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To Study the Cytotoxic effect of bleaching powder (Calcium hypochlorite) on *Drosophila melanogaster*

Shantanu Sonune[1], and Sangeeta Sinha*[2]

[1]Shantanu Sonune , P G Student, Department of Zoology, Nowrosjee Wadia College, Pune, shantanusonune8490@gmail.com
*[2]Sangeeta Sinha, Assistant Professor, Department of Zoology, Nowrosjee Wadia College, Pune, sangeeta.amma@gmail.com

Abstract: ***Drosophila melanogaster*** **(fruit fly) can prove an ideal model organism for the toxicological studies and risk assessment, as it bears striking similarities to various systems in human body. *Drosophila* is also useful for studying potential toxic effects of different organic and inorganic compounds. Bleaching powder, an inorganic compound is frequently used for cleaning and disinfection; bleaches kill or control most types of bacteria, flies etc. In the present study bleaching Powder, calcium hypochlorite was investigated for its cytotoxic potential and its effect on biological molecules in drosophila. Various concentrations (0, 2.5, 5, 7.5, and 10%) of the bleaching powder were mixed with food and fed to the flies. Biochemical assays (Protein and Lipids) were performed to check the amount of oxidative stress caused to biomolecules. The present study reveals that prolonged exposure to bleaching powder at higher concentration causes toxicity which in turn affects the hatching and life cycle of drosophila. Concentration dependent significant increase in lipid peroxidation and hydro peroxides; Protein carbonyl and sulphydryls were observed in treated flies. Long term exposure to bleaching powder can have significant effect on the survival of flies' population even if its concentration is low and it may also reflect population adaptive capacity.**

Index Terms: **Drosophila, Bleaching Powder, Lipid peroxidation, Protein oxidation**

I. INTRODUCTION

Pesticides are widely used are persistent to most of the pests but they cause important problems for non-target living beings by means of food chain. Many studies have indicated that insecticides and degradation products have carcinogenic effects on humans and other living beings (Swarup, et al., 2013). Active contents and metabolites of these chemical materials disperse into the environment and harm other living beings than the target organism (Inamdar, et al., 2013; Rand, 2010). Chlorine is the major component present in the form of calcium hypochlorite $Ca(ClO)_2$ seen in bleaching powder. Calcium carbonate and chlorine gas when bleaching powder is exposed in air to it reacts with carbon dioxide gas present in air To disinfect water and to kill diseases causing pathogens such as bacteria, viruses and protozoans chlorine is widely used now a days. Many civic bodies and government departments are found sprinkling bleaching powder as flies repellent due to lack of awareness.

The *Drosophila melanogaster* (fruit fly) is one of the most extensively studied model organisms. The model at various development stages, such as embryo, larva, and adult fly, has been used to test the toxicity of chemicals, including industrial volatile organic compounds, heavy metals and anesthetic gases (Rand, et al., 2013). Fruit flies have been used to explore the mechanisms and genetics underlying the susceptibility to ethanol intoxication, to document the olfactory avoidance behavior ,to identify neurotransmitter pathways affected by volatile fungal toxins (Inamdar, et al., 2013) and to quantify the changes in the metabolic rate during exposure to components of gasoline[6,] . It is recognized as a powerful biological model, among many kinds of animal models in the cytotoxic studies. Using drosophila as model system, many studies show chemical toxicity are closely related to metabolic rate, cell size , feeding preferences and body mass (Pappus, et al., 2018; Yiwen, et al., 2021) . This is due to its highly dynamic and well-characterized morphology changes during the process of embryogenesis.

Animal behaviour depends upon the integrated processes at the different levels, which is susceptible to disruption by a broad spectrum of chemicals and environmental, stresses (Asante-Duah K., 2017). Behavioral changes are therefore considered an important indicator of chemical toxicity because it reflects the alteration in integrated physiological condition. Behavioral changes in water flea, *Daphnia magna* due to chemical have

been investigated in environmental toxicity tests in continuous water quality monitoring (Kieu, et al., 2001; Tyagi, et al., 2007).

In the present study, we investigated the potential toxicity of a bleaching powder in a fruit fly *D. melanogaster* model. This model has an advantage over others as it raises few ethical concerns and has served as a unique and powerful model to study toxicity of various chemicals, screening synthetic and natural compounds with respect to diseases and cure.(Kim, et al., 2013). Particularly, we investigated the behavioral changes and damage to lipids (peroxides and hydro peroxides) and proteins (carbonyls and sulphydryls) on *D. melanogaster* exposed to bleaching powder

II. MATERIAL AND METHOD

A. Drosophila Culture

The wild-type Drosophila melanogaster strain was maintained in the laboratory on a standard cornmeal, yeast, dextrose, and agar medium at 25°C (Ng et al., 2019). Eggs were collected from these flies by shaking them without anesthesia into bottles containing an approximately 2 cm layer of fermenting fresh baker's yeast supplemented with sucrose The egg collection were kept in the dark for 8 hours at 25ºC. After removing the parental flies, the egg collection bottles were taken back to 25ºC where they remained at a relative humidity of 65% for the rest of their development. 72 h larvae were collected after 3 days by washing them out the bottles with tap water through a fine-meshed stainless steel strainer.

B. Preparation of bleaching powder

Bleaching Powder was purchased from Himedia, India. Different concentrations (0, 2.5%, 5%, 7.5% and 10%) of bleaching powder were used for the experiment.

C. Estimation of percent mortality

The larvae were transferred to vials (20 larvae/vial) containing 0.5 g of *Drosophila* Instant Medium (Carolina Biological Supply Co, NC, USA) prepared with the solutions of the test compounds, Bleaching powder at 0 to 10 % concentration were used. Five replications were made for each concentration in five independent experiments for each bleaching powder. The experimental vials were kept at 25ºC and at a relative humidity of 65%. The surviving flies were collected from the vials on days 10 to 12 after egg laying and shaken into a flask containing 70% ethanol to quantify mortality.

Mortality formula of a defined population, over a specified period of time, is:

$$\frac{\text{Death occurring during a given time period}}{\text{Size of the population among which death occur}} \times 100$$

D. Quantitating oxidative damage to biomolecules

(i) Oxidative damage to lipids in drosophila induced by bleaching powder at different concentration was measured in terms of nmoles of malondialdehyde equivalents formed (Devasagayam, 1986) and expressed as nmoles of TBARS/100 mg tissue and Lipid hydro peroxides (Anna, et al., 2000) was measured in terms of nmoles of LOOH equivalents formed, and expressed as nmoles of LOOH formed / mg protein.

(ii) Oxidative damage to proteins carbonyls is based on the reaction of carbonyl groups with 2, 4-dinitrophenylhydrazine (DNPH) to form a 2,4- dinitrophenylhydrazone, which can be measured at 366 nm. Amount of carbonyls formed was expressed as nmoles of protein carbonyls formed/100 mg tissue (Palamanda and Kehrer, 1992) and protein sulphydryls were quantitated using Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid) and expressed as nmoles of protein sulphydryls/100 mg tissue (Ellmann, 1959).

E. Statistical analyses

The LC50's were calculated using logistic regression with all five replications of every concentration. LC50's obtained from the five experiments were analyzed with a two-way ANOVA. Experiments were repeated at least five times and data presented is average of these replicates.

III. RESULT AND DISCUSSION

From many decades man's battle with insects to control and eradication of structure dwelling insects has been focused on chemical poisons (Mawdsley, 2011). Many chemicals acts as insecticides and has long lasting residual insecticidal properties (VoPham, et al., 2017), DDT, a well-known effective insecticide, having residual insecticidal properties for many days. Other insecticides may be highly toxic to insects, but are also highly toxic to humans, pets and warm blooded animals generally, or have very serious ecological impact (Wang, et al., 2021). *Drosophila is* best known multicellular eukaryote model organism which helps to study the interactions between genes and environmental clues simultaneously. Recently, there have been successful attempts to use this species to investigate the effect of a certain type of diet on viability and lifespan (Erkosar et al., 2013; Khan et al., 2017). Radia et al, 2015 have shown Drosophila as an excellent model system to evaluate lethal concentration and their effect of different chemical or bioactive substances such as, growth and molting disruption effects of azadirachtin. The effect of Aspirin and acetaldehyde on longitivity and metamorphosis duration was studied by Duygu keser and Ayla Karata., 2012.

Drosophila is reared on yeast-agar-corn meal medium for three generations and them these flies were transferred into Drosophila culture bottles containing different concentrations of the bleaching powder., this gives an advantage that progeny get exposed throughout there lifecycle to bleaching powder and

control was reared in medium without bleaching powder the same method used by Mathew Endre et al.,2018.In the present study, an attempt has been made to document the short term toxicity of commonly used commercial bleaching powder to the *Drosophila melanogaster.*

Morphological Changes in Drosophila Life cycle

Drosophila exposed at different concentrations of bleaching powder ranging from 0 to 10 % showed significant morphological changes in its life cycle. Control drosophila showed no significant change in the morphology and percent mortality. Bleaching powder (2.5%) showed significant mortality and the size of emerged larva was almost half compared to control larva. Bleaching powder (5 %) showed significant mortality and the size of emerged larva was almost 3 times reduced compared to control larva. At 7.5% Bleaching powder significant mortality was observed at the larval forms than the control larval forms. Also, the delay in the emergence of larval forms was observed compared to control. Interestingly at 10% of bleaching powder the life cycle of drosophila was ceased at the stage of eggs which was observed till 15 days. This may be due to inhibitory effect of bleaching powder on gonadal development. Imbalanced endocrine system or inhibition of ovarian development or deformities in oviposition organs results in the inhibition of oviposition (Phoebe et al., 2002). Gireesh Nadda et al., 2005 reported reduced fecundity rate was observed by the effect of beta-cyfluthrin,

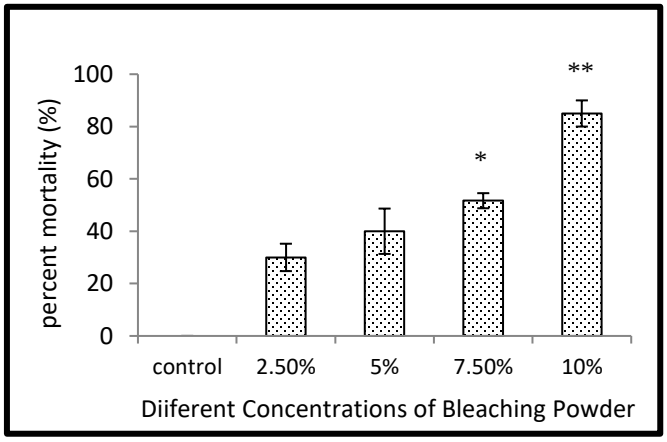
At the concentration 10% of bleaching powder the eggs were not able to hatch from the egg this is may be due to direct impact of bleaching powder various tissue such as follicular cells, trophocytes, perifollicular cells and oocyte themselves (Soltani et al., 2016) or hormonal imbalance (Gireesh Nadda, 2005). In the present study it was observed that the egg hatching process and ovicidal action was decreased due to accumulation of bleaching powder in eggs results in their direct death. The eggs which laid but do not hatched, are may be result of inappropriate incorporation of the yolk so that the embryo failed to complete metamorphosis or may be due antifeedant effect of bleaching powder resulted in weak and non -viable egg production

To measure and evaluate the median lethal concentration (LC_{50}) and percent mortality

LC_{50} is defined as the lethal concentration at which 50 % of the population if killed in a given period of time. There can be wide range of tolerance to toxic agents among different population of a species which should be taken into account. Variability in acute toxicity depends on the size, age and condition of the test species along with experimental factors such as changes in water quality and test species (Sadeghi et al., 2018).

In the following experiment the drosophila was treated with different concentrations of bleaching powder ranging from 0 to 10 %. At the different concentrations of bleaching powder viz 0, 2.5, 5, 7.5 and 10% percent mortality were 0, 30, 40, 52 and 85 % respectively. Figure 1 shows the mortality rates and LD50's for bleaching powder was 7.35% (Figure 1). On the other hand, the ANOVA results show significant differences between control ($F = 38.21$, $p = 0.0013$) and bleaching powder interaction ($F = 9.62$, $p = 0.0032$). Results revealed an increase in mortality rates directly proportional to increase in different concentrations of bleaching powder (Figure 1). The similar result was observed by insecticide beta cyfluthrin on drosophila studies (Gireesh Naada et al., 2005). Results revealed a sigmoid type curve with an increase in mortality rates directly proportional to all different concentrations of bleaching powder.

Figure 1: Percent mortality of drosophila melanogaster exposed to different concentrations of bleaching powder



All experiments were repeated at least five times and data presented is average of these replicates. Results are mean ± SE of 20 drosophila used in each group. ** Statistically significant ($p<0.001$) compared to control group; * statistically significant ($p<0.05$) compared to control group

Oxidative damage to lipids:

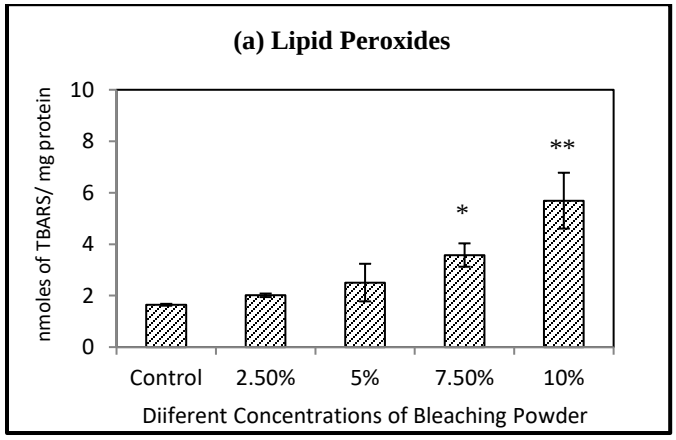
Formation of reactive oxygen species (ROS) causes tissue dysfunction and damage in a number of pathological conditions. ROS oxidize the lipids to generate hydro peroxides and peroxides. The lipid peroxidation (LPO) products are highly reactive which causes alterations in cell signalling, protein and DNA damage, and cytotoxicity (Ramana et al., 2013).

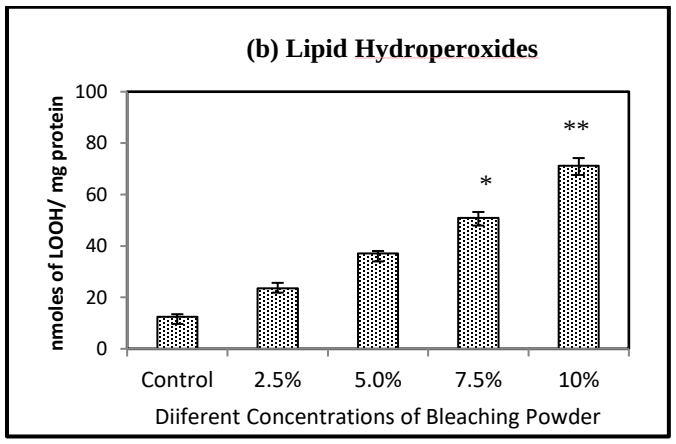
Oxidative damage caused to lipids due to bleaching powder was estimated in terms of thiobarbituric acid reactive substances (TBARS) and is expressed as nmoles of TBARS formed/mg protein. The lipid peroxidation (amount of TBARS/mg) was significantly ($p<0.001$; 10 %) increased with increasing concentration of bleaching powder as compared to control. Amount of lipid peroxidation was (1.6 ± 0.04 ; 2.0 ± 0.07; 2.51 ± 0.7; 3.58 ± 0.4 and 5.7 ± 1.0 nmoles of TBARS formed/mg protein at bleaching powder concentration at control, 2.5%, 5% , 7.5% , 10 %, respectively (Figure 2a). Products of lipid

peroxidation as a factor in the toxic effect of silver nanoparticles (Patrycja et al., 2020).

Lipid hydro peroxides (LOOHs) are prominent non-radical intermediates of lipid peroxidation whose identification can often provide valuable mechanistic information, i.e whether a primary reaction is mediated by singlet oxygen or oxyradicals (Girotti ; 1998; Antonio et al., 2014) . Amount of LOOH formed /mg protein (Figure 2b) was significantly ($p<0.05$) increased with an increase in the concentrations of the bleaching powder. At 10 % concentration of bleaching powder the amount of lipid hydro peroxides formed were 5.7 times more (71.23 ± 2.9 nmoles of LOOH formed/ mg protein; $p<0.001$) and at 7.5 % lipid hydro peroxides were 4.1 times more (51 ± 2.2 nmoles of LOOH formed/ mg protein; $p<0.05$) compared to control (12.4 ± 1.1 nmoles of LOOH formed/ mg protein) respectively.

Figure 2: Effect of bleaching Powder on the amount of Lipid damage under different conditions





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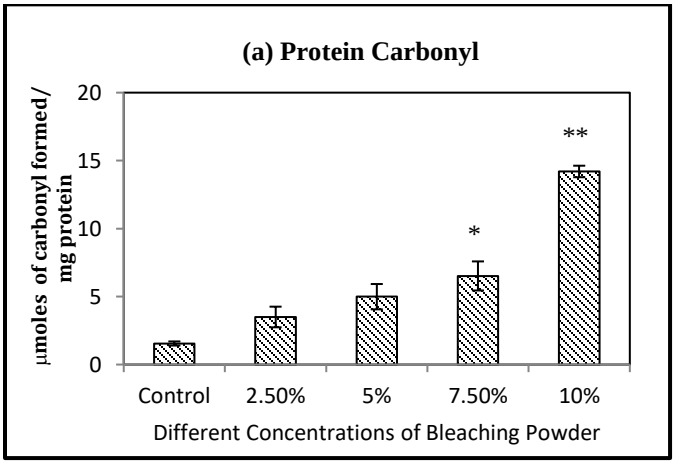
Oxidative damage to proteins

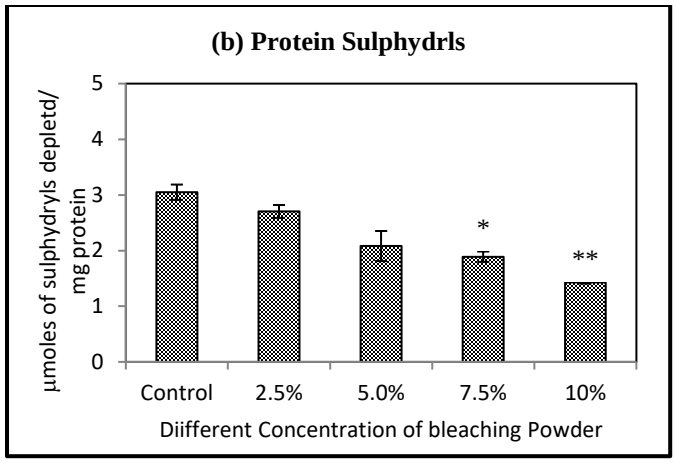
Reactive oxygen species (ROS) may either react directly with some amino acid residues or lead to oxidative cleavage of the protein backbone. (Vaishali et al., 2017) Therefore most often used procedure to detect protein carbonyls is after their derivatization with DNPH.(2,4-Dinitrophenylhydrazine).

Amount of protein carbonyl was significantly ($p<0.001$; 10 %) increased in concentration dependent manner with an increase in concentration of bleaching powder as compared to control. It was found that protein carbonyl concentration was 1.6 ± 0.15; 3.5 ± 0.8; 4.9 ± 0.9; 6.5 ± 1.0 and 14.20 ± 0.4 µmoles of protein carbonyl formed/mg protein at bleaching powder concentration at control, 2.5% , 5% , 7.5 % , 10 %, respectively (Figure 3a). Similar result was observed by Suhel Parvez and Sheikh Raisuddin (2005) as oxidative stress-inducing pesticides in freshwater fish Channa punctate.

Sulfhydryl (SH) is ubiquitous in peptides and proteins throughout the body. Molecules with SH groups are referred to as thiols or mercaptans (Nordberg et al., 2015). Proteins containing Cys residues are abundant throughout the body, both in enzymes, organelles, and in intracellular and extracellular membranes. Since most SH groups are important for the function or structure of numerous proteins. Presence of intact disulfide linkages appears to be necessary to the physiological behavior of other active principles such as insulin, the appearance of free -SH groups coinciding with loss in activity (Brandes et al., 2009). At 10 % concentration of bleaching powder the amount protein sulphydryl formed was 2.1 times significantly ($p<0.001$) decreased (1.4 ± 0.02 µmoles of protein sulphyryl depleted/mg protein) and at 7.5 % amount of protein sulphydryls were 1.8 times significantly ($p<0.05$) less (1.7 ± 0.2 µmoles of protein sulphyryl depleted/mg protein) compared to control (3.1 ± 0.1 µmoles of protein sulphyryl depleted/mg protein) respectively(Figure 3b). Ajsuvakova et al., 2020 also observed sulfhydryl groups as targets of mercury toxicity.

Figure 3: Effect of bleaching Powder on the amount of Protein damage under different conditions





All experiments were repeated at least five times and data presented is average of these replicates. Results are mean ± SE of 20 drosophila used in each group. ** Statistically significant ($p<0.001$) compared to control group; *statistically significant ($p<0.05$) compared to control group.

CONCLUSION

The present study indicates that bleaching powder induced alterations in the Lipid and Proteins biomolecules of the drosophila at acute concentration of bleaching powder. These alterations can be considered as a tool for biomonitoring of toxic substances in the environment. Further studies are needed to understand the risk of bleaching powder using different end points. This novel study will provide such effects can be risky as they could damage the demographic structure of the population in non-target species.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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