# SODIUM BENZOATE: FOOD PRESERVATIVE OR TOXIN? A DROSOPHILA MELANOGASTER PERSPECTIVE

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**Keywords:**

Developmental Toxicology, Drosophila melanogaster as a Model Organism, Neurobehavioral Alterations, Sublethal Toxicity Assessment, Food Preservative-Induced Oxidative Stress.


**Introduction:**

In the realm of modern food production, food additives play a crucial role in enhancing taste, maintaining texture, and ensuring product longevity. Among these, sodium benzoate (SB) stands out as a widely used synthetic preservative, particularly in acidic food items like sodas, sauces, jams, and pickles. Although it has received a "Generally Recognized as Safe" (GRAS) classification from the FDA, growing concerns about its long-term effects on health have sparked intense scientific scrutiny. Sodium benzoate is prized for its antimicrobial properties, which help inhibit the growth of yeasts and moulds, making it an essential component in the food, cosmetic, and pharmaceutical industries.

However, its potential links to genotoxicity, behavioural disorders, and metabolic disturbances have raised red flags. As processed food consumption increases globally, the need for a rigorous assessment of additive safety has become paramount. This project investigates the impact of sodium benzoate on biological systems, using the fruit fly, *Drosophila melanogaster*, as a model organism. Owing to its genetic similarity to mammals, rapid lifecycle, and ease of maintenance, *Drosophila* serves as

a powerful tool to explore biochemical and physiological changes induced by chemical exposure.

By examining the effects of sodium benzoate on *Drosophila*'s development, metabolism, and reproduction, this study aims to provide valuable insights into the broader implications of food preservative consumption on human health. Understanding these effects at a molecular and cellular level can contribute to improved regulatory practices and the development of safer food alternatives.

**Objectives:**

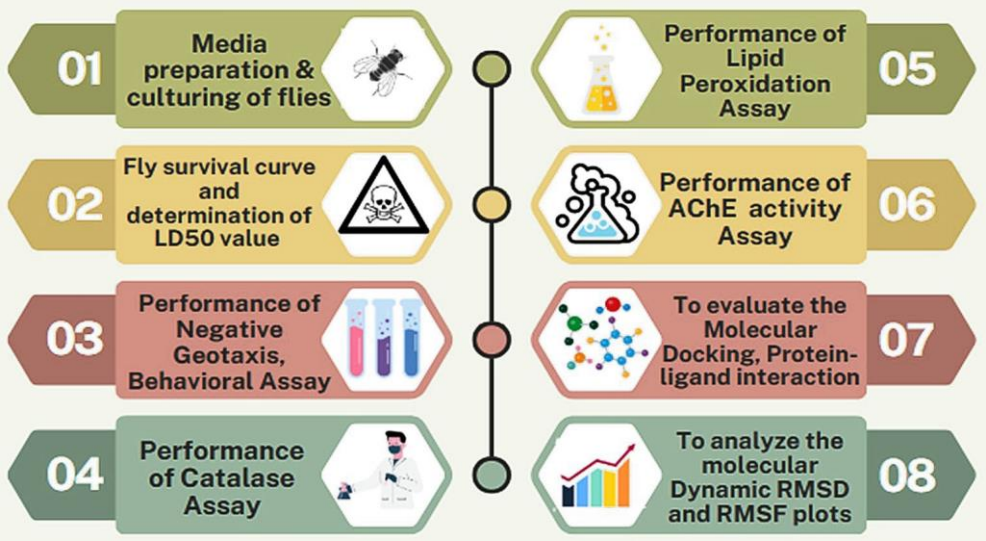
1. To test the Lethal Dose, $LD_{50}$ value of Sodium Benzoate on *Drosophila melanogaster.*

2. To test the neurotoxicity of sodium benzoate by behavioural assay (Negative geotaxis assay).

3. To test the oxidative stress and neurotoxicity of Sodium Benzoate by performing various biochemical assays.

4. To perform in silico molecular docking and dynamic studies to evaluate the interaction of sodium benzoate and a reference ligand with the target protein, acetylcholinesterase (AChE).

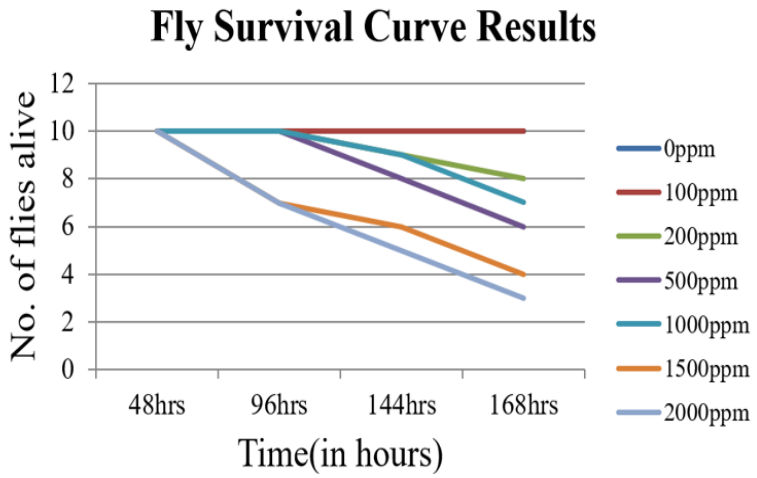
**Methodology:**



1) Fly Survival Curve: A fly survival curve tracks the lifespan of Drosophila melanogaster, highlighting mortality patterns, genetic or environmental influences.
2) Lethal dose, $LD_{50}$: LD50 (lethal dose 50%) for Drosophila melanogaster refers to the dose of a stressor (e.g., toxin, drug) at which 50% of the fly population dies. It quantifies toxicity or stress resistance in lifespan studies.
3) Negative Geotaxis: Negative geotaxis in Drosophila melanogaster is their instinctive upward climbing response when displaced downward. It is commonly used to assess locomotor ability, aging, and neurodegenerative effects.
4) Catalase Assay: A catalase assay measures the enzyme activity that breaks down hydrogen peroxide, reflecting oxidative stress resistance and antioxidant capacity in Drosophila melanogaster.
5) Lipid Peroxidation: A lipid peroxidation assay with MDA (malondialdehyde) quantifies the levels of MDA, a byproduct of lipid degradation, in Drosophila melanogaster. MDA is detected using thiobarbituric acid (TBARS), forming a pink chromogen that can be measured spectrophotometrically, providing an indication of oxidative damage to lipids.
6) Acetylcholinesterase (AChE) Assay: Measures acetylcholinesterase activity, assessing its ability to hydrolyze acetylcholine into choline and acetate, often using colorimetric or spectrophotometric methods to evaluate neurotoxicity or enzyme inhibition.
7) Molecular docking studies: A computational docking method was used using PyRx to evaluate the interaction of the food additive (ligand) with the target protein. Using AutoDock-Vina, the docking studies were performed, and based on the vina scores and interactive ligand behaviors, the resulting docked complexes were examined.
8) Ligplot visualization: Ligplot is a tool that is extensively used for the visualization of protein-ligand interactions. It generates schematic representations of hydrogen bonds, hydrophobic interactions, amino acid residues, and other important information for critical analysis between a ligand and its surrounding residues.

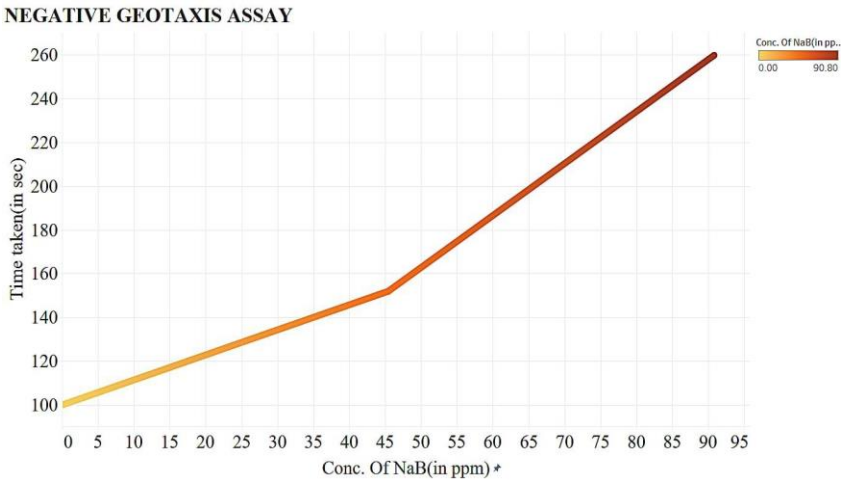
**Result and Conclusion:**

Fly Survival curve:



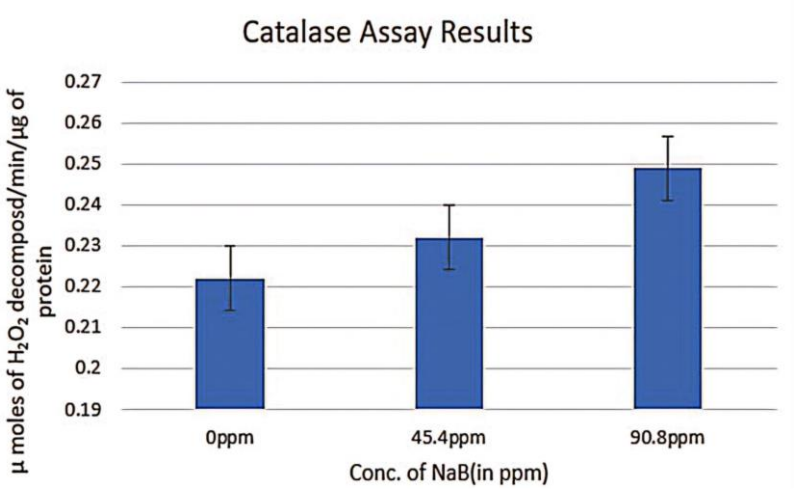

Sodium benzoate (NaB) had a definite concentration-dependent toxic effect on flies, and survival decreased as NaB concentration increased. For example, only 5 out of 10 flies survived at 24 hours at 500 ppm NaB, whereas all 10 survived at 0 ppm. The LD50 value, determined with AAT BioQuest software, was 454 ppm—defined as the concentration at which 50% of the flies would be killed. This value corresponded with the survival data and Fig. 6.1, highlighting NaB's neurotoxicity.

2) Negative geotaxis assay:



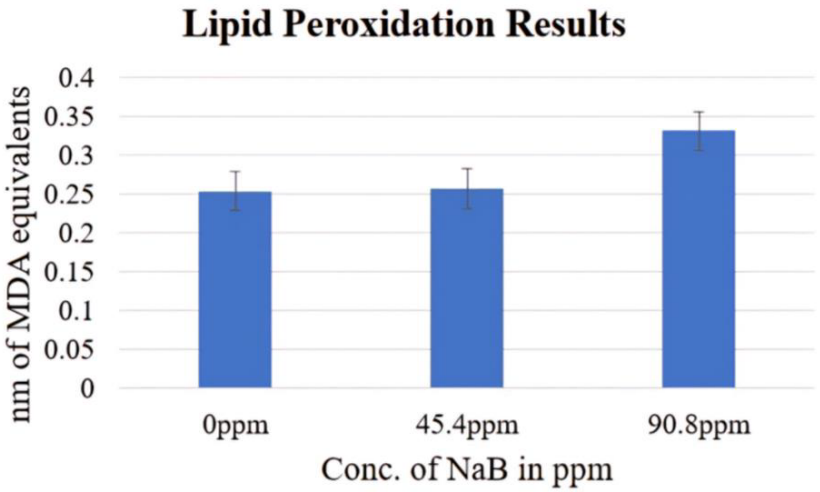
To test the impact of sodium benzoate (NaB) on locomotion, Drosophila melanogaster were treated with different NaB concentrations and analyzed by a negative geotaxis assay. Flies treated with higher concentrations of NaB exhibited longer climbing times, reflecting disrupted motor function. Mild climbing impairment was noted at 45.4 ppm, and 90 ppm resulted in pronounced locomotor dysfunction, with climbing times almost double that of controls. These findings show a concentration-dependent impairment of neuromuscular function, illustrating NaB's neurotoxic action on motor coordination.

3) Catalase Assay:



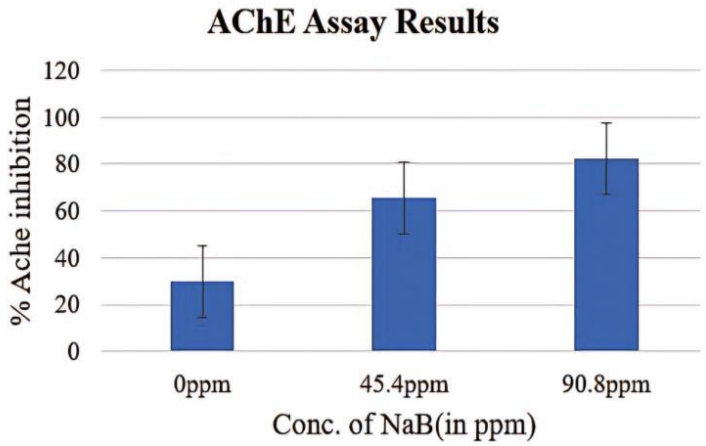

To determine oxidative stress, catalase activity in Drosophila melanogaster treated with different concentrations of sodium benzoate (NaB) was determined by measuring $H_2O_2$ degradation. Results indicated concentration-dependent reduction in catalase activity. At 0 ppm NaB, there was normal activity with 0.22 μg $H_2O_2$. There was mild reduction at 45.4 ppm, reflecting mild oxidative stress, whereas at 90.8 ppm, catalase activity was drastically reduced, resulting in $H_2O_2$ accumulation. At higher concentrations, the activity of the enzyme declined to almost half that of the control. These results indicate NaB inhibits antioxidant defense by enhancing oxidative stress in a dose-dependent way

4) Lipid Peroxidation Assay:




Lipid peroxidation in Drosophila melanogaster treated with different levels of sodium benzoate (NaB) was evaluated by observing the level of malondialdehyde (MDA). At 0 ppm NaB, control MDA was 0.25 nmol, which signified normal oxidative status. Increment in MDA at 45.4 ppm corresponded to moderate oxidative stress, whereas extreme rise at 90.8 ppm signified widespread lipid damage as an outcome of severe oxidative stress. These findings show the direct relationship of NaB concentration and lipid peroxidation and the ability of NaB to cause cellular injury by weakening the integrity of membranes by oxidative actions.

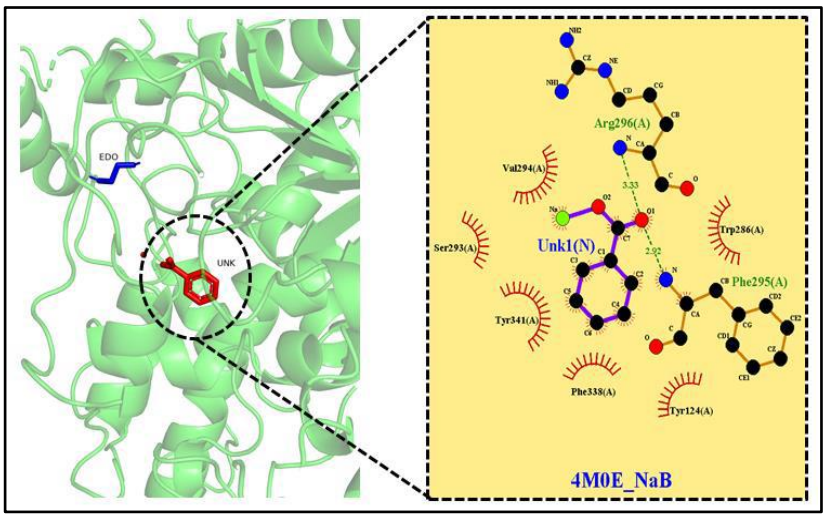
5) Acetylcholinesterase Assay:




Acetylcholinesterase (AChE) activity in brain homogenates of Drosophila melanogaster was reduced with increased concentration (NaB), with a definite dose-dependent inhibition. AChE activity was inhibited by about 70% at 45.4 ppm, while

90.8 ppm inhibited it by about 85%, although the rate was slowed as a result of enzyme saturation. This indicates that NaB causes significant interference with cholinergic transmission through the inhibition of AChE, resulting in possible neural dysfunction. These observations underscore NaB's neurotoxic properties and the need to maintain monitoring of its concentration to appreciate its action on neural health and biochemical pathways.

6) Results from molecular docking studies:




Molecular docking experiments showed that sodium benzoate (NaB) binds to the active site of acetylcholinesterase (AChE) with stable conformations having high binding affinity, as reflected by low Vina scores. NaB had comparable binding energies and interaction patterns with the known ligand EDO, implying it could be mimicking EDO's function within AChE's catalytic site. Ligplot analysis established interactions with major residues such as Tyr124(A), reinforcing this similarity. Considering EDO's well-documented pro-oxidant activity, NaB is also implicated in the promotion of oxidative stress. Such in-silico results are supported by experimental findings, suggesting evidence of neurotoxic action by NaB through the inhibition of AChE activity and oxidative stress induction.

**Project Outcome & Industry Relevance:**

By utilizing Drosophila melanogaster as a model organism, the study provides insights into the neurotoxic effects of sodium benzoate, a widely used food preservative. The research demonstrates how exposure to sodium benzoate can lead to oxidative stress, impaired neurotransmission, and cellular damage, as evidenced by reduced catalase activity, acetylcholinesterase inhibition, and increased lipid peroxidation markers.

Contributions to the Field of Study:

The project advances neurotoxicology by establishing Drosophila melanogaster as a reliable model for studying chemical-induced neurodegeneration.

It highlights the biochemical pathways affected by sodium benzoate, such as oxidative stress and synaptic dysfunction, which are relevant to understanding broader neurodegenerative conditions. The findings also raise concerns about the safety limits of sodium benzoate in food products, suggesting the need for regulatory reassessment.

Real-World Applications:

1. Food Industry: The study could inform manufacturers and regulators about safer limits for sodium benzoate usage and encourage the exploration of alternative preservatives.

2. Healthcare: Insights into oxidative stress and neurotransmission impairment may contribute to developing therapeutic interventions for neurodegenerative diseases.

3. Regulatory Bodies: Agencies like FDA or FSSAI might use this data to refine guidelines on acceptable daily intake levels for sodium benzoate.

Overall, this project bridges experimental research with practical applications, emphasizing the importance of evaluating long-term health risks associated with common food additives.

**Working Model Vs. Simulation/Study:**

The project utilized *Drosophila melanogaster* (fruit flies) as a physical model organism to study the neurotoxic effects of sodium benzoate. Laboratory experiments were conducted to assess survival rates, behavioral changes (negative geotaxis assay), and biochemical parameters such as catalase activity, lipid peroxidation, and acetylcholinesterase inhibition. These experiments involved culturing and subculturing flies, preparing media, and conducting assays using physical tools like microplate readers.

The project also incorporated in silico molecular docking studies, where computational simulations were performed to analyze the interaction between sodium benzoate and acetylcholinesterase (AChE) protein at the molecular level. This theoretical component complemented the experimental findings by providing insights into the biochemical mechanisms of neurotoxicity

Thus, the project was a combination of **physical experimentation** and **computational modeling**, making it a multidisciplinary study that integrated hands-on biological research with theoretical simulations.

**Project Outcomes and Learnings:**

**Key Outcomes of the Project:**

1. The project demonstrated that sodium benzoate induces neurotoxicity in *Drosophila melanogaster* by causing oxidative stress, impairing neurotransmission, and damaging cellular structures.
2. Behavioral tests revealed significant neuromuscular impairments, as evidenced by the negative geotaxis assay.
3. Biochemical assays showed reduced catalase activity (indicating oxidative stress), increased lipid peroxidation (cell membrane damage), and inhibited acetylcholinesterase activity (synaptic dysfunction).
4. Molecular docking studies confirmed strong interactions between sodium benzoate and acetylcholinesterase, further validating its neurotoxic potential.

**Learnings from the Process:**

- Designing the project emphasized the importance of selecting a suitable model organism (*Drosophila melanogaster*) for studying toxicological effects.
- Implementing the experiments required mastering techniques like culturing flies, conducting biochemical assays, and performing molecular docking simulations.
- Analyzing data underscored the value of integrating experimental and computational approaches to derive comprehensive insights into chemical toxicity.

**Future Scope:**

1. Developmental Neurotoxicity: Assess the impact of sodium benzoate exposure during different developmental stages of *Drosophila* to identify critical windows of vulnerability.
2. Genetic Background: Examine how different genetic backgrounds in *Drosophila* populations influence susceptibility to sodium benzoate-induced neurotoxicity.
3. Antioxidant Supplementation: Evaluate the protective effects of antioxidant supplementation (e.g., Vitamin E, Selenium) against sodium benzoate-induced oxidative stress and neurodegeneration.
4. Advanced Imaging Techniques: Employ advanced microscopy (e.g., confocal microscopy) to visualize cellular and structural changes in the Drosophila brain following sodium benzoate exposure.
5. Metabolomics Analysis: Conduct metabolomics studies to identify metabolic biomarkers associated with sodium benzoate exposure and neurotoxicity.