created large surpluses and gave rise to complex societies, cities, urbanization and increase the social and gender inequalities that came with the discovery of Agriculture. Total antioxidant capacity is used to

assess the amount of free radicals scavenged by a test solution, being used to evaluate the antioxidant capacity of biological samples (Bartosz, 2010; Pinchuk *et al.,* 2012).

This study was designed to evaluate the survival rate, levels of total antioxidant capacity and Malondialdehyde in *Drosophila melanogaster* exposed to bronze borne soil samples collected from (Igun foundry site, Environmental) and University of Benin soil sample.

The analysis of data obtained in this study shows that the soil sample used to detect the levels of total antioxidant capacity and Malondialdehyde in factory bronze borne soil sample and control soil sample were matched, this eliminates any potential confounding effects from the study. Nutritional status of groups used was similar and the nutritional status observed in the groups of participants was pertinent to the confounding influence of nutrition on the data obtained in this study. Results demonstrated that oxidative stress damage plays a role in governing the lifespan of *Drosophila* during normal metabolism and under conditions of environmental stress (Fleming *et al.,* 1992).

From the survival study, it was observed that flies fed with varying concentration of bronze borne soil sample resulted in high death rate in factory group than the control group, which may be due to the presence of the component of bronze and heavy metal concentration (Selenium, copper and zinc) present in the factory soil sample. This suggest that there was a lower survival of flies fed with the bronze borne soil sample due to oxidative stress than the regular meal administered to the control group. From this, it can be deduced that bronze borne soil sample had a modulatory effect on the survival rate of flies exposed to it. The survival rate of *Drosophila* indicated that Uniben soil sample used as control has the lowest death rate, while *Drosophila* fed with environmental and factory soil sample has the highest death rate.

In this study, it was observed that the total antioxidant capacity has a varying effect on the *Drosophila melanogaster* in Uniben and control soil sample which indicates no significant difference, but a statistical difference compared to factory and environmental soil sample. It implies a higher level and accumulation of oxidative stress in factory soil sample as a result of exposure of *Drosophila* to it (Sotibran *et al.,* 2011). Total antioxidant is necessary because it evaluates antioxidants response against free radicals produced in a given diseases such as Diabetes, Alzheimer, Cancer, Parkinson diseases and other diseases (Sies, 2007). It deduced excessive reactive oxygen species generation which triggers programmed cell death that attacks the DNA causing oxidative damage through base modification, strand break and DNA protein crosslink supported by Akram *et al.,* (2019).

High level of Malondialdehyde was observed in environmental soil sample compared to other groups, which indicates possible high exposure of free radicals, toxic molecules, lipid peroxidation and oxidative stress compared to other group (Uniben, Factory and Negative control). However, the increase of Malondialdehyde support the hypothesis of increase oxidative stress in the body (Weiss and Landauer, 2014). Meanwhile, decrease level of Malondialdehyde was observed in factory soil sample which indicate no significant difference and a varying low lipid peroxidation and free radical exposure in *Drosophila melanogaster.*

Conclusion

From the study, it can be observed that the survival rate of *Drosophila melanogaster* on synthetic medium containing the soil concentrations shows a significant reduction in their growth and development or metamorphosis for most soil extracts. The survival rate of *Drosophila melanogaster* decreased with exposure to the concentration of bronze borne soil sample with high level of oxidative stress.

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