Toxicity of Formaldehyde and Formalin against the oriental latrine fly Chrysomya megacephala (Fabricius,1794) (Diptera: Calliphoridae)

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DEDICATED

TO

My parents and family members

SUPERVISORS CERTIFICATE

This is to certify that the work embodied in this dissertation entitled: "[Toxicity of Formaldehyde and Formalin against the oriental latrine fly *Chrysomya megacephala*(Fabricius,1794) (Diptera:Calliphoridae).was carried out by the author under my supervision. The dissertation is the result of the candidate's own research work and is, to the best of my knowledge, an original contribution to the field.

I further certify that this dissertation is suitable for submission in partial fulfillment of the requirements for the degree of Master of Philosophy at the Dhaka University.

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DECLARATION

I hereby declare that the present research is the result of my own original research and has not been previously submitted, either in whole or in part, for the award of any degree, diploma, or prize at any university or institution. All sources of information have been duly acknowledged.

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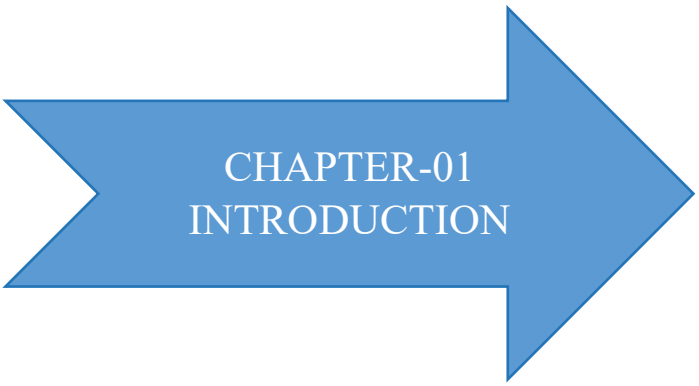
— The Author

ABSTRACT

The present study investigated the toxicological effects of formalin and formaldehyde on the blow fly *C. megacephala*, a species of considerable forensic and ecological significance. Five formalin concentrations (0.001, 0.010, 0.100, 0.200, and 0.300 ml/g) were administered via contaminated food to assess dose-dependent mortality and effects on growth and development. Mortality data revealed a progressive increase in response to higher doses, with mean values of 1.788 ± 0.223% at 0.001 ml, 2.400 ± 0.731% at 0.010 ml, 6.611 ± 0.418% at 0.100 ml, 7.179 ± 0.336% at 0.200 ml, and 9.478 ± 0.977% at 0.300 ml. One-way ANOVA confirmed highly significant differences among dose groups ($F = 236.925$, $p < 0.001$), and Duncan's Multiple Range Test (DMRT) revealed stepwise separation of mortality rates, indicating strong dose-dependent toxicity. Morphometric analyses showed substantial reductions in growth parameters across increasing concentrations and treatment volumes. Total body length decreased from 4.708 ± 3.938 mm at 0.001 ml to 3.075 ± 1.387 mm at 0.300 ml. Similarly, head length declined from 0.896 ± 0.672 mm to 0.800 ± 0.352 mm, thorax length from 1.504 ± 1.390 mm to 1.000 ± 0.605 mm, abdomen length from 2.279 ± 1.914 mm to 1.475 ± 0.739 mm, wing length from 3.008 ± 1.811 mm to 2.192 ± 1.829 mm, wing span from 2.292 ± 0.900 mm to 1.667 ± 0.489 mm, larval length from 3.833 ± 3.075 mm to 3.983 ± 3.547 mm, and pupal length from 4.500 ± 3.529 mm to 4.067 ± 3.479 mm. DMRT analyses confirmed that higher doses resulted in significantly smaller morphometric traits, reflecting clear dose- and volume-dependent growth inhibition. Histological examinations revealed progressive tissue damage corresponding to dose intensity. Low doses induced mild cytoplasmic vacuolization and disorganization, whereas intermediate doses caused epithelial disruption and reduced cellular integrity. At the highest concentrations, extensive necrosis, muscular degeneration, and severe tissue disruption were observed. These findings corroborate the cytotoxic mechanisms of formalin, including protein denaturation, oxidative stress, and disruption of physiological systems. Overall, formalin exposure significantly affects the mortality, development, and morphological traits of *C. megacephala* in a dose-dependent manner. The study underscores the need for further research on sublethal and behavioral effects, as well as the potential environmental risks associated with formalin pollution.



Introduction

1.1 Background

1.1.1 Physicochemical Characteristics and Industrial Applications

A highly reactive aldehyde, Formaldehyde (HCHO) subsist as a gas which is colorless with a pungent and dyspnoeic aesthesis. The aqueous solution of formaldehyde, commonly known as formalin, which typically contains 37–40% formaldehyde stabilized with methanol (Xu et al., 2017). Though for its poisonous nature, Formaldehyde is essential in numerous industrial and research laboratory processes. It serves not only a crucial raw material for the manufacture of phenol-formaldehyde but also urea-formaldehyde resins, plastics, textiles, and adhesives. In the medical field, it is extensively used for preservation of tissue, embalming, sterilization because of its antimicrobial and properties of crosslinking (Fenech et al., 2016). The scientific community relies on Formaldehyde for its remarkable fixative abilities, which stabilize proteins and nucleic acids, making it ideal for histological and molecular studies. Additionally, Formaldehyde is used as an anti-contaminant in artificial diets for insect breeding and various microbiological experiments. However, its volatility has contributed significantly to indoor air pollution, especially in occupational settings such as general anatomy laboratories, histopathology units, funeral homes, and certain industrial plants (Tang et al., 2009).

1.1.2 Atmospherical Chemistry and Biological science Significance

Formaldehyde plays a important role in atmospheric chemistry as a key of intermediate in the oxidation of hydrocarbons. It participates in photochemical reactions that produce radicals, promoting the formation of tropospheric ozone and secondary organic aerosols. These atmospheric processes exacerbate air pollution, contributing to respiratory illnesses in urban populations.

In addition to anthropogenic sources, Formaldehyde is produced naturally in small amounts through metabolic pathways in plants and animals. However, industrial and vehicular emissions have dramatically elevated their environmental concentration. Given its high reactivity, Formaldehyde in the environment can interact with other pollutants, forming more toxic derivatives that increase the ecological burden.

1.1.3 Human Health Toxicity: Acute and Chronic Effects

Formaldehyde is well-documented for causing both acute and chronic toxic effects, posing significant health risks. Short-term exposure through inhalation typically causes eye irritation, nasal mucosa, and throat, coughing, sneezing, lacrimation, and headaches (Xu et al., 2017). In sensitive individuals, it may provoke allergic contact dermatitis and asthma-like respiratory hypersensitivity (Tang et al., 2009).

At the molecular level, Formaldehyde exerts genotoxic effects by forming DNA–protein cross-links and DNA adducts, causing mutations and chromosomal aberrations (Le et al., 1990). Experimental studies have demonstrated its capacity to repair and interfere with DNA replication mechanisms, starring to genomic instability (Nafei & Auerbacke, 1964). Chronic exposure has also been associated with neurotoxicity, hematotoxicity, and immunosuppression. Nilsson et al. (1998) reported tissue-specific toxic effects, particularly targeting the gonads, gastrointestinal system, and respiratory epithelium.

1.1.4 Carcinogenicity and Epidemiological Evidence

The carcinogenic agent of Formaldehyde has been widely investigated. The (IARC) International Agency for Research on Cancer,(1995) classified the Formaldehyde as a Group 2A probable human as carcinogen, citing epidemiological evidence linking occupational exposure to increased incidences of nasopharyngeal cancer, leukemia, and sinonasal cancer (Soysal & Munzi et al., 2007). Formaldehyde's carcinogenicity is primarily attributed to its genotoxic properties, which induce DNA mutations and chromosomal instability in target tissues.

Although a very little amounts of Formaldehyde are naturally present among human metabolism—produced during amino acid catabolism and methyl group oxidation—exogenous exposure significantly raises the risk of malignant transformation (Tang et al., 2009). Epidemiological studies on workers in funeral homes, anatomy labs, and wood product manufacturing plants have consistently reported increased cancer risk compared to the general population.

1.1.5 Public Health Concerns and Food Safety

Beyond occupational settings, formaldehyde contamination in food represents - public health concern which is significant, especially in developing countries such as Bangladesh. The illegal use of formalin , prolong the shelf life of fish, meat, fruits, and vegetables, posing chronic health risks to consumers (Xu et al., 2017).

1.1.6 Occupational Exposure and Safety Regulations

Formaldehyde is widely used in research labs, hospitals and different industrial and manufacturer companies, which is very terrible and hazarders for the people who works this place. A long-term use or inhalation of the chemical as vapors may occur different types of disease like respiratory, bronchitis as well as neuro diseases. (Tang et al.,2009). Despite the recommendation of safety rules were exists, the enforcement is limited.

1.1.7 Toxicity in Insects and Forensic Implications

The research of toxic effect of formaldehyde and formalin on insect is very limited then vertebrates. Previous studies revealed a very significant finding of formaldehyde elicited consequence in gene mutation and DNA replication in insect and gene models. (Nafei &Auerback,1964; Le et al.,1990). A very recent research revealed its inauspicious effects on organic process, life time and motor abilities imply latent disturbance of key biological process (Duang et al.)

1.1.8 Future Directions

Though research on formaldehyde on human toxicity is going on for decades, research gap is remaining in understanding its use and impacts. Investigating tissue-specific changes, developmental stage susceptibility, and dose–response relationships in blow flies could enhance forensic applications and help assess environmental risks. Furthermore, given the increasing misuse of formalin in food preservation and its widespread occupational exposure, multidisciplinary research combining toxicology, forensic entomology, and environmental health is urgently needed. Such studies will

provide valuable information for policy-making, environmental monitoring, and public health risk assessments.

1.2 Sources of Formaldehyde

There are two sources of how Formaldehyde enters the environment. Both natural and human sources produce Formaldehyde. Naturally, Formaldehyde is formed when the oxidation of hydrocarbons occurs in the troposphere. The reaction of OH radicals with hydrocarbon and ozone forms formaldehyde, and a series of reactions occur in the formation of carbon monoxide, hydrogen, and water. Besides terpenes and isoprene, which are spread around by foliage, and treated with OH, they form Formaldehyde.
Some artificial sources are also present in the environment, such as industrial uses, off-gassing from building materials, and consumer products. The primary artificial sources include a range of fuels such as wood, plastics, plywood, chipboard, cigarette smoke, wood-burning stoves, fireplaces, power plants, and fabrics (Jermini C et al., 1976; Kitchens JF et al., 1976; Baker DC, 1994).

The development of the wood furniture industry with urea-formaldehyde glue, which is a widely used adhesive for furniture production. The rate of using urea-urea-formaldehyde glue as a synthetic material is increasing, as the use of wood materials is increasing here in indoor and outdoor living spaces. As a result, the widespread use of urea-formaldehyde as a synthetic material brings air pollution and also acts as one of the most common contaminants in the indoor environment. Soysal A. and Demiral Y. (2007) and Marutzky R. (1994. Both indoor and outdoor air have different concentration levels.

Endogenous Formaldehyde is a product produced through biological processes in the human body, resulting from numerous catabolic reactions. Sometimes it is found that it comes into being via demethylation of N-methyl, O-methyl, methyl, and S-methyl compounds during metabolism. It is excreted from the body through urine and stool, by metabolizing formic acid, and catalyzed by formaldehyde dehydrogenase enzymes in the liver, or excreted through respiration. By converting to carbon dioxide. (Usanmaz SE, et al.,2002).

Formaldehyde may be found in human bodies in tiny amounts. Firstly, it may be derived after the metabolism of glycine, serine, choline, and methionine, and then it is found here in human cells. (Unsaldi E,2010 and IPCS,1989),The excretion process of Formaldehyde from the body is through urine and stool. Formic acid is metabolized here, catalyzed by the formaldehyde dehydrogenase enzyme, and then converted into carbon dioxide after being excreted through respiration.(Usanmaz SE .,2002,Eells JT,1981 and Koivusalo M.,1982).In a nonenzymic manner, Formaldehyde tends to combine strongly with nucleic acids, unsaturated fatty acids, and proteins, leading to an inflammatory reaction and allergy. This results in cytotoxicity and a mutagenic effect, as evidenced by the denaturation of protein production. Besides, Formaldehyde also shows function detection in tissues and antimicrobial activity.
(Usanmaz SE,2002; Bolt HM,1987; and Heck H,1999)

1.3 Uses of Formaldehyde

A limited amount of formalin recommended by the European Commission can be used as animal food preservation or as a decontamination treatment (Anonymous,2004). Long-term use of this stabilizing agent may cause in a toxic effect on different organs of the animal body (Webster et al.,2009). In Bangladesh, some people use this preservative to protect fish, meat, vegetables, and fruits from decay, but they are often unaware of the long-term effects of Formaldehyde or formalin.
In the storage of biological samples and mummification, it is used because it hardens proteins and prevents decomposition. As a disinfectant, Formaldehyde is also used for its properties to kill microorganisms. (Schlink K. et al.,1999).In the field of Medicine, Formaldehyde is used for the determination of cadavers and their long-term storage, and also used in histology and pathology during the fixation of tissues. It is also used in the preservation of certain drugs, in the structure of dental coatings, for the treatment of persistent cystitis, and, notably, formalin solution is utilized in hemodialysis. (Smith AE,1992; Khanzadeh FA,1994; Cohen BI,1998; and Sarnak MJ, et al.,1999.).The use of Formaldehyde has great significance for human beings. Below are some uses of formaldehyde-containing products.

In agriculture, Formaldehyde is used in Grain preservation, seed dressing, soil disinfection, rot protection of feed, and nitrogen fertilizer. The industry of cleaning agent-formaldehyde is used here as a preservative in soaps and detergents. In the cosmetic industry, Formaldehyde is used as a preservative in soaps, deodorants, shampoos, etc. In the food industry, it is used for the preservation of dried foods, disinfection of containers, preservation of fish, certain oils, fats, etc. In Medicine, disinfection, sterilization, and preservation of preparations are used. The leather and textile industry utilizes additive tanning agents, such as Formaldehyde, which provide crease resistance, dimensional stability, and flame resistance, as well as binders in textile printing. Sugar and wood industry-Infection inhibitor in producing juices, and also used in the wood industry as a preservative.

1.4 Harmful effects of formalin

Due to its far-flung use ,Researchers identified significant harmful effects on animal health as well as human. The odor of the chemical is very suffocating, which can be detected in very low concentrations. Its vapor is known to be an eye and skin irritant in humans. Irritation of the mucosa in the eyes and upper respiratory tract is a common effect of Formaldehyde. Some studied groups were at risk of the effects of Formaldehyde, which are mentioned here. The workers who are working at the production stage of Formaldehyde. Furniture industry workers who are working with formaldehyde-containing products like chipboard, plywood, MDF, lacquer, fire retardants, etc. Medicine and veterinary lab: anatomy, pathology, and histology staff. Dentists and nurses who are sterilizing dialysis equipment and other supplies. Paper industry: workers who work in paper, paper products, and recycling. Rosenstock L, et al (2005), Tanaka K et al.,2003 and Pecka I. et al., (2001).

According to researchers, Industrial area workers who are exposed to formaldehyde production or use have shown an increase in the number of people dying from blood cancer, brain cancer, and colon cancer compared to other working people. Shaham J. et al., 1996, and Schlink K. et al., 1999. Besides, there is some use of formaldehyde-containing products at home and in the workplace, such as wall paint, furniture, deodorants, and cleaning products, as well as environmental factors like fuel oil, exhaust gas, cigarette smoke, and wood burning, which are responsible for increasing

the impact of Formaldehyde. Experimental studies show that Formaldehyde is carcinogenic, which has harmful effects on many systems such as the nervous system, respiratory system, and digestive system. (Smith AE,1992; Usanmaz SE, et al.,2002; Zararsiz I. et al.,2006). Besides, the adverse effect of Formaldehyde on the reproductive system, such as damage to germinal cells, which is the cause of fertility problems, disrupts the structure of the testicle and decreases sperm count as well as the serum testosterone levels. (Chowdhury AR. et al.,1992, Thrasher JD,2001, and Özen OA, et al.,2005). It is called a mutagenic, teratogenic, genotoxic, carcinogenic, and embryotoxic agent that includes chromosomal errors, gene mutations, single-chain fractures, sister chromatid exchange, and cell changes. Shaham J, 1996, and Heck H, et al., 1996. In a very low concentration, respiratory system toxicity may occur, also causing a burning sensation in the nose and throat, coughing, wheezing, difficulty in breathing, and acute effects. Pneumonia, pulmonary edema, and inflammation may occur in higher concentrations. (Shaham J,1996; Heck H, and Casanova M, 1999; Blair A,1990; and Kriebel D,2001. It is also observed that the workers who are exposed to Formaldehyde have a mortality rate that is almost 30% higher.(Halperin WE, et al.,1983 and Hayes RB., et al 1986.).The toxic effects of Formaldehyde have been reported on the central nervous system, eyes, skin, and respiratory system. (Chowdhury AR,1992; Thrasher JD,2001; Özen OA,2005). Oral ingestion of Formaldehyde causes a local corrosive effect in the upper part of the gastrointestinal system.

Necrosis, perforation, and bleeding develop after the following symptoms: nausea, severe diarrhea, and abdominal pain. Then, circulatory failure and severe metabolic acidosis occur and result in death within a few days [1]. In addition, some studies have found that Formaldehyde inhibits the activity of certain enzymes and increases the activity of others [13, 34].

After combines with DNA, RNA protein and unsaturated fatty acid it performs formaldehyde performs in a nonenzymatic way. (Bolt HM, 1987). The neurotoxic effects of Formaldehyde are Insomnia, depression, headache, dizziness and loss of appetite and long-term exposure can lead to mood disorders, behavioral disorders, and epilepsy. (Kilburn KH,1987; Stroup NE,1986; Kilburn KH,1994)

1.5 The Blow fly (*C. megacephala*)

The blow fly species *C. megacephala,* commonly known as Oriental Latrine Fly, is classified as Order: Diptera, Family Calliphoridae. Blow flies, which is why Blow flies play a very important role in forensic entomology as any dead body at first attacked by them. The Family Calliphoridea is divided into two subfamilies: the Calliphorinae and Chrysomylinae. Subfamily Calliphorinae is also divided into species *Calliphora* and *Lucilla*, as well as Chrysomylinae, which includes the *Crysomya* spp.

Accidental myiasis (Brundage et al, .2009), caused by the Blow fly, which is associated with some public health issues; As their body is characterized by a greenish–blue metallic box-like appearance. The color of thorax and abdomen show a metallic blue-green color and have yellow genes on the cheeks. According to their larval instar, they are getting different size and are more thickly developed in the final stage (Jason et al., 2001). Blow flies have large, red eyes, close together in males and apart in females (Byrd et al.,2001). The posterior end of segmented appendages(cercus) of the male is longer than that of the female(Chaiwong et al., 2008)

C. megacephala consents to high and warm atmospheres, showing a connection between higher temperatures and a higher capability of producing offspring. Fruitfulness brings down in territories with a high density of hatching, where numerous individuals in one small territory go after a similar food source.

1.6 Why formaldehyde and formalin has been chosen for the study

Formaldehyde and formalin were chosen for the present study on the toxicity on blue flies (*Calliphora* spp.) for several important scientific and practical reasons. Formaldehyde and formalin were selected for this study due to their widespread use as chemical preservatives and disinfectants, as well as their known toxic effects on various organisms. Despite their common application in medical, industrial, and forensic environments, there is limited research on their impact on insects like the blue fly (*Calliphora* spp.), which play a key role in decomposition and forensic investigations. Besides the toxic impact on insect also shows the harmful effect on human. The toxicological effects of low and high concentrations of formaldehyde and

formalin on insects are not well -documented. Here the present study helps fill a gap in knowledge and provides data that can guide safer use of these chemicals. Investigating the toxicity of these substances on blue flies helps fill a knowledge gap and may provide valuable insights into environmental safety, pest control, and forensic entomology. This species was selected due to its ecological and forensic significance, as well as its frequent exposure to chemically treated organic matter in urban environments.

1.7 Aim of the research

This systematic review aims to update the scientific evidence on the relationship between occupational exposure to Formaldehyde and formalin. The main aim of the research is to determine the toxicity of Formaldehyde and formalin on *C. megacephala.* (The blowfly*)* to evaluate the harmful impact of the mentioned chemicals on the mortality of Blow fly.

1.8 Objectives

Here ,the research mainly evaluated the toxicity of formalin and formaldehyde on *C. megacephala* as well as the changes of size and development also observed. Theobjectives are here described below:

1. To observe the impact of Formaldehyde and formalin on size and development in larvae, pupa and adult of C. *megacephala*.
2.The mortality rate of adult *C. megacephala*. upon exposure to varying concentrations of Formaldehyde and formalin.
3. Behavioral changes (e.g., flight ability, feeding response, grooming behavior) in adult C. megacephala treated with Formaldehyde and formalin also to be determined.
4. Histological changes in major internal organs, alimentary canal. Flight muscles, Malpighian tubules, fat bodies, Accessory glands
and ovaries to be investigate.
5. To compare the differential effects of Formaldehyde and formalin (individually and in combination) on physiological and anatomical features of the adult flies.

6. To provide a scientific basis for understanding the ecological risks of formaldehyde/formalin contamination in decomposing organic matter that attracts flies in open environments.

CHAPTER-02
REVIEW OF LITERATURE

REVIEW OF LITERATURE

A search of the literature on formalin and formaldehyde and the impact on blow flies reveals that some work was carried out in other countries, but very little is available here in Bangladesh. To understand the current state of knowledge and identify gaps for future investigation, relevant literature has been reviewed in alignment with the objectives of the present study. Although formaldehyde is naturally present in the environment, it is also released through various human activities. It is reported to exert toxicity on multiple biological systems, including the CNS, eyes and skin reproductive organs (such as testes and menstrual function), and the respiratory system. Among them some of the reviewed literature is mentioned below.

Ajasa (2025) reported that formaldehyde and formaldehyde-releasing preservatives are present in various personal care products—such as lotions, body washes, and conditioners—often marketed to Black women. The article highlights growing concerns over long-term exposure to these toxic chemicals, which are linked to cancer and other health risks, and emphasizes the need for stricter regulations and transparency in cosmetic labeling in the United States.

Columbia University Mailman School of Public Health (2025), The study revealed that many of the commonly used products contained formaldehyde-releasing preservatives. Unlike earlier research relying on generalized surveys, this approach provided more accurate, product-specific exposure data.

Erickson (2025) reports that the U.S. Environmental Protection Agency's final risk evaluation, released on January 2, 2025, concluded that formaldehyde poses unreasonable health risks to both workers and consumers. The evaluation—building on the contested IRIS cancer assessment—found risks not only from long-term inhalation (linked to cancer), but also from noncancer effects such as skin sensitization, eye irritation, and allergic responses. These findings could lead to new regulatory restrictions under the Toxic Substances Control Act (TSCA), despite strong pushback from chemical manufacturers and political opposition.

Sun et al. (2025) reviewed the dual role of formaldehyde in food processing. While recognized for its toxic effects—including carcinogenicity, neurotoxicity, and genetic damage—formaldehyde is also involved in forming both harmful by-products (e.g., heterocyclic amines, N-nitrosamines) and aromatic flavor compounds. The study highlights the chemical pathways through which formaldehyde contributes to these outcomes and suggests strategies for reducing its risks while preserving flavor development in foods.

According to **Enuneku, A., et al. (2025)**, this survey analyses attribute and impermanent trends of carbon monoxide (CO) and tropospheric formaldehyde (HCHO) concentrations over Nigeria, between 2018 and 2023. The authors analyzed that pollutant fluctuations and potential public health risks increased there. The level of formaldehyde in Troposphere pretending a concern rise in urban areas. Which is the indication of respiratory diseases and different type of health disease issues. The paper emphasizes the utility of GEE and satellite data for real-time air quality monitoring, especially in data-scarce regions like sub-Saharan Africa, and calls for urgent policy responses to mitigate air pollution risks.

The **Environmental Protection Agency (2025)** released its final risk evaluation for formaldehyde under the Toxic Substances Control Act (TSCA). The evaluation, based on the best available science and weight of evidence, concluded that formaldehyde poses an unreasonable risk of injury to human health. This determination was made without regard to economic or other non-risk factors and included risks to potentially exposed or susceptible subpopulations. As required under TSCA, the EPA must now initiate risk management measures to address these identified risks.

Mazurkiewicz, W. et al.(2024) reviewed and discussed the harmful effects of environmental formaldehyde exposure on human health. It mainly enters the body through inhalation and is linked to cancer (especially nasopharyngeal cancer and leukemia), asthma, and brain disorders. Workers in healthcare, factories, and construction are most at risk. The study stresses the need for proper education, protective measures, and exposure control to reduce health risks.

According to **Lerner, S., & Shaw, A. (2024)** formaldehyde is the top toxic air pollutant linked to cancer in the U.S., yet regulatory action has been minimal. Despite strong scientific evidence and warnings from EPA scientists, political and industry pressures have delayed stricter controls. As a result, millions of Americans remain at risk, especially those living near industrial sites that emit formaldehyde.

Rieckher et al. (2024) reported that formaldehyde, produced both metabolically and environmentally, causes DNA damage during development and aging. The survey revealed life-stage–specific DNA repair mechanisms and demonstration that N-acetyl-L-cysteine (NAC) can decrease the toxicity of formaldehyde.

Wang and Zhung (2023) Their research explained that formaldehyde is very active in biological process such as metabolism of living organism and at the same time by involving biological processes they develop diseases like heart disease and cancer.

Rease A. (2023) added that formaldehyde exposure adversely affects the skin, mucous membranes, and respiratory system, and is capable of crossing the blood-brain barrier. This raises concern about its neurotoxic potential, including a possible role in cognitive decline and memory loss associated with Alzheimer's disease. A rat model study investigating chronic occupational exposure revealed impacts on learning, anxiety, and memory functions.

La Torre, G. et. Al.(2023) investigates two key links related to formaldehyde exposure: first, its role in causing respiratory irritation, particularly asthma; and second, its potential to contribute to the development of cancers. Formaldehyde, commonly used in industrial products and found in tobacco smoke, is recognized as both a respiratory irritant and a human carcinogen by the International Agency for Research on Cancer.

Kang, D. S., et al. (2022) investigated the fabrication of flame-resistant bacterial celluloses (BCs) using milk-derived proteins—whey protein isolate (WPI) and casein—via physical entrapment and crosslinking. The researchers optimized production parameters, including protein content, method, citric acid concentration,

and crosslinker: catalyst molar ratio, using thermogravimetric analysis at 1000 °C. The most effective formulations used 50 wt.% WPI or casein and 10% (w/v) citric acid, achieving the highest residual mass. Structural analyses confirmed successful integration of proteins without disrupting the BC nanostructure. The flame resistance remained after three washes, and the durability, softness, and stability of the resulting flame-resistant BCs were comparable to cowhide leather of similar thickness. The findings offer a promising, protein-based approach for producing durable flame-retardant BC materials.

Herrero, M. et al. (2022) investigated the presence of formaldehyde in both eco-friendly and conventional clothing for pregnant women, babies, and toddlers in Catalonia, Spain. Formaldehyde was detected in 20% of samples, with surprisingly higher levels in eco-friendly garments. Although health risks were within acceptable limits, the study highlights that babies are more vulnerable to exposure. Washing clothes before use was found to eliminate detectable formaldehyde levels, suggesting a simple strategy to reduce dermal exposure.

Hua et al. (2021), explained the toxicity of formaldehyde on *Drossophilla as* well as the harmful effect sensory and neuro activity and behavioral changes.

Protano et al. (2021) conducted a systematic review evaluating the link between occupational formaldehyde exposure and cancer incidence. Although formaldehyde was officially classified as a carcinogen in 2004, further research has investigated associations with specific cancers, including leukemia, lung cancer, nasopharyngeal cancer, and non-Hodgkin lymphoma. While some studies found weak associations, the authors concluded that more rigorous, large-scale studies are required to clarify the carcinogenic risks and to minimize confounding due to co-exposure to other harmful substances.

Tarvares Dias, M. (2021) explores formalin's multifaceted impact in aquaculture, particularly focusing on its toxicity, physiological stress, histopathological alterations, and antiparasitic efficacy in both freshwater and marine fish. The study notes that acute toxicity levels (LC50–96h) vary significantly across species (from 0.1 to 640.0

mg/L). The compound induces disruptions in metabolism, respiration, and hematological parameters, with organ-specific damage particularly affecting the gills, liver, kidney, and spleen. On the therapeutic side, formalin baths are effective against protozoans and monogeneans infecting external fish surfaces. However, its use near toxic thresholds in many species necessitates careful regulation and monitoring.

According to **Dubey & Das (2021)** formaldehyde is an extremely toxic, flammable, and colorless gas that is slightly denser than air at room temperature. Its sharp, pungent odor is detectable at low concentrations but fails to provide sufficient warning at harmful exposure levels, especially for sensitive individuals. Human exposure can occur through inhalation, dermal contact, and ingestion, leading to irritation of the skin, eyes, and respiratory tract. Notably, industrial workers exposed to formaldehyde have shown increased risks of developing leukemia. In their review, Dubey and Das evaluated formaldehyde's physicochemical characteristics, toxicity, reproductive implications, and treatment approaches.

Dan et al. (2020), Dugheri et al. (2021), J. Lam et al. (2021), and Wartew (1983) collectively noted that formaldehyde exposure results in common symptoms such as eye, nose, and throat irritation, as well as coughing, wheezing, and shortness of breath. Chronic exposure is associated with diminished lung function and a higher risk of developing long-term respiratory diseases.

According to **Kang, D. S. et al.(2021) f**ormaldehyde is a highly reactive and toxic chemical classified as a Group 1 carcinogen by the International Agency for Research on Cancer, mainly linked to nasopharyngeal cancer and leukemia. However, its connection to leukemia remains debated. This study used a network-based analysis of formaldehyde-related genes to explore the biological mechanisms behind this association. Findings suggest that formaldehyde induces oxidative stress, leading to genetic changes that disrupt the blood-forming (hematopoietic) system, potentially causing leukemia. The study also identifies key genes involved in this process, providing a basis for further experimental research on formaldehyde's leukemogenic effects.

Utuh & Ugwoha (2021) reviewed the systemic effects of formaldehyde exposure on the human body, emphasizing its impact across multiple organ systems. The article outlines respiratory, neurological, dermatological, carcinogenic, and reproductive effects, highlighting formaldehyde as a potent environmental and occupational hazard. It stresses the importance of exposure prevention, proper ventilation, and adherence to safety guidelines to mitigate health risks in both industrial and domestic settings.

Dubey, S.K., & Das, P. (2021) provide a comprehensive overview of formaldehyde as a hazardous gas, emphasizing its environmental distribution, toxicological profile, and health risks. It details sources of formaldehyde emissions—both anthropogenic (e.g., industrial processes, combustion, and building materials) and natural. The authors examine its environmental persistence, noting its volatility and potential to contaminate indoor air. Human exposure occurs primarily through inhalation, leading to acute effects like eye, nose, and throat irritation, and chronic effects such as respiratory illnesses, DNA damage, and cancer, especially nasopharyngeal cancer and leukemia. The chapter also includes a detailed risk assessment framework, encompassing exposure routes, dose-response data, and regulatory thresholds set by global health agencies. It underscores the importance of monitoring, regulation, and public awareness in mitigating formaldehyde-related hazards.

Asare-Donkor, N. K. et al. (2020) done research in beauty salons in Ghana to asses health risk for the employees and clients. They found that the level of indoor formaldehyde exceeded (Determined by WHO and EPA, safety limit) here. Poor ventilation and the products were used for straightening hair is very carcinogenic and hazardous. Their recommendation was to improve ventilation and use of safer products in salons.

Subasi N.T. (2020), explained that formaldehyde is a simple highly reactive, also found in living animals, which is used industrial sector, medicine, laboratory and so on. Though the use of formaldehyde is wide it can easily do harm and lead to do diseases.

Dan et al. (2020), found that research has extensively documented its toxic effects on multiple organ systems, highlighting its genotoxic and carcinogenic properties. The review also explores decontamination methods and strategies to mitigate the harmful impact of formaldehyde exposure. Based on this evidence, the authors emphasize the need for strict protective measures when handling this hazardous chemical.

Bernardini et al. (2020) mentioned formaldehyde as leading to broad human exposure which is a xenobiotic air pollutant with widespread environmental distribution,. Their review of in vitro and in vivo studies from 2013 to 2019 demonstrated formaldehyde's toxic effects across multiple organ systems, including the lungs, upper respiratory tract, brain, and bone marrow. Cellular studies further confirmed its role in inducing inflammation, oxidative stress, and genotoxicity, with particular emphasis on its impact on apoptosis and cell viability.

This systematic review by **Tesfaye et al. (2020)** revealed here the formaldehyde exposure on the oxidative stress and carcinogenic potential . Formaldehyde contributes to cellular damage through the generation of reactive oxygen species (ROS), leading to DNA-protein crosslinking, lipid peroxidation, and chromosomal instability also highlighted by the author.

Dan, S. et al. (2020) evaluates the toxicological effects of formaldehyde (FA) on various organ systems. The authors compile evidence highlighting FA's role in causing respiratory tract irritation, carcinogenicity, neurotoxicity, and oxidative stress. The review identifies vulnerable occupational groups, such as laboratory workers and embalmers, and emphasizes the importance of personal protective equipment and ventilation. The study calls for stricter regulations and public health strategies to reduce long-term exposure and mitigate health risks.

According to **Ai et al. (2019)**, this study reveals that formaldehyde, traditionally known as a toxic environmental chemical, also functions as an endogenous signaling molecule involved in memory regulation in both mice and humans. The researchers found that physiological levels of formaldehyde are generated during neuronal activity and are essential for learning and memory via modulation of N-methyl-D-

aspartate (NMDA) receptor activity, particularly through a specific cysteine residue (C232) on the NR1 subunit.

Reingruber & Pontel (2018) found two terrible characteristics of formaldehyde which may occur danger for genome integrity. The dual role is as they are natures toxic agent and metabolic byproduct of living organism. Experiment on mice brought a result including a terrible disfunction of different organ and cancer. Rujis-alfs syndrome and cancers with BRCA2 mutations also may occur by Formaldehyde.

According to **Reichel, C. (2018)**, this article reviews research linking formaldehyde exposure to leukemia, especially myeloid leukemia. It emphasizes that formaldehyde is a Group 1 carcinogen and highlights evidence from occupational studies, dose-response data, and biological mechanisms like DNA-protein crosslinking and oxidative damage. It also discusses uncertainties about low-dose effects and the role of exposure intensity, duration, and genetics in leukemia risk.

According to Sarsilmaz, M. (2018), this review outlines the toxicological profile of formaldehyde (FA), a widespread environmental and occupational pollutant, emphasizing its carcinogenic and neurotoxic effects. Formaldehyde exposure, common in workplaces and residential environments, is linked to oxidative stress, lipid peroxidation, behavioral disturbances, and prefrontal cortex damage in experimental models. The review highlights the antioxidant and neuroprotective properties of melatonin (M) and Nigella sativa (NS), demonstrating their ameliorative effects against FA-induced damage in rats. It concludes by recommending further clinical studies to evaluate dosage, application routes, and long-term benefits of these agents in mitigating formaldehyde toxicity.

According to Zhang, L. (Ed.). (2018), this book particularly highlights formaldehyde-related public health concerns in countries with high production and usage, such as the United States and China. The book aims to raise awareness and promote interdisciplinary research, making it valuable for environmental health scientists, occupational safety experts, policymakers, students, and anyone affected by formaldehyde exposure.

Afrin, M. et al. (2016) investigated the toxic effects of formaldehyde (FA) on the liver of Swiss albino mice, focusing on biochemical and histological changes. Mice were divided into three groups: control, oral FA exposure, and intraperitoneal FA injection. These exposure groups were further divided and treated with FA for 30 days (oral) and 10 days (intraperitoneal).

Kundu, S., & Gangrade, P. (2015). Assessed the toxic effects of formaldehyde vapors on 100 first-year medical students in a dissection hall at the Government. Medical College, Raigarh. The embalming solution used contained 37% formaldehyde. Students with no prior exposure reported symptoms like respiratory irritation, eye and nasal discomfort, and skin reactions. The study concludes that formaldehyde can cause acute sensitivity and recommends preventive measures such as improved ventilation and protective gear.

According to **Arici, S., Karaman, S., Kaya, Z., et al. (2014)**, this experimental study investigated the neurotoxic effects of acute oral formaldehyde (FA) exposure in rabbits. Sixteen rabbits were divided into two groups, with one receiving 40 mg/kg/day FA for five days. Brain tissue analysis revealed that FA exposure caused significant DNA damage, increased apoptosis (↑ Bax, caspase-3, TUNEL), and reduced anti-apoptotic Bcl-2 expression. Elevated GFAP levels also indicated neurotoxicity. The study concludes that FA induces neuronal injury and apoptosis in the central nervous system.

According to **Abdu et al. (2014**),The neurotoxic effect of Formaldehyde in widely used areas like hospitals and industry were discussed here. Depending on dose and time duration can increase the symptoms of the lethargy and neurotoxic effect and brain cancer may eliminate. Recommendation of safety measures, assurance of proper ventilation of the following places also mentioned here.

According to **İnci, M. et al. (2013)**. This review discusses the toxic effects of formaldehyde on the urinary system. Formaldehyde, a water-soluble compound with widespread industrial and medical use, is metabolized into formic acid and excreted through urine, feces, or respiration. Due to occupational exposure—especially among

medical professionals and industrial workers—formaldehyde poses health risks, including to the urinary system. The authors emphasize that while complete protection is difficult, preventive measures can help reduce its harmful effects. This study examined the health effects of exposure to formalin-treated cadavers among medical students, faculty, and workers at the Alexandria Faculty of Medicine. It found that exposure to formalin vapors in dissection rooms led to a range of acute symptoms, including eye, nose, and throat irritation, headache, cough, and fatigue. The severity and frequency of symptoms were associated with exposure duration and lack of ventilation. The authors stress the importance of improving laboratory ventilation systems and using safer preservation alternatives to reduce formaldehyde-related health risks in academic settings.

Raja, D. S., & Sultana, B. (2012) assessed that Formaldehyde, widely used in anatomy labs, causes unpleasant odors and immediate symptoms like nausea and eye irritation. Prolonged exposure can lead to serious health issues, including dermatitis, birth defects, and cancer. Preventive measures are needed to protect exposed individuals.

Duong et al. (2011) conducted a systematic review of human and animal studies on the reproductive and developmental toxicity of formaldehyde. The review found significant associations between formaldehyde exposure and increased risks of spontaneous abortion and adverse pregnancy outcomes in women. Animal studies showed reproductive toxicity, particularly in males. Proposed mechanisms include genotoxicity, oxidative stress, hormonal disruption, apoptosis, and epigenetic changes. The authors emphasize the need for well-designed molecular epidemiological studies to clarify these effects. According to Zhang, L. et. al.(2010), Formaldehyde (FA) is a widely present environmental toxin linked to oxidative stress and carcinogenic effects, particularly in the brain and respiratory system. Experimental studies show that FA reduces antioxidant enzyme activity and causes behavioral and physiological damage in rats. Antioxidants like melatonin and Nigella sativa (NS) have shown protective effects against FA-induced oxidative damage. Given the widespread exposure to FA, especially in workplaces, further research and clinical trials are needed to evaluate the therapeutic potential of melatonin and NS in humans.

Loyed Ll. (2009) observed that formaldehyde works best as a poison for flies when the solution does not contain formic acid and only small amounts of methylamine. For it to be most effective, the formaldehyde should be clear and have no fishy smell. To improve its effect, a small amount of weak alkali should be added. This helps neutralize any acid already present or that might form during use.

According to **Songur, A. et.al. (2009)**, Formaldehyde (FA), FA is highly soluble in water and organic solvents. Its cytotoxicity arises mainly from forming strong DNA–protein and amino acid cross-links, contributing to tissue damage and cellular dysfunction.

Day et al. (2008) emphasized that accurate approximation of the post-mortem interval (PMI) in forensic entomology relies heavily on proper preservation of insect evidence. Their study investigated how different preservation methods affect the integrity of larvae(3rd instar) of *Calliphora augur* and *Lucilia cuprina*. Preservation treatments included different concentrations of ethanol, the solution of Kahle's , and 10% formalin, applied in triplicate.

According to **David, D., & Arkeman, H. (2008)**, this study investigated the toxic effects of oral formaldehyde exposure on the stomach lining of rats. Thirty female Sprague-Dawley rats were divided into three groups: a control group, a group receiving 4 mg/L formaldehyde, and another receiving 6 mg/L in their drinking water for 12 weeks. Histological analysis showed a significant reduction in the thickness of the gastric mucosa, especially in the higher-dose group. Both treatment groups exhibited vacuolar degeneration and necrosis in the gastric fundus glands. The results indicate that long-term formaldehyde ingestion can cause dose-dependent damage to the stomach lining in rats.

Karunantre and Hwshan (2004) reported that formalin negatively affects fly visitation and reduces the development of maggot masses on fish carcasses. Their study demonstrated that the use of formalin the preservative which is extend the life of shelf of fish influences all developmental stages of blow flies, including colonization, maggot growth, and the rate of carcass decomposition. Compared to

control samples, formalin-treated carcasses produced significantly fewer pupae and adult flies. However, the significant effect of formalin into the overall decay of the fish carcasses, suggesting that its impact is more pronounced during the initial stages of decomposition but diminishes over time. In an early observation published in the Indian Medical Gazette in 1913, Huston described formaldehyde as an effective insecticide against flies. He noted that simply exposing flies to formaldehyde in shallow dishes was ineffective. Instead, a more successful method involved mixing formaldehyde with milk and water to create a bait solution, which, when ingested by flies, proved lethal.

Marks J. (2003) similarly describes formaldehyde as a colorless, flammable gas with a strong odor, often compared to the smell of pickles. Naturally produced in small amounts by humans, animals, and plants, it is also commonly found in building materials and household products as a preservative. While low-level exposure does not typically cause health issues in most individuals, it can still trigger irritation of the eyes, throat, respiratory tract, and skin, especially in sensitive individuals. Prolonged exposure to high concentrations has been linked to more severe health outcomes, including an elevated risk of cancer. This supports the classification of formaldehyde as a dangerous environmental and occupational hazard.

According to **Pandey, C.K. et al.(2000)**, Formalin (40% formaldehyde in water) is a toxic, corrosive substance that can cause severe damage to multiple body systems if ingested. Symptoms include gastrointestinal bleeding, metabolic acidosis, unconsciousness, and respiratory distress. There is no specific antidote, and treatment focuses on supportive care and managing organ failure through a multidisciplinary approach.

This clinical study by **Pandey et al. (2000)** discusses the toxic effects of accidental or intentional ingestion of formalin in humans. The ingestion causes severe metabolic acidosis, gastrointestinal corrosion, and potentially fatal multi-organ failure. The paper emphasizes the need for early diagnosis and aggressive supportive care,

including fluid resuscitation, correction of acidosis, and renal support. The study underscores formalin's high toxicity when ingested and the challenges in clinical management of such poisonings.

Rach, J. J. et al. (1997). Formalin was applied here on both warmwater and cool water species eggs which were carried fungal infections. Here they observed at a specific developmental stage a very special dose of formalin was effective and safe to control, whereas improper use of formalin may reduce hatch rates of egg.

Howe et al. (1995) investigated the effects of paraformaldehyde precipitation in stored formalin solutions used in fish hatcheries. They found that moderate amounts of paraformaldehyde did not increase toxicity or reduce antifungal efficacy in treatments of rainbow trout and channel catfish. Both the clear and bottom fractions of the precipitated formalin were equally effective and safe. However, formalin with heavy paraformaldehyde buildup—especially after long-term freezing—should not be used. Proper storage at room temperature prevents this issue.

Fischer (1905) investigated the toxic effects of formaldehyde and formalin in animals through various exposure routes. Inhalation of formaldehyde caused bronchitis and pneumonia, while ingestion of formalin could lead to sudden death and severe gastritis. Intraperitoneal and muscular injections resulted in inflammation of internal organs, including peritonitis, myositis, and damage to the liver, kidneys, and lungs. Formalin exposure caused cloudy swelling, fatty degeneration, and necrosis in parenchymatous organs. It also triggered a strong inflammatory response, beginning with eosinophilic infiltration followed by other leukocytes. Chronic exposure led to persistent organ damage and systemic toxicity. The study concluded that formalin is a potent irritant and systemic toxicant affecting multiple organ systems.

According to **Chinabut, S. et. al. (1988)**. A static bioassay was used to determine the 96-hour LC_{50} values of formalin in three freshwater fish species: silver barb *(Puntius gonionotus*), common carp (*Cyprinus carpio*), and snakehead fish (*Channa striatus*), which were found to be 67–80 ppm, 106–128 ppm, and 147–166 ppm, respectively. The highest toxicity was observed within the first 24 hours of exposure, and neither

pH nor water hardness significantly influenced toxicity levels. An 8-week exposure experiment with common carp fry revealed significant reductions in growth at 75 ppm formalin, though no histological changes were observed in major organs. However, silver barb exposed to formalin showed hyperplasia of gill lamellae and fatty degeneration in liver tissue, while common carp and snakehead fish did not exhibit such changes.

CHAPTER-03
MATERIALS AND METHODS

MATERIALS AND METHODS

3.1 Materials

3.1.1 Location and Duration

The study location was at the animal garden of department of Zoology, Dhaka university from June 2024 to January 2025.Here the primary rearing, treatment of different doses and observation were conducted. Entomology laboratory were performed for dosing, analysis and histology and analysis.

3.1.2 Materials for the collection of *C. megacephala*

Adult specimens of *C. megacephala* were collected from Ananda Bazar, a local market area characterized by abundant organic waste and decomposing animal matter—ideal breeding and feeding sites for blowflies. The collection was conducted during daylight hours when adult flies are most active, particularly around meat and fish stalls where formalin-treated products are frequently present.

The objective of this collection was to obtain a sufficient number of healthy, field-active adult flies for the establishment and maintenance of a laboratory stock culture. This culture was used as a controlled population for various experimental purposes, including toxicological testing, developmental studies, and histopathological analyses involving exposure to formalin-contaminated food sources.

1. Insect Collecting Net

An insect collecting net was used to capture adult *C. megacephala* from the field. The net facilitated the collection of live, undamaged flies for use in laboratory rearing and experimentation.

2. Cotton Ball

Sterile cotton balls were soaked in a sugar solution and placed inside the rearing cages as a feeding source for adult flies. In some cases, cotton balls were also used to provide moisture or serve as oviposition substrates.

3. Bovine Liver

Fresh bovine liver was used as a source of protein and oviposition medium for gravid female flies. It was also employed as a medium to administer various concentrations of formalin to larvae in toxicity experiments. The liver was weighed and mixed with formalin in specific doses based on wet weight (ml/g).

4. Forceps

Dissecting forceps were used for handling delicate insect specimens during collection, transfer, and dissection. They were especially useful during the preparation of larvae, pupae, and adults for morphological and histological examination.

5. Net Cage

Standard-size rearing cages (16×16×16 cm) constructed of fine mesh netting were used to house adult flies under laboratory conditions. These cages allowed adequate ventilation while preventing escape and contamination. They also provided a controlled environment for mating, oviposition, and egg collection.

3.1.3 Chemicals

1. Formalin

Formalin (37–40% aqueous solution of formaldehyde) was used as a primary chemical treatment to assess its toxicological effects on different developmental stages of *C. megacephala*. Various concentrations were prepared by diluting formalin with distilled water and mixing with bovine liver to expose larvae through feeding.

2. Alcohol

As a preservative and sterilizing agent, Ethanol (70%) was used . It was employed for cleaning instruments such as forceps and dissecting tools before and after use, and for preserving insect specimens for morphological or histological analysis.

3. Glucose

Glucose solution was prepared and soaked into cotton balls to serve as a carbohydrate source for adult flies during rearing. It maintained the energy levels of flies and promoted longevity and mating activity.

4. Dettol

Dettol (antiseptic liquid) was used for disinfecting laboratory surfaces, hands, and equipment to maintain aseptic conditions during insect handling, dissection, and rearing processes. It helped minimize microbial contamination in rearing cages and specimen preparation areas.

3.1.4 Equipment

A variety of laboratory equipment was employed throughout the course of the study to ensure the successful execution of experiments involving the collection, treatment, observation, and preservation of *C. megacephala* at various developmental stages. Each piece of equipment was selected to fulfill specific methodological functions in rearing, dissection, chemical exposure, and histological procedures.

1. Microwave Oven

A microwave oven was used in certain stages of sample processing, such as the rapid drying of glassware, filter papers, and other materials when traditional air drying was not feasible. In addition, it aided in mild sterilization of non-flammable laboratory items.

2. Other Small Items Used in the Laboratory

These included commonly used tools such as dissecting scissors, pins, fine blades, scalpels, glass rods, droppers, labels, adhesive tapes, and measuring spoons. They played a supporting role in dissection, labeling, transferring small samples, and preparing solutions.

3. Beakers

Beakers made of borosilicate glass in different volumes (50 mL to 500 mL) were essential for solution preparation, mixing formalin with bovine liver, holding insect samples, and measuring chemicals. These were also used as temporary containers during feeding trials and sample storage.

4. Deionized Water

Deionized water was used throughout the study for preparing physiological saline solution (0.9% NaCl), diluting chemicals such as formalin and alcohol, washing dissection tools, and rinsing insect samples. It ensured purity in all chemical preparations and prevented interference from ionic contaminants.

5. Sample Preserving Containers

Airtight glass or high-quality plastic containers were used to preserve larval, pupal, and adult specimens post-treatment. These containers were labeled with sample information and stored in appropriate conditions for either histological processing or further analysis.

6. Forceps

Fine stainless steel forceps were utilized to handle larvae, pupae, and adult flies during transfer, dissection, and microscopic observation. They allowed precision and minimized physical damage to delicate tissues.

7. Filter Paper

It was used for multiple purposes like filtering liquids, to absorb moisture from sample tissues as well as for drying dissected specimens. To reduce spare chemical residues from samples, filter paper also used here.

8. Funnel

Glass or plastic made funnel were used for transferring liquids, chemicals or for preparing doses for treatment.

9. Gloves

As the formalin and formaldehyde are very toxic, disposable gloves were used for personal protection. It was used during handling chemicals and preserved samples.

10. Petri Dishes

In various purposes sterile petri dishes were used, such as to place samples, tissues and so on.

11. Pipettes

Pipettes were used to measure and dispense small volumes of liquid chemicals .To prepare formalin doses they were used as well as to prepare histology slide preparation they used.

12. Suction Pump

A hand-held or electric suction pump was utilized for removing excess liquid during washing or dissection processes. It provided better visibility of tissues and reduced moisture that could interfere with sample preservation or microscopic examination.

13. Tissues and Cotton Balls

Absorbent tissues were used for cleaning laboratory benches, wiping glassware, and blotting samples. Cotton balls soaked in glucose solution were provided as a carbohydrate source for adult flies. They were also used for maintaining humidity in rearing containers.

14. Water Glasses

Simple water glasses were repurposed as temporary holding containers for flies or chemical solutions during short-term procedures. Their transparent walls allowed easy monitoring of sample condition.

15. Plastic Jars

Lidded plastic jars were used for storing and transporting bovine liver, rearing larvae, and preserving treated insects. Their versatility made them useful at various stages of the research, especially during field-to-lab sample handling.

16. Sweeping Net

A standard entomological sweeping net, made with fine mesh and a long handle, was used to capture live Chrysomya megacephala adults from the field. This tool enabled efficient and non-lethal collection of target species from open environments for laboratory experimentation.

17. Vernier Scale

Used for measuring samples.

3.1.5 Materials for Histopathology Study

This segment of the research involved the histopathological examination of Chrysomya megacephala specimens exposed to varying concentrations of formalin. To conduct accurate tissue processing and microscopic analysis, specialized equipment and histological chemicals were utilized as described below.

A. Equipment

1. Fine Forceps

Fine-tipped forceps were used for handling small and delicate tissue samples during dissection and transfer to minimize physical damage prior to fixation and staining.

2. Dropper

Glass and plastic droppers were employed to apply small volumes of solutions such as fixatives, stains, and dehydrating agents onto tissue samples or slides with precision.

3. Eppendorf Microtubes

Sterile microcentrifuge tubes (1.5–2.0 mL) were used for storing small tissue specimens, fixatives, or reagents during sample processing. These tubes helped maintain the sterility and containment of sensitive biological material.

4. Fine Forceps for Dissection

A second set of ultra-fine forceps specifically designated for dissection was used during the longitudinal cutting and internal examination of larval, pupal, and adult tissues.

5. Micropipette

Adjustable micropipettes with variable volume settings were used to accurately dispense minute quantities of chemical reagents such as stains, alcohol, and xylene onto slides or into containers.

6. Microscope

A compound light microscope was used for observing the stained tissue sections at various magnifications. It enabled the identification of cellular and sub-cellular alterations caused by formalin exposure.

7. Petri Dishes

Sterile Petri dishes served as temporary holding containers for dissected organs or tissues during washing, staining, or mounting procedures.

8. Slides

Clean, dust-free glass slides were used to mount stained tissue samples. Proper labeling and handling ensured the integrity of prepared slides for microscopic analysis.

9. Surgical Scissors

Small surgical-grade scissors were used for dissecting the insect body and isolating internal organs, especially the midgut, fat body, and salivary glands, for histopathological evaluation.

10.Tubes

Plastic or glass tubes were used for storing prepared solutions, holding samples during dehydration, and facilitating reagent exposure during tissue processing steps.

11. Tissues

Laboratory-grade absorbent tissues were employed to blot excess liquids, clean slides, and dry glassware during histological work.

B. Chemicals

1. Canada Balsam

A natural resin used as a mounting medium to permanently fix stained tissue sections between the slide and cover slip. It preserved tissue transparency and ensured long-term stability.

2. 70% Ethyl Alcohol

Ethanol at 70% concentration was used as a dehydrating agent and fixative for tissue preservation. It also served in the graded alcohol dehydration series for slide preparation.

3. Xylene

Xylene was used as a clearing agent to remove alcohol from tissue samples and improve the refractive index before mounting. It enhanced slide transparency and stain visibility.

4. Hematoxylin

Hematoxylin, a basic dye, was used for nuclear staining. It imparted a blue to purple color to the nuclei, enabling clear observation of nuclear structures and histological patterns.

5. Eosin

Eosin, an acidic dye, was used as a counterstain to hematoxylin. It stained cytoplasmic components and connective tissues pink, providing contrast and detail to the histological sections.

Figure 1. ***C. megacephala*.**

Figure 2. Sweeping net.

Abiotic material

1. Water

Biotic material

1. Blow fly

3.1.6 Descriptive of Biotic materials

Blow fly

C. megacephala, commonly known as blow flies or Oriental latrine flies. Classification as phylum-Arthropoda, class-Insect, Order-Diptera and family-Calliphorid. This species is widely distributed in the world including Bangladesh and having greenish blue metallic box like body.

Figure 3. ***C. megacephala***

Figure 4. Preparation of doses.

Figure 5. Preparation of treatment.

3.2 Methods

3.2.1 Preparation of doses of formaldehyde and formalin

Preparation and Application of Formalin Solutions for Toxicity Testing

The toxicological effects of formalin on various developmental stages of the blow fly *C. megacephala*, formalin solutions of different concentrations were prepared and applied in a controlled laboratory setting. The experimental procedure was designed to simulate real-world scenarios where flies may come into contact with formalin-contaminated food substances, such as preserved meat in markets.

1. Preparation of Formalin Solutions

Commercially available formaldehyde (typically containing 37% formaldehyde by weight) was diluted with tap water to prepare formalin solutions of varying strengths. This method mirrors common informal practices observed in food preservation, where concentrated formaldehyde is mixed with water to create working solutions.

Four formalin concentrations were prepared by mixing formaldehyde with tap water in the following proportions:

20% Formalin: 20 ml of formaldehyde + 80 ml of tap water

40% Formalin: 40 ml of formaldehyde + 60 ml of tap water

60% Formalin: 60 ml of formaldehyde + 40 ml of tap water

80% Formalin: 80 ml of formaldehyde + 20 ml of tap water

Each mixture was prepared in a clean measuring cylinder and thoroughly stirred to ensure complete homogenization. These formalin solutions were stored in airtight

glass containers and used for preparing treated food baits for the fly exposure experiments.

2. Preparation of Formalin-Treated Liver Bait

To deliver formalin to the flies via ingestion, fresh bovine liver was used as the food substrate. The method of exposure followed the protocol by Gitunga (2001), with formalin doses prepared on a wet weight basis (w/w), where specific volumes of formalin solution were added to 1 gram of liver.

The following concentrations of formalin were used in the feeding assay:

0.3 ml/g

0.2 ml/g

0.1 ml/g

0.01 ml/g

0.001 ml/g

For the highest dose, 0.3 ml of selected formalin dose was added to 1 gram of fresh bovine liver and mixed thoroughly to ensure even distribution. The lower doses were prepared by serially diluting the volume of formalin while maintaining the liver weight constant at 1 gram for each treatment group. A control group was included where liver samples were left untreated with formalin to serve as a baseline for comparison.

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3. Experimental Exposure and Observation

The treated liver samples were placed in feeding trays and introduced into separate insect rearing cages, each containing a fixed number of adult Chrysomya megacephala. Flies were allowed to feed freely on the treated bait under standardized laboratory conditions:

Temperature: 26 ± 2°C

Relative Humidity: 70 ± 5%

Photoperiod: 12 hours light : 12 hours dark (12L:12D)

Each treatment group, including the control, was monitored continuously over a 72-hour observation period. Mortality was recorded at 24-hour intervals—i.e., at 24, 48, and 72 hours. Dead individuals were counted and removed during each observation point to prevent microbial decomposition and interference with further analysis.

3.2.2 Collection and Rearing of Flies

C. megacephala flies were reared in the laboratory of the Animal Garden, Department of Zoology, University of Dhaka. Wooden cages, each measuring 16 × 16 × 16 cm, were used for rearing. The floor of each cage was made of wood, and the other sides were covered with fine mesh netting. A circular hole on the front side, fitted with a net sleeve, was used for removing flies and adding food.

Newly emerged adult flies were fed with glucose, sugar-soaked cotton, and fresh or treated bovine liver. These food sources helped in adult fly development and egg-laying. About 10 adult flies were placed in each cage, selected from the existing laboratory colony. For experiments, adults that were three days old were used.

The larvae were fed only fresh bovine liver. When they stopped feeding and reached the pupal stage, wood dust was added to help them pupate. Each larva was then placed into a separate beaker with wood dust. The beakers were covered with cloth held by a plastic band and moved into an incubator.

Pupae were also kept in plastic containers with sterilized netting for air flow. These were maintained at 28 ± 2°C with 68 ± 5% humidity until adult flies emerged. Room

temperature was recorded daily with thermometers and ranged between 25°C and 32°C. Humidity remained around 70–80%.

The flies were checked every day. Emerging adults were collected regularly, and their food was replaced whenever needed.

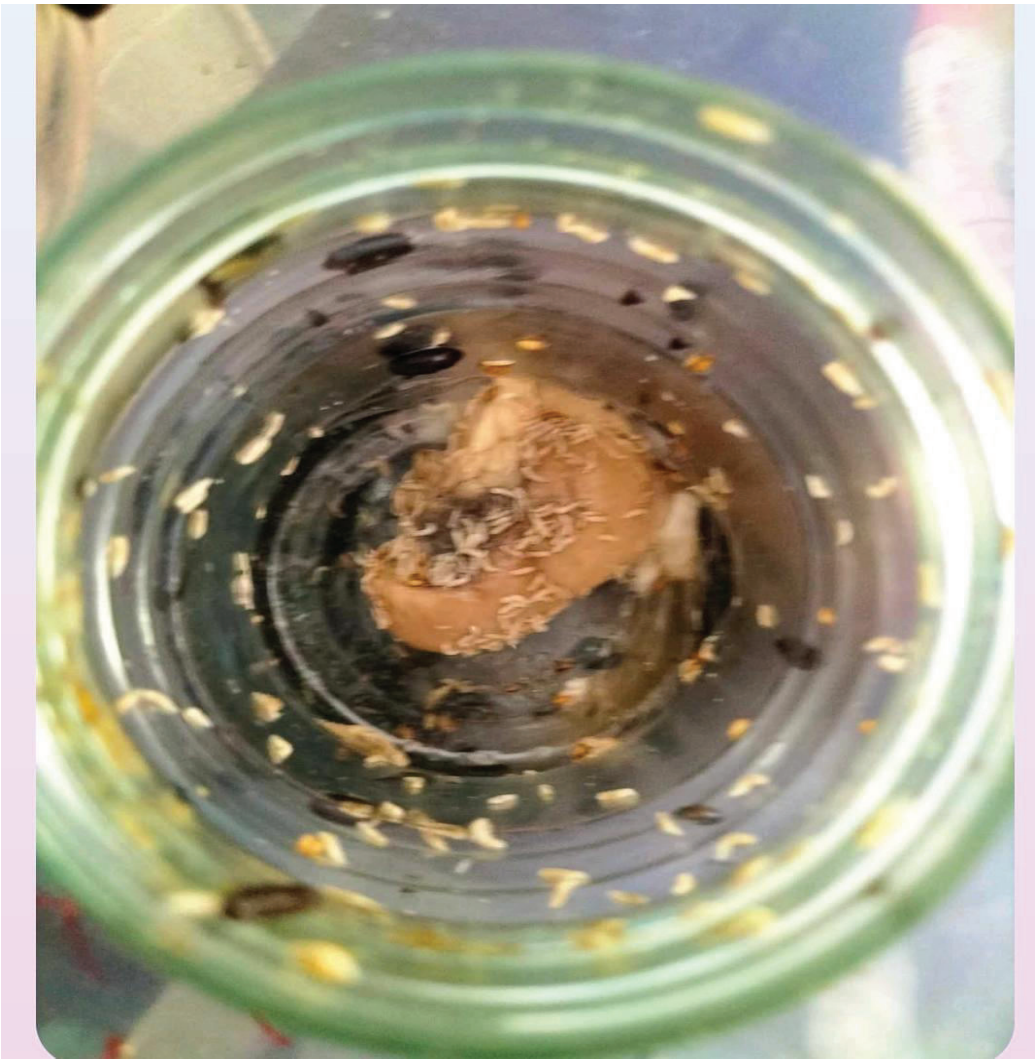

Figure 6. Larva and pupa formed after treatment.

Figure 7. Larvae & pupa after treatment given.

Figure 8. Incubator for maintaining optimum temperature(25±2℃).

3.2.3 Sample collection

Blow fly (larvae, pupa and Adult) were collected from applied prepared doses (Formaldehyde and formalin) culture.After collecting insects and their developmental stages, they were stored in separate vial in 70% Alcohol.

Figure 9. Collected samples.

Dissection and Preservation

After 72 hours, the samples of the flies were dissected and washed in a physiological saline solution prepared by dissolving 0.9 g of NaCl in 100 ml of distilled water. The tissues were preserved in 70% ethanol in 1.5 ml microcentrifuge tubes, and subsequently dehydrated through 90% and 100% ethanol.

3.2.4 Histological Procedure

The dehydrated tissues were cleared in xylene and embedded in paraffin wax at 80°C. After cooling, the wax blocks were trimmed and sectioned. Tissue ribbons were affixed to glass slides using Mayer's albumin and treated with xylene. Gradual rehydration was performed using descending concentrations of alcohol (100%, 90%, 70%, 50%) followed by distilled water. Staining was carried out with Hematoxylin and Eosin. Slides were then dehydrated with ascending alcohol concentrations (70%, 90%, 100%), cleared in xylene, and mounted with Canada balsam. The slides were examined with a microscope at 10X,20X and 40Xmagnification to assess the changes of tissues.

The microscopic examination of plant and animal cells and tissues is referred to as histology (McRae et al., 2009). After dissection and preservation, the following steps were carried out for histological study:

a) Xylene-to remove alcohol

The dissected tissue were placed here in xylene for almost 10 minutes for removing alcohol.

b) Saturation with Wax:

To allow impregnation, tissues were then soaked in a mixture of both xylene and wax.

c) Embedding in Wax:

Later on ,the tissues were placed in incubator for 30 minutes in incubator which was maintained 80°C and were embedded in paraffin wax(embedded).

d) Preparation of Blocks:

A paper boat was made coated its inner side with glycerinate the boat placed in a water bath. then after eliminated water bubbles with a hot needle, the tissue was placed longitudinally in the wax. The process was done in room temperature.

e) Trimming and Sectioning:

After getting cool and solid the wax, it is called block., which was fixed onto a holder for microtomy. The blade of the microtome was adjusted at a 45° angle to trim the wax block into thin ribbons.

f) Attaching to Slides (Affixing):

The ribbons from the wax block were mounted on glass slides using Mayer's Albumen, which is a solution composed of 50 ml egg white, 50 ml glycerin, and 5 ml sodium salicylate. To prepare the mounting solution, 3–5 drops of Mayer's Albumen were mixed with 20 cc of distilled water.

g) Slide Stretching:

After applying Mayer's Albumen to the slides, the wax ribbons were carefully stretched onto them for further processing.

Deparaffinization: To eliminate wax from tissue samples, slides were immersed in xylene for 5 to 10 minutes.

h)Hydration: The slides underwent a rehydration process by being sequentially transferred through 100%, 90%, 70%, and 50% ethanol. Afterward, they were rinsed in distilled water to remove residual alcohol.

I)Staining: Hydrated tissue slides were stained with Hematoxylin, followed by a rinse under running water for 30 minutes. This process oxidized the nuclei, making them appear blue.

j)Differentiation with Acid Water: Slides were then counterstained with Eosin for about 5 minutes. This stained the cytoplasm pink, enhancing visibility under the microscope.

k)Dehydration: The slides were again treated with ascending alcohol concentrations (70%, 90%, and 100%) for approximately 5 minutes each to dehydrate the tissues.

l)Clearing: To remove any remaining alcohol, slides were placed in xylene for another 5 to 10 minutes.

m)Mounting: Coverslips were placed on the slides using Canada balsam as the mounting medium. The slides were left to dry at room temperature.

n)Labelling and Microscopy: After drying, the prepared slides were labeled and examined under a microscope using 20x and 40x magnifications to observe tissue structure and identify any damaged areas.

Figure 10. Paper boat making.

Figure 11. Waxing in Paper boat for sample.

Figure 12. Block making.

Figure 13. Microtomy for expected slide.

Figure 14. Microscopic observation.

Figure 15. Vernier scale for estimate size of the samples.

3.3 Data Analysis

i) Mortality Calculation

The mortality data were adjusted using Abbott's formula (Abbott, 1925)

Corrected Mortality (%) = ((T - C) / (100 - C)) × 100

Where,

T = mortality percentage in the treatment group

C = mortality percentage in the control group

The total mortality of blowflies (both control and treated groups) within 72 hours was analyzed using a one-way ANOVA in IBM SPSS Statistics (version 26.0). Significant differences between groups were determined using Duncan's Multiple Range Test (DMRT).

The results were expressed as Mean ± Standard Error (SE) of mortality for blowflies exposed to treatment (both through diet and injection).

ii) Histological Analysis

For easier interpretation, larva, pupa and adult blowfly tissue samples were collected.

iii)Size and developmental analysis

Formalin exposure significantly affected the size and development of Chrysomya megacephala across all life stages. Larvae and pupae showed reduced body size and delayed growth, with visible deformities and extended development time. Adults emerging from treated groups exhibited smaller sizes and malformations, indicating long-term physiological damage. These findings confirm that formalin causes dose-dependent developmental toxicity, making growth parameters reliable indicators of environmental chemical stress.

CHAPTER-04
RESULTS

Results

To determine the toxic effects of formalin and formaldehyde exposure on the developmental stages of insects, experimental treatments were conducted on larvae, pupae, and adult *C. megacephala*. The insects were exposed to different concentrations of formalin through feeding and environmental contact. The study was carried out in controlled laboratory conditions within the Entomology Laboratory at the University of Dhaka. The purpose of this study was to assess the dose-dependent effects of formaldehyde on insect mortality, growth inhibition, and physiological damage.

The concentrations of formalin used in this study were prepared by diluting commercially available 37% formaldehyde solution to achieve varying levels of toxicity. The tested doses included 0.3, 0.2, 0.1, 0.01, and 0.001 ml/g (wet weight basis), applied directly to bovine liver used as bait. Observations were made on larval development, pupal formation, and adult emergence, as well as overall survival rate and behavioral changes.

Histological analysis was performed to evaluate tissue damage in different life stages of the insects. Treated specimens were dissected and preserved in buffered formalin, followed by standard staining techniques. Morphological changes were recorded under light microscopy. Insects exposed to higher doses exhibited severe disruption in tissue structure, delayed pupation, and increased mortality rates. At the highest concentration (0.3 ml/g), mortality reached 100% in larvae within 24 hours of exposure, and no pupae or adults emerged.

Data analysis indicated a clear correlation between increasing formalin concentration and toxic effects, including cellular necrosis, gut wall damage, and failure in metamorphosis. The toxic effects were quantified based on percentage mortality and developmental delay, and results were expressed as mean ± standard deviation. These findings confirm that formalin and formaldehyde have strong dose-dependent toxicity in insects, supporting their classification as hazardous environmental chemicals.

4.1 Effect of Treatments on Mortality of *C. megacephala*

Descriptive Statistics

The descriptive statistics for correct mortality rate across varying dose levels (.001, .010, .100, .200, and .300 ml) reveal a clear upward trend in mortality rates as the dose increases. At the lowest dose of .001 ml, the mean mortality rate is 1.7875, with a relatively small standard deviation of 0.22321, indicating low variability among samples. As the dose rises to .010 ml, the mean mortality rate increases to 2.4000, though variability also increases (SD = 0.73095). At .100 ml, the mean sharply rises to 6.6105, followed by 7.1788 at .200 ml, and finally peaks at 9.4783 at .300 ml. The standard deviations for the higher doses are moderate, suggesting consistent increases in mortality with dose escalation. The 95% confidence intervals confirm the reliability of these means, with no overlap between the lowest and highest dose levels, indicating a significant dose-dependent effect.

Table 1. Descriptive Statistics and One-Way ANOVA of Corrected Mortality (%) of *C. megacephala* at Different Treatment Levels

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
.001	8	1.7875	.22321	.07892	1.6009	1.9741	1.50
.010	8	2.4000	.73095	.25843	1.7889	3.0111	2.00
.100	8	6.6105	.41746	.14759	6.2615	6.9595	6.00
.200	8	7.1788	.33566	.11867	6.8981	7.4594	7.00
.300	8	9.4783	.97739	.34556	8.6612	10.2954	8.00
Total	40	5.4910	3.03378	.47968	4.5208	6.4613	1.50

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	346.164	4	86.541	236.925	.000
Within Groups	12.784	35	.365		
Total	358.948	39			

ANOVA Results

The one-way ANOVA for correct_mortality_rate4 shows a highly significant difference between the dose groups, with an F-value of 236.925 and a p-value of less than 0.001. This extremely low significance level confirms that the observed differences in mortality rates are not due to random chance but are strongly associated with the dose levels administered. The between-groups sum of squares (346.164) far exceeds the within-groups sum of squares (12.784), meaning most of the variation in mortality can be explained by differences between doses rather than within the same dose group. This statistical evidence supports the hypothesis that mortality rate is dose-dependent.

Table 2. Duncan's Multiple Range Test (DMRT) for Mean Corrected Mortality (%) of *C. megacephala* Across Different Dose Levels.

Dose_ml	N	Subset for alpha = 0.05		
		1	2	3
.001	8	1.7875		
.010	8	2.4000		
.100	8		6.6105	
.200	8		7.1788	
.300	8			9.4783
Sig.		.050	.068	1.000

Duncan Post-Hoc Test

The Duncan multiple range test further breaks down the differences between dose groups. The results place the smallest doses (.001 and .010 ml) into the same subset, suggesting no statistically significant difference between them at $\alpha = 0.05$. However, higher doses (.100, .200, and .300 ml) form distinct subsets, with increasing mortality rates as dose increases. This stepwise separation in the Duncan test supports the conclusion from the ANOVA that the mortality rate escalates significantly with dosage, particularly beyond the 0.010 ml level.

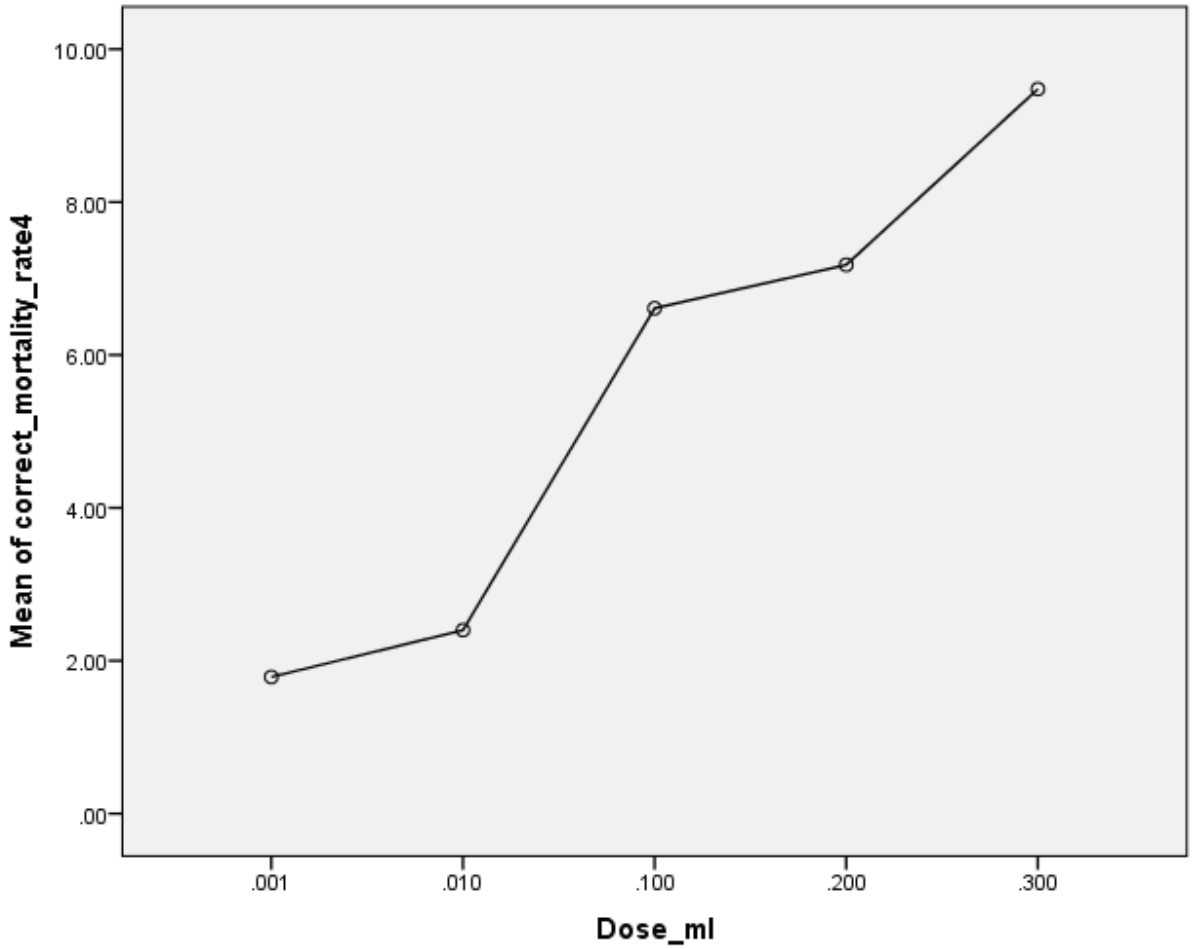

Figure 16. Dose response curve derived from DMRTs.

Graph Interpretation

The plotted graph of correct_mortality_rate4 against the administered dose clearly illustrates a positive dose–response relationship. At the lowest dose of 0.001 ml, the mortality rate is minimal, and the line rises gradually through 0.010 ml. However, after crossing the 0.100 ml dose level, the slope of the curve steepens considerably, indicating a sharp increase in mortality rates. This visual pattern mirrors the statistical findings, where lower doses produce marginal effects while higher doses induce much greater mortality. The curve's shape suggests a possible threshold effect, where toxicity remains low until a certain concentration is reached, beyond which lethal effects escalate rapidly. The nearly linear rise in the upper range (.100 to .300 ml) also reinforces the idea that increased exposure proportionally increases mortality, making the graph a powerful visual confirmation of the dose-dependent nature of the response.

4.2 Effect of Treatments on Size and Development Traits of *C. megacephala*

The effects of different formalin doses and treatment volumes on total body length, head length, thorax length, and wing length of Chrysomya megacephala are summarized in the data tables and visually represented in Figure 2. Across all measured parameters, considerable variation was observed among treated groups, with trends indicating dose- and volume-dependent morphological changes.

For total body length, the control group consistently exhibited the highest mean value of 9.00 mm. Treated groups showed marked reductions in body length, particularly at higher formalin doses and volumes. At the 80 ml volume, the total body length declined to as low as 1.33 mm at the 0.3 ml dose, whereas in the 20 ml group at the same dose, it was 4.66 mm. Notably, the highest mean among treated groups occurred at the 0.01 ml dose in the 20 ml group (10.0 mm), possibly indicating a mild hormetic response at very low concentrations.

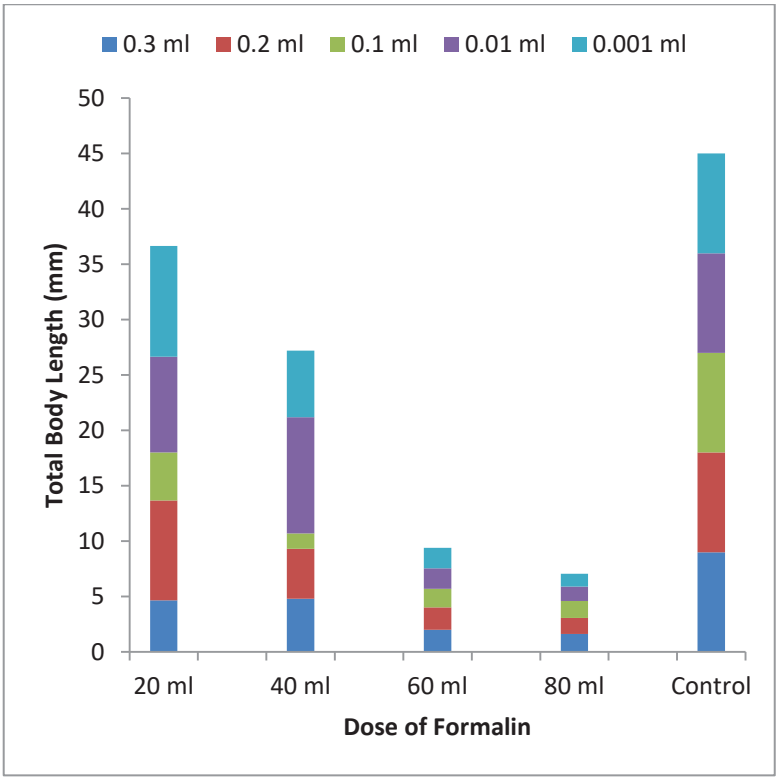

Figure 17. Effect of different doses of Formalin on the Total Body Length of *C. megacephala*

Head length also decreased with increasing treatment intensity. While the control group maintained a stable mean of 2.00 mm, treated individuals showed progressive reductions, with the lowest head length (0.4333 mm) observed at the 0.3 ml dose under 80 ml treatment conditions. Moderate values were found at lower doses and treatment volumes.

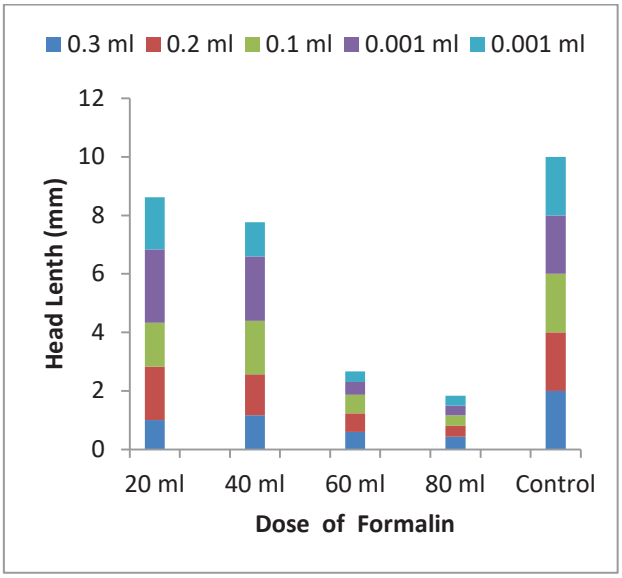

Figure 1. Effect of different doses of Formalin on Head Length (mm). of *C. megacephala*

The same declining trend was evident for thorax length. Control flies measured 2.00 mm on average, whereas treatment groups experienced dose-dependent reduction. The lowest mean thorax length (0.5666 mm) occurred at the 0.3 ml dose with 60 ml volume, reflecting the compounding impact of both factors.

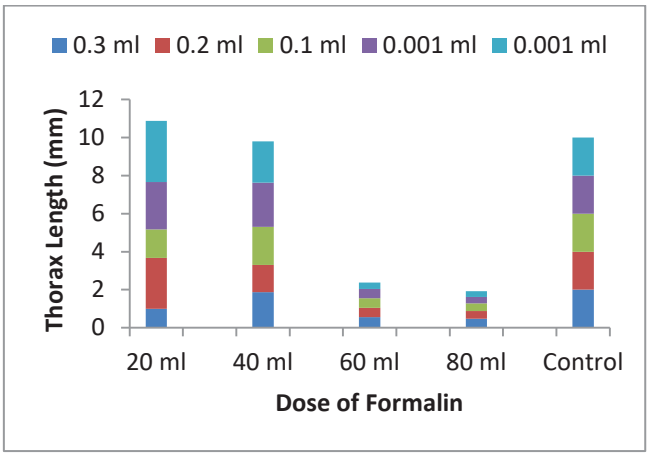

Figure 19. Effect of different doses of Formalin on Thorax Length of *C. megacephala*

Wing length followed a comparable decreasing pattern. Control individuals showed a consistent mean wing length of 6.00 mm, while treated groups recorded significantly shorter wings. The smallest wings were observed in the 0.3 ml, 60 ml group (0.1155 mm), suggesting that wing development is highly sensitive to formalin exposure.

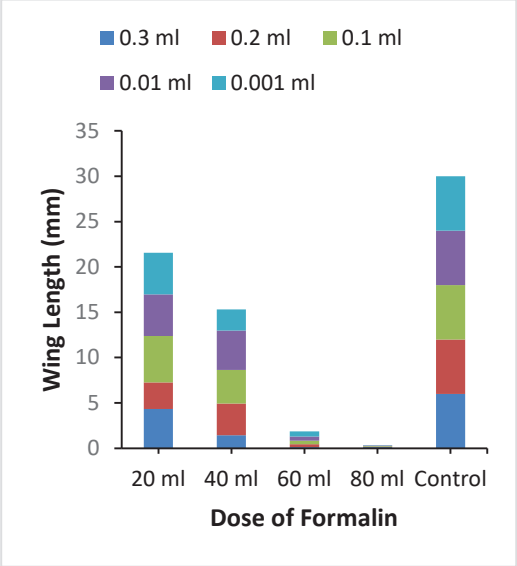

Figure 20. Effect of different doses of Formalin on Wings Length of *C. megacephala*

These findings are visually represented in Figure 2, which displays the dose- and volume-dependent variations in all four morphometric parameters. The graph clearly illustrates that increasing formalin concentrations and exposure volumes are associated with reduced body size and appendage development, although some fluctuations occur at lower doses.

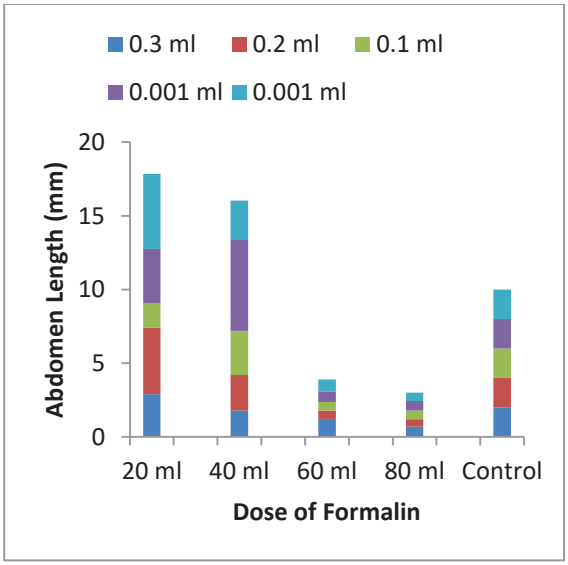

Figure 21. Effect of different doses of Formalin on Abdomen Length of *C. megacephala*

Effects of formalin dose (0.001 to 0.3 ml) and treatment volume (20 ml, 40 ml, 60 ml, 80 ml) on body size parameters of Chrysomya megacephala, including total body length, head length, thorax length, and wing length (mean values).

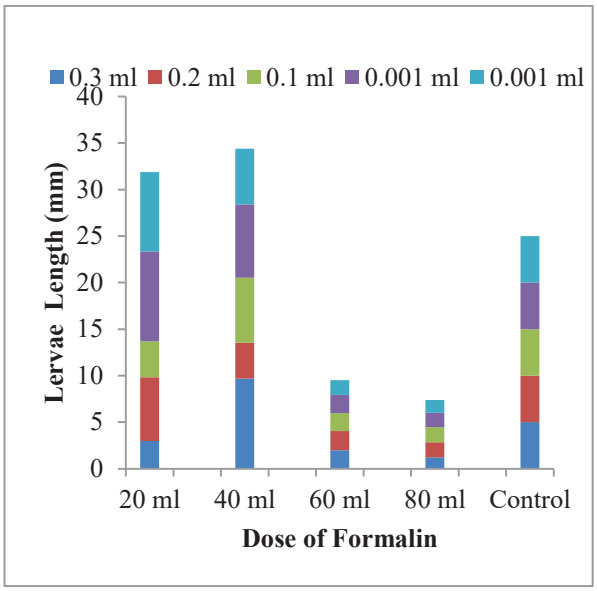

Figure 22. Effect of different doses of Formalin on Larvae Length of *C. megacephala*

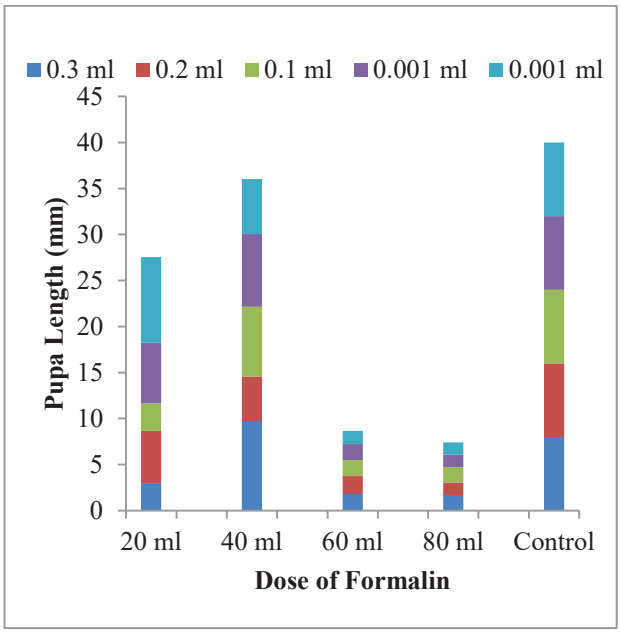

Figure 23. Effect of different doses of Formalin on Pupa Length of *C. megacephala*

4.2.1 Statistical Analysis on Size and Development Traits of *C. megacephala*

Trait evaluated: Total Body Length (mm)

Table 3. Descriptive Statistics, ANOVA, and Duncan's Multiple Range Test for Total Body Length (mm) of Blowflies in Response to Different Treatment Doses

(a) Descriptive Statistics

Dose (ml)	N	Mean	Std. Deviation	Std. Error	95% CI Lower	95% CI Upper	Minimum	Maximum
0.001	12	4.7083	3.93826	1.13688	2.2061	7.2106	1.00	11.00
0.010	12	5.5333	4.34309	1.25374	2.7739	8.2928	1.10	11.00
0.100	12	3.4250	2.16254	0.62427	2.0510	4.7990	1.10	7.50
0.200	12	4.2417	3.14743	0.90858	2.2419	6.2414	1.30	10.00
0.300	12	3.0750	1.38703	0.40040	2.1937	3.9563	1.50	5.00
Total	60	4.1967	3.20624	0.41392	3.3684	5.0249	1.00	11.00

(b) One-Way ANOVA

Source	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	46.849	4	11.712	1.151	0.342
Within Groups	559.670	55	10.176		
Total	606.519	59			

(c) Duncan's Multiple Range Test (DMRT)

Dependent Variable: Total Body Length (mm)

Dose (ml)	N	Subset for α = 0.05
0.300	12	3.0750
0.100	12	3.4250
0.200	12	4.2417
0.001	12	4.7083
0.010	12	5.5333

Sig.: 0.097

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 12.000

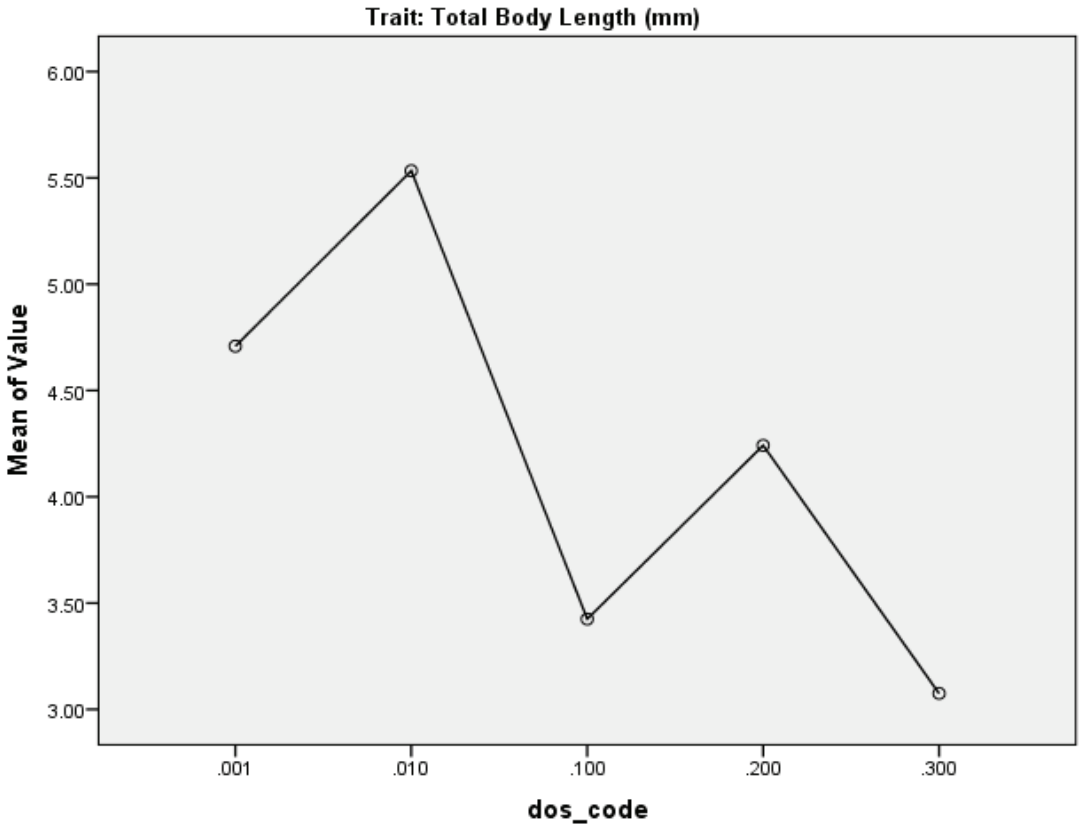

Figure 24. Dose response curve on total body length derived from DMRTs.

Trait evaluated: Head Length (mm)

Table 4. Descriptive Statistics, ANOVA, and Duncan's Multiple Range Test for Head Length (mm) of Blowflies in Response to Different Treatment Doses

(a) Descriptive Statistics

Dose (ml)	N	Mean	Std. Deviation	Std. Error	95% CI Lower	95% CI Upper	Minimum	Maximum
0.001	12	.8958	.67165	.19389	.4691	1.3226	.20	2.00
0.010	12	1.3667	1.06287	.30682	.6913	2.0420	.30	3.00
0.100	12	1.0750	.70081	.20231	.6297	1.5203	.30	2.00
0.200	12	1.0250	.75453	.21781	.5456	1.5044	.30	3.00
0.300	12	.8000	.35162	.10150	.5766	1.0234	.40	1.50
Total	60	1.0325	.74389	.09604	.8403	1.2247	.20	3.00

(b) One-Way ANOVA

Source	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.235	4	.559	1.011	.410
Within Groups	30.414	55	.553		
Total	32.649	59			

(c) Duncan's Multiple Range Test (DMRT)

Dependent Variable: Head Length (mm)

Dose (ml)	N	Subset for α = 0.05
.300	12	.8000
.001	12	.8958
.200	12	1.0250
.100	12	1.0750
.010	12	1.3667

Sig.: 0.101

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 12.000

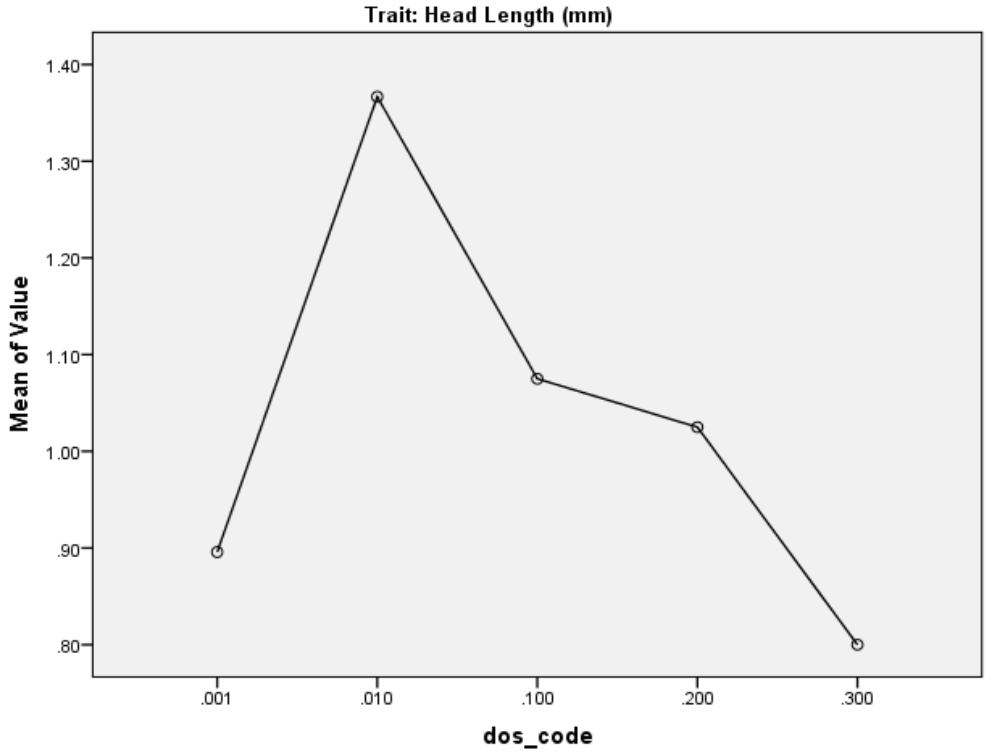

Figure 25. Dose response curve on Head Length (mm) derived from DMRTs.

Trait evaluated: Thorax Length (mm)

Table 5. Descriptive Statistics, ANOVA, and Duncan's Multiple Range Test for Thorax Length (mm) of Blowflies in Response to Different Treatment Doses

(a) Descriptive Statistics

Dose (ml)	N	Mean	Std. Deviation	Std. Error	95% CI Lower	95% CI Upper	Minimum	Maximum
.001	12	1.5042	1.38965	.40116	.6212	2.3871	.20	4.15
.010	12	1.4125	1.10168	.31803	.7125	2.1125	.30	3.00
.100	12	1.1000	.76752	.22156	.6123	1.5877	.30	2.50
.200	12	1.2500	1.01646	.29343	.6042	1.8958	.40	3.00
.300	12	1.0000	.60453	.17451	.6159	1.3841	.40	2.00
Total	60	1.2533	.99634	.12863	.9960	1.5107	.20	4.15

(b) One-Way ANOVA

Source	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	2.111	4	.528	.514	.726
Within Groups	56.458	55	1.027		
Total	58.569	59			

(c) Duncan's Multiple Range Test (DMRT)

Dependent Variable: Thorax Length (mm)

Dose (ml)	N	Subset for α = 0.05
0.300	12	1.0000
0.100	12	1.1000
0.200	12	1.2500
0.001	12	1.4125
0.010	12	1.5042

Sig.: .286

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 12.000

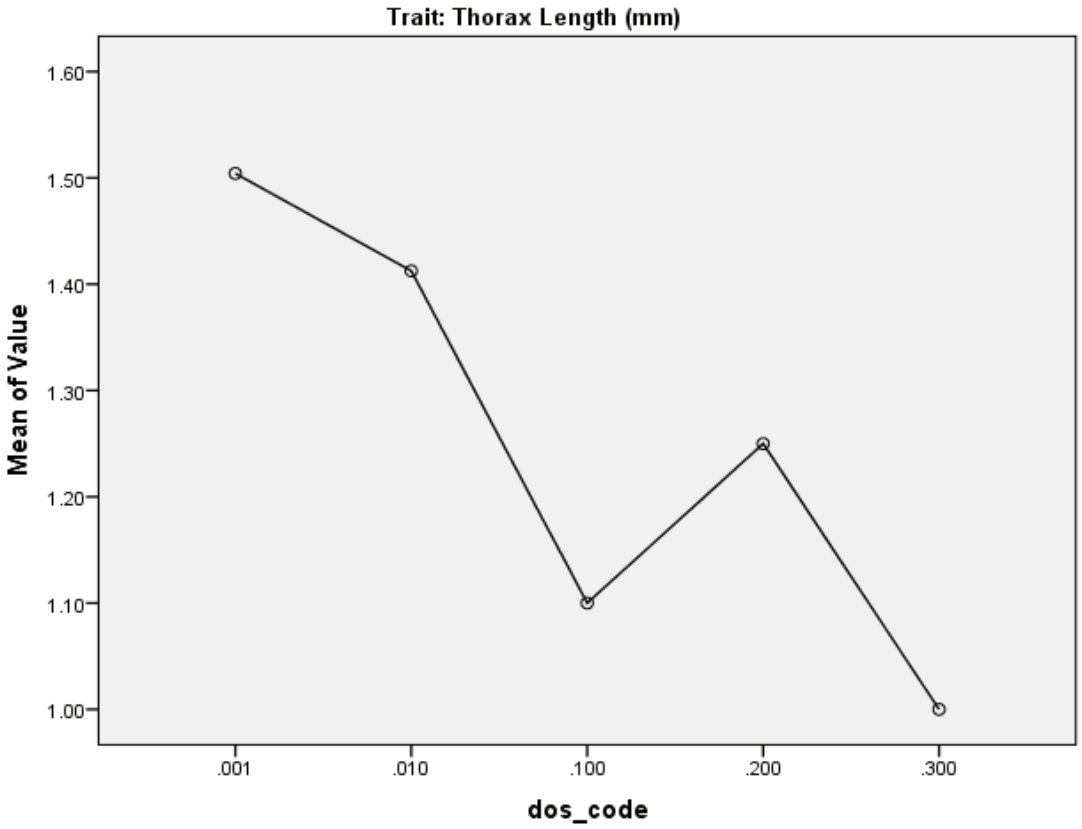

Figure 26. Dose response curve on Thorax Length (mm) derived from DMRTs.

Trait evaluated: Abdomen Length (mm)

Table 6. Descriptive Statistics, ANOVA, and Duncan's Multiple Range Test for Abdomen Length (mm) of Blowflies in Response to Different Treatment Doses

(a) Descriptive Statistics

Dose (ml)	N	Mean	Std. Deviation	Std. Error	95% CI Lower	95% CI Upper	Minimum	Maximum
0.001	12	2.2792	1.91352	.55239	1.0634	3.4950	.40	5.25
0.010	12	2.8000	2.41473	.69707	1.2658	4.3342	.50	6.50
0.100	12	1.2917	.91000	.26270	.7135	1.8699	.50	3.00
0.200	12	1.9967	1.80740	.52175	.8483	3.1450	.40	6.00
0.300	12	1.4750	.73870	.21325	1.0057	1.9443	.60	3.00
Total	60	1.9685	1.71408	.22129	1.5257	2.4113	.40	6.50

(b) One-Way ANOVA

Source	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	17.884	4	4.471	1.582	.192
Within Groups	155.462	55	2.827		
Total	173.347	59			

(c) Duncan's Multiple Range Test (DMRT)

Dependent Variable: Abdomen Length (mm)

Dose (ml)	N	Subset for α = 0.05
0.100	12	1.2917
0.300	12	1.4750
0.200	12	1.9967
0.001	12	2.2792
0.010	12	2.8000
Sig.		.053

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 12.000

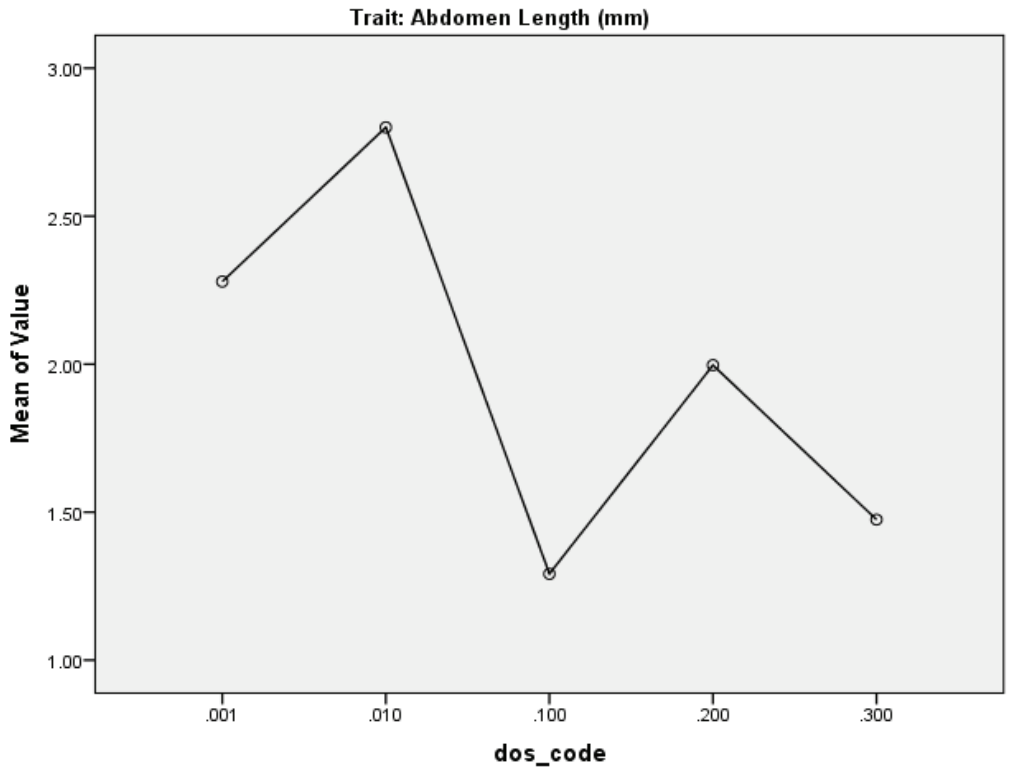

Figure 27. Dose response curve on Abdomen Length (mm) derived from DMRTs.

Trait evaluated: Wing Length (mm)

Table 7. Descriptive Statistics, ANOVA, and Duncan's Multiple Range Test for Wing Length (mm) of Blowflies in Response to Different Treatment Doses

(a) Descriptive Statistics

Dose (ml)	N	Mean	Std. Deviation	Std. Error	95% CI Lower	95% CI Upper	Minimum	Maximum
0.001	12	3.0083	1.81081	.52273	1.8578	4.1589	.30	5.30
0.010	12	3.4250	1.97628	.57050	2.1693	4.6807	.30	5.50
0.100	12	2.6167	1.46028	.42155	1.6888	3.5445	.30	4.10
0.200	12	3.0167	1.65135	.47670	1.9674	4.0659	.40	5.00
0.300	12	2.1917	1.82879	.52793	1.0297	3.3536	.50	5.20
Total	60	2.8517	1.74536	.22532	2.4008	3.3025	.30	5.50

(b) One-Way ANOVA

Source	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	10.456	4	2.614	.849	.500
Within Groups	169.274	55	3.078		
Total	179.730	59			

(c) Duncan's Multiple Range Test (DMRT)

Dependent Variable: Wing Length (mm)

Dose (ml)	N	Subset for α = 0.05
0.300	12	2.1917
0.100	12	2.6167
0.200	12	3.0083
0.001	12	3.0167
0.010	12	3.4250

Sig.: 0.131

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 12.000

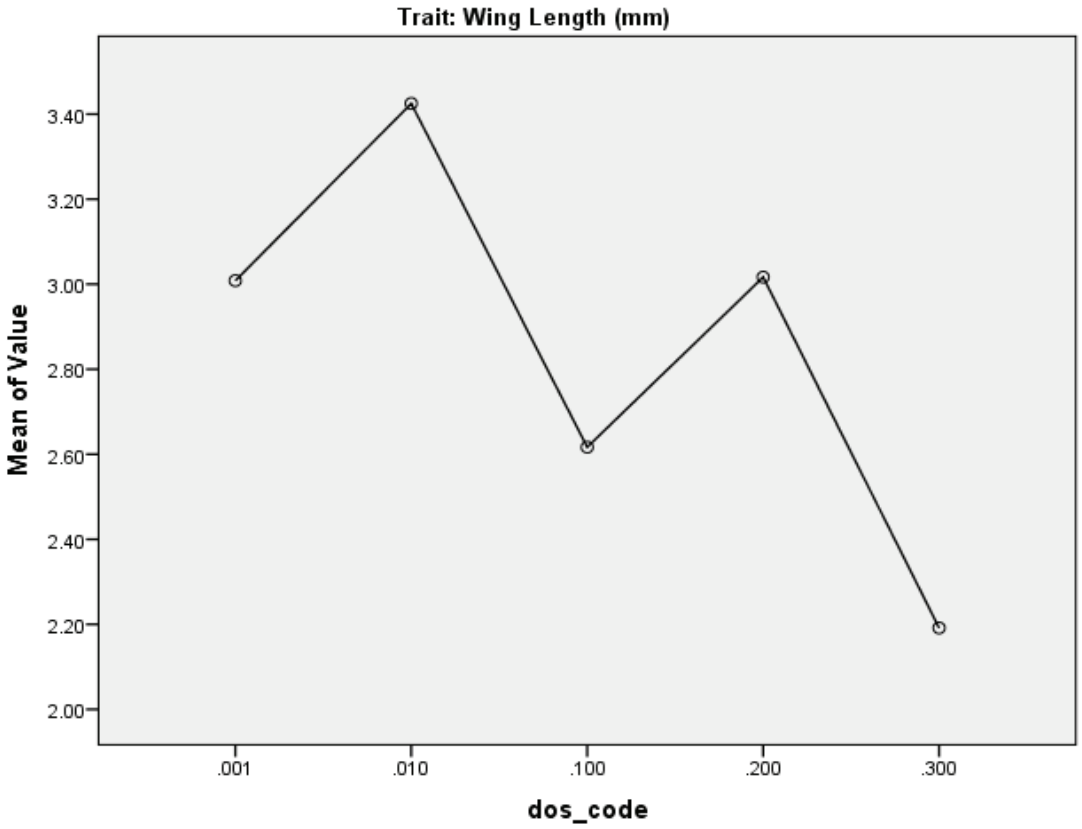

Figure 28. Dose response curve on Wing Length (mm) derived from DMRTs.

Trait evaluated: Larval Length (mm)

Table 8. Descriptive Statistics, ANOVA, and Duncan's Multiple Range Test for Larva Length (mm) of Blowflies in Response to Different Treatment Doses

(a) Descriptive Statistics

Dose (ml)	N	Mean	Std. Deviation	Std. Error	95% CI Lower	95% CI Upper	Minimum	Maximum
0.001	12	3.8333	3.07522	.88774	1.8794	5.7872	1.20	10.00
0.010	12	4.8167	3.54909	1.02453	2.5617	7.0717	1.50	10.10
0.100	12	3.5917	2.31102	.66714	2.1233	5.0600	1.20	8.00
0.200	12	3.6083	2.13731	.61699	2.2503	4.9663	1.50	7.00
0.300	12	3.9833	3.54653	1.02380	1.7300	6.2367	.20	10.10
Total	60	3.9667	2.91708	.37659	3.2131	4.7202	.20	10.10

(b) One-Way ANOVA

Source	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	12.115	4	3.029	.340	.850
Within Groups	489.938	55	8.908		
Total	502.053	59			

(c) Duncan's Multiple Range Test (DMRT)

Dependent Variable: Larva Length (mm)

Dose (ml)	N	Subset for α = 0.05
.100	12	3.5917
.200	12	3.6083
.001	12	3.8333
.300	12	3.9833
.010	12	4.8167

Sig.: 0.380

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 12.000

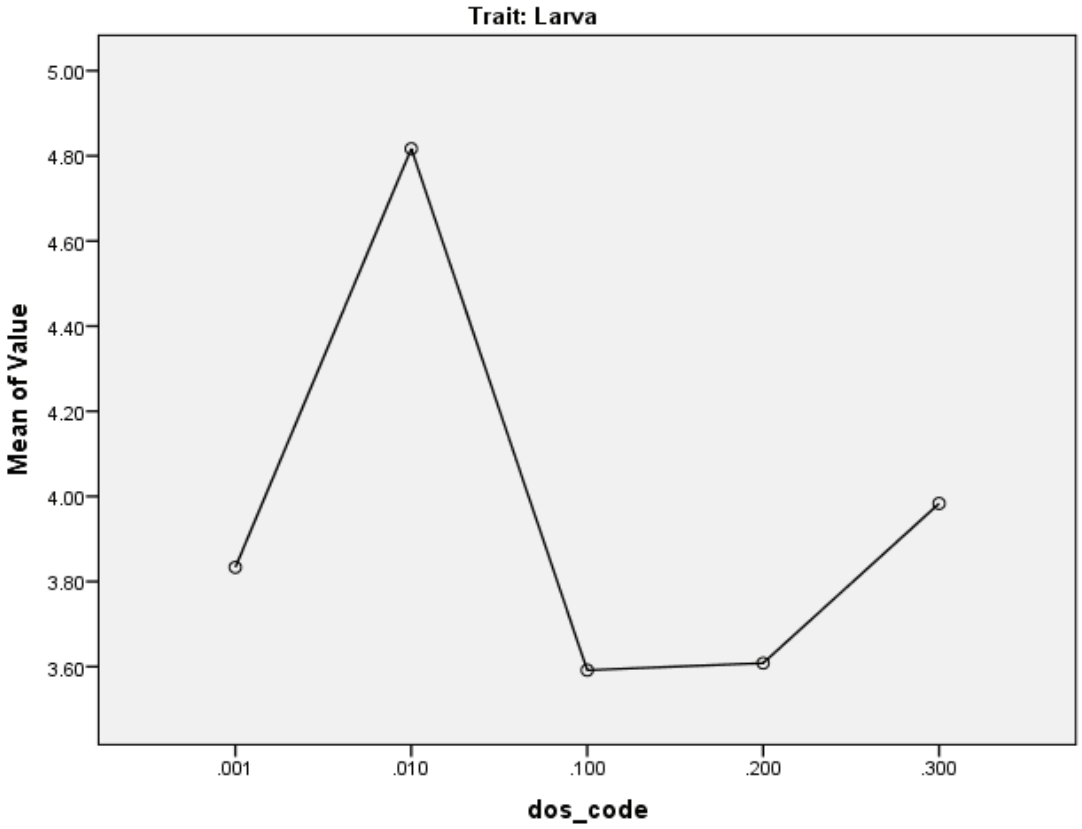

Figure 30. Dose response curve on Larva length derived from DMRTs.

Trait evaluated: Pupal Length (mm)

Table 09. Descriptive Statistics, ANOVA, and Duncan's Multiple Range Test for Pupal Length (mm) of Blowflies in Response to Different Treatment Doses

(a) Descriptive Statistics

Dose (ml)	N	Mean	Std. Deviation	Std. Error	95% CI Lower	95% CI Upper	Minimum	Maximum
0.001	12	4.5000	3.52884	1.01869	2.2579	6.7421	1.00	10.00
0.010	12	4.4250	3.01093	.86918	2.5119	6.3381	1.30	8.10
0.100	12	3.3333	2.71204	.78290	1.6102	5.0565	.50	8.00
0.200	12	3.4583	2.07910	.60018	2.1373	4.7793	1.20	7.00
0.300	12	4.0667	3.47938	1.00441	1.8560	6.2774	1.20	10.20
Total	60	3.9567	2.94667	.38041	3.1955	4.7179	.50	10.20

(b) One-Way ANOVA

Source	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	13.962	4	3.491	.385	.818
Within Groups	498.325	55	9.060		
Total	512.287	59			

(c) Duncan's Multiple Range Test (DMRT)

Dependent Variable: Total Body Length (mm)

Dose (ml)	N	Subset for α = 0.05
.100	12	3.3333
.200	12	3.4583
.300	12	4.0667
.010	12	4.4250
.001	12	4.5000

Sig.: 0.407

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 12.000

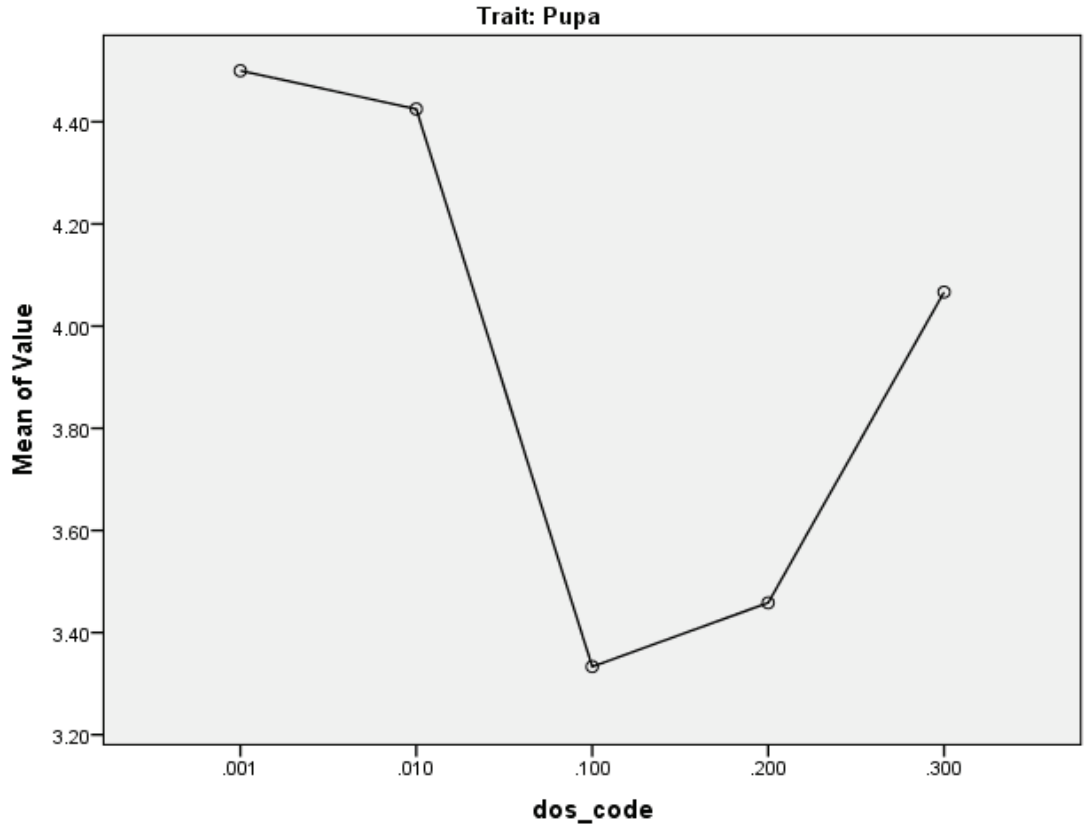

Figure 31. Dose response curve on Pupal length derived from DMRTs.

4.3 Histological Effect of Treatments on *C. megacephala*

The larval muscles stretched in long bundles, but they appeared swollen and disrupted. Cytoplasmic vacuoles sparkled like tiny blisters, scattered across the cells. The nuclei were irregular—some shriveled, some completely broken down. The gut epithelium, normally smooth, was torn and thinning. He whispered to himself, “So even at the very beginning, formalin begins its work—corrupting growth at the cellular core.”

He moved on to the pupal slide, the stage of transformation. Here, the story should have been one of rebirth—cells reorganizing, old tissues breaking down to form the new adult body. But instead, the histological scene looked chaotic. Disintegration ruled the field. The epithelial lining was fragmented; necrotic spots spread across the tissue. Instead of a clean remodeling, there was disarray—like a book whose pages had been scattered by the wind. It was as though the metamorphosis had been interrupted, trapped in confusion by the toxic presence of formalin.

After lifted the adult slide into place. This was the end of the journey—the fully developed fly. Yet, the tissues told a darker story. The powerful flight muscles, usually rich with striations and strength, were fragmented and collapsed. The epithelial layers were weak and distorted, offering no clear architecture. Necrosis ran deep. Even in the reproductive tissues, degeneration was evident, hinting at a future where life could no longer continue.

staring at the three slides laid out in a row.

Larva—disturbed beginnings.

Pupa—broken transformation.

Adult—shattered completion.

Each stage bore scars of exposure, each tissue whispered of a life altered by chemical intrusion. The histology of larva, pupa, and adult of C. megacephala reveals a progressive sequence of cellular destruction under formalin exposure—early vacuolization, disrupted metamorphosis, and ultimate tissue collapse in adulthood. Life itself becomes fragile when its smallest structures are torn apart.

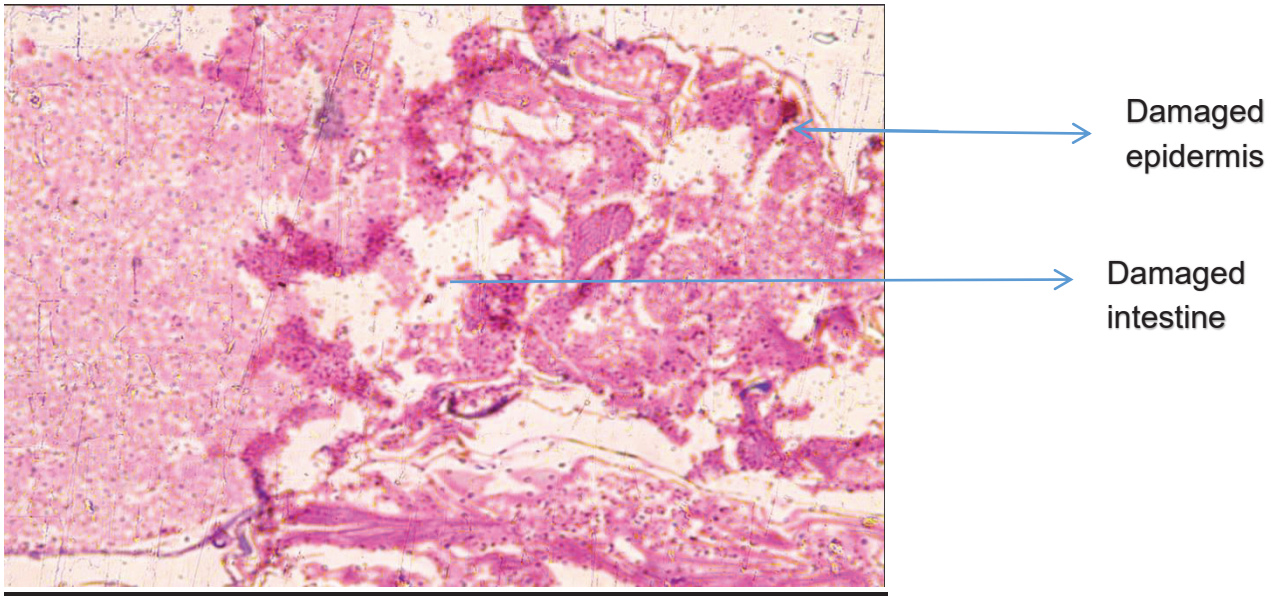

Figure 32. C.S. of pupa of C. *megacephala of 0.3 ml/g dose. (10X)*

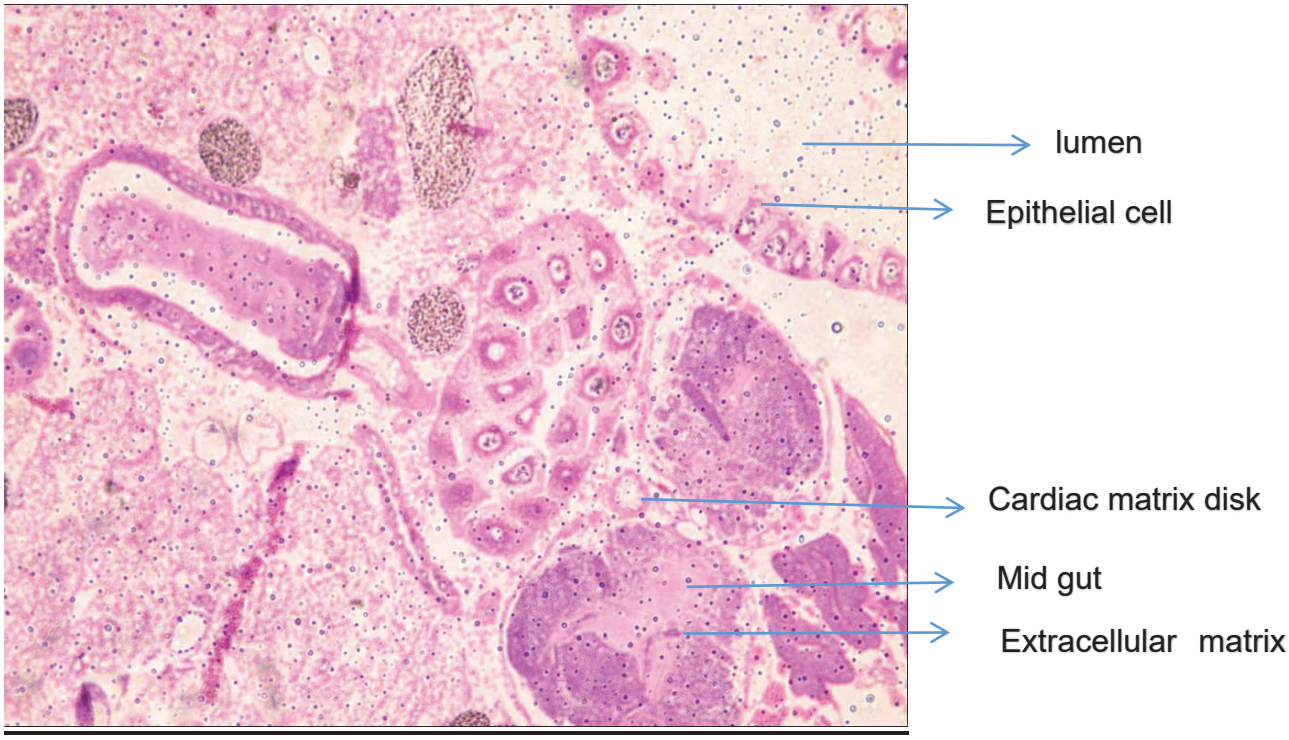

Figure 33. L.S. of pupa of *C. megacephala* 0.1 ml/g dose. (10X)

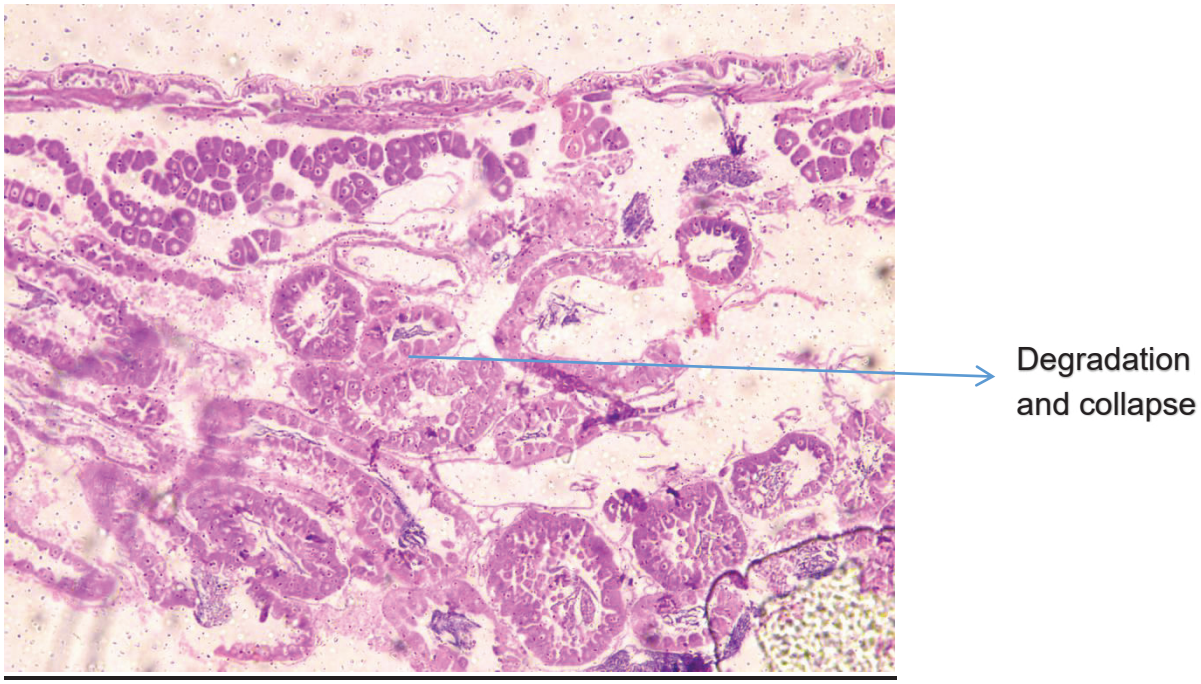

Figure 34. L.S. of pupa of *C. megacephala* at -0.3ml/g dose. (10X)

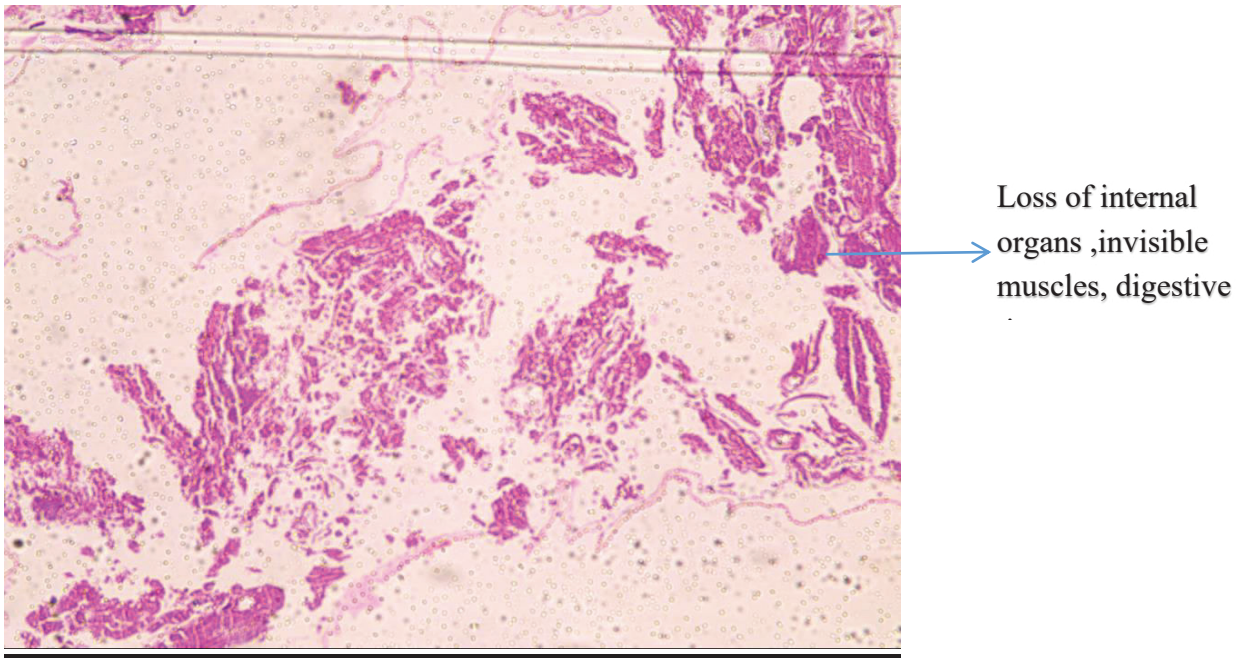

Figure 35. L.S. of larvae of *C. megacephala* 0.3 ml/g.(10X)

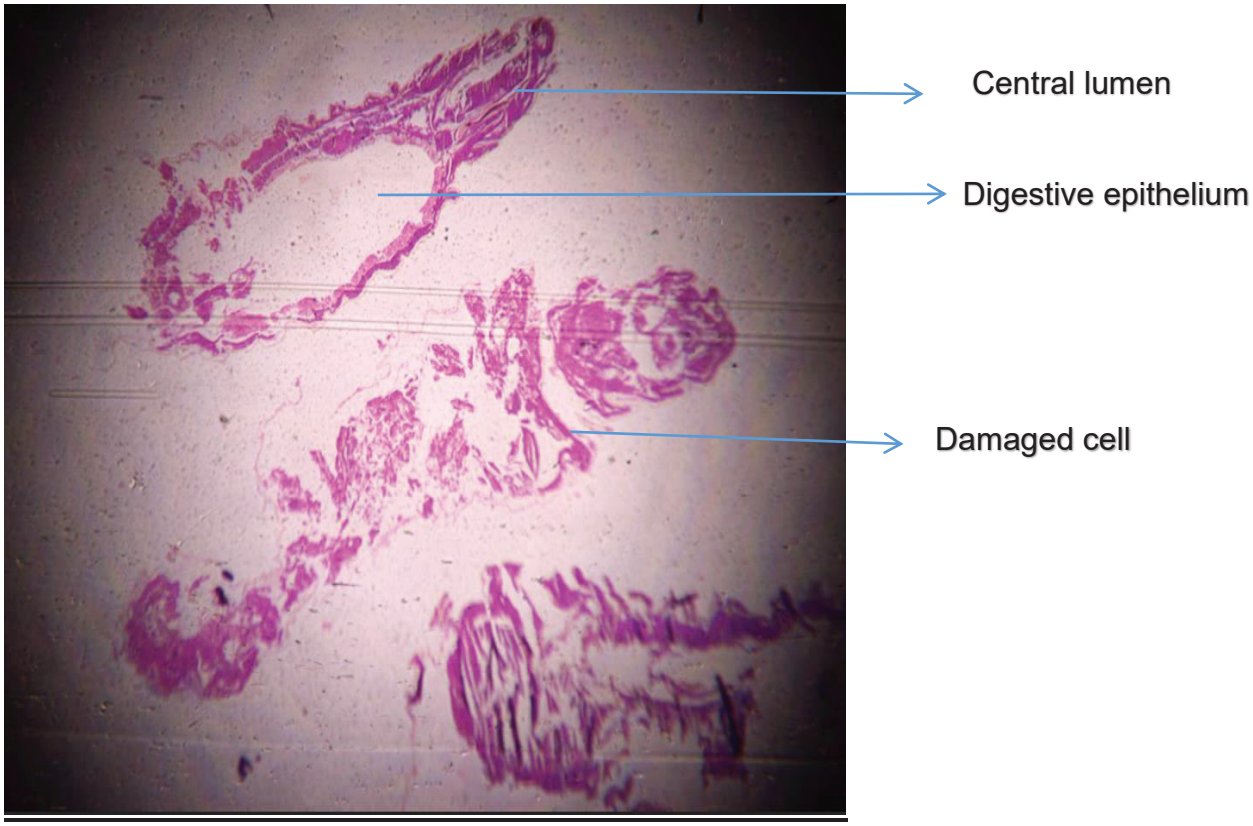

Figure 36. L.S. of larvae of *C. megacephala* 0.3 ml/g. (40X)

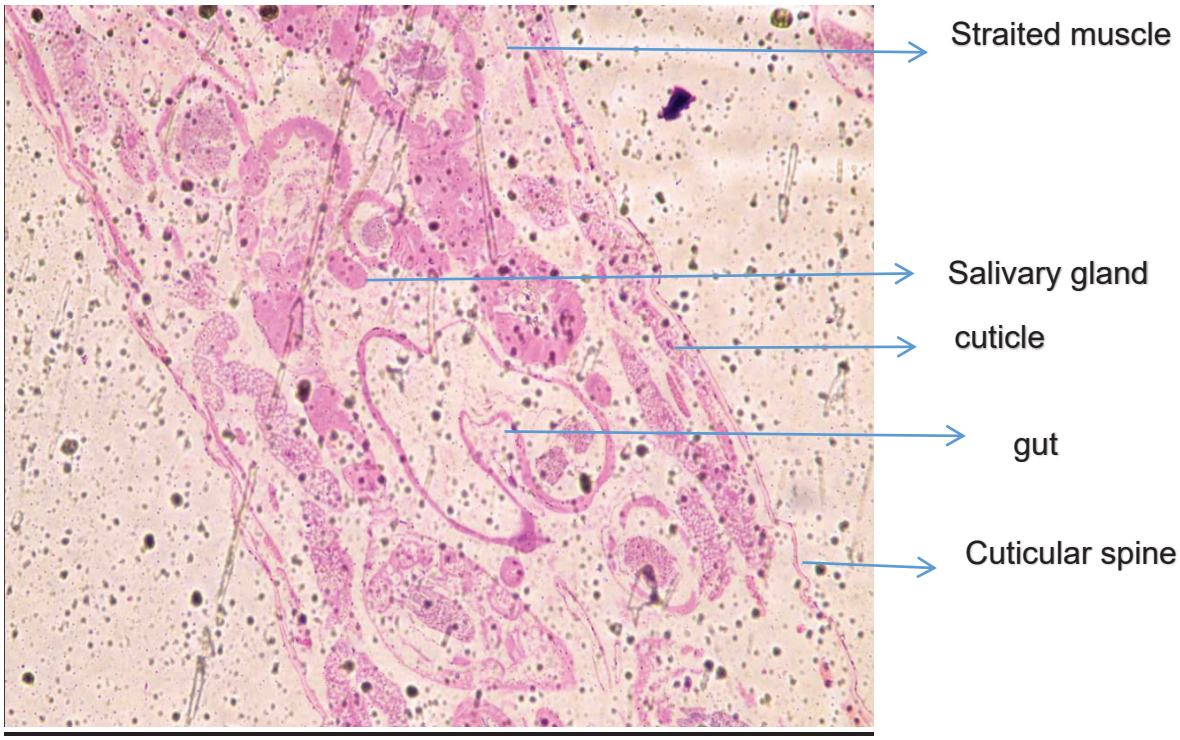

Figure 37. L.S. of pupa of *C. megacephala* 0.2 1ml/g. (10X)

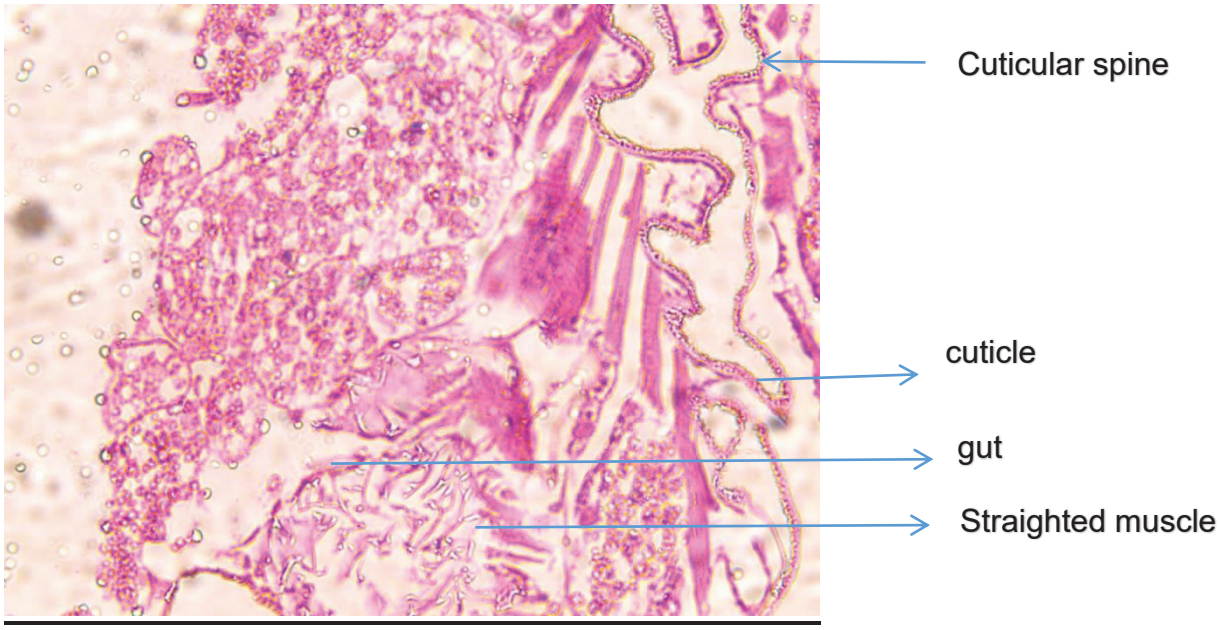

Figure 38. L.S. of adult of C. megacephala at a dose of 0.2 ml/g (40X).

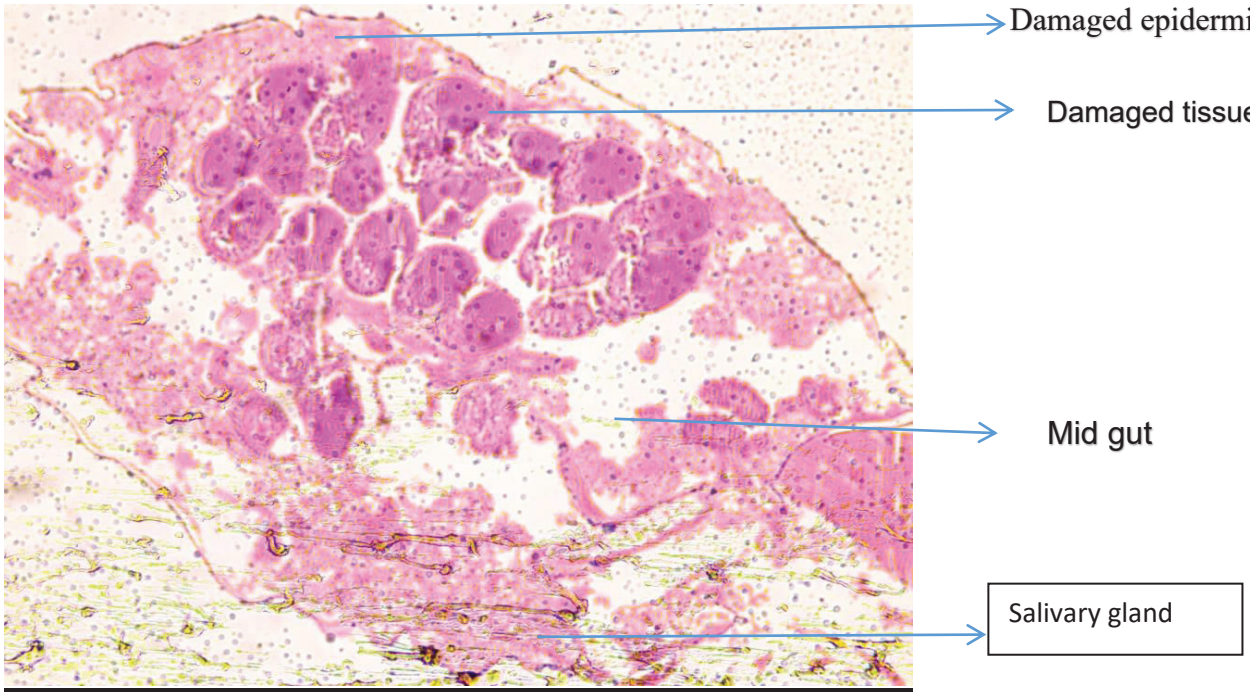

Figure 39. L.S. of Adult of *C. megacephala* 0.3 ml/g. (10X)

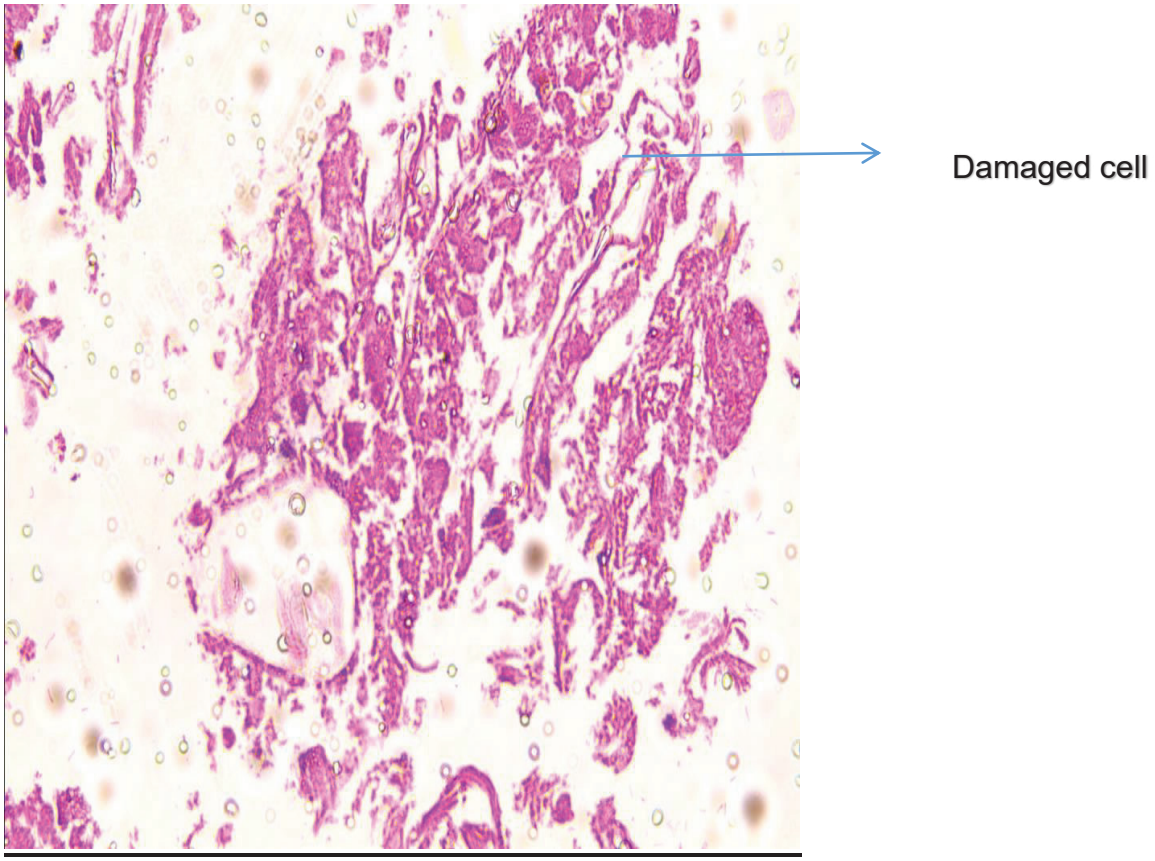

Figure 40. L.S. of larvae of *C. megacephala* 0.3 (40X).

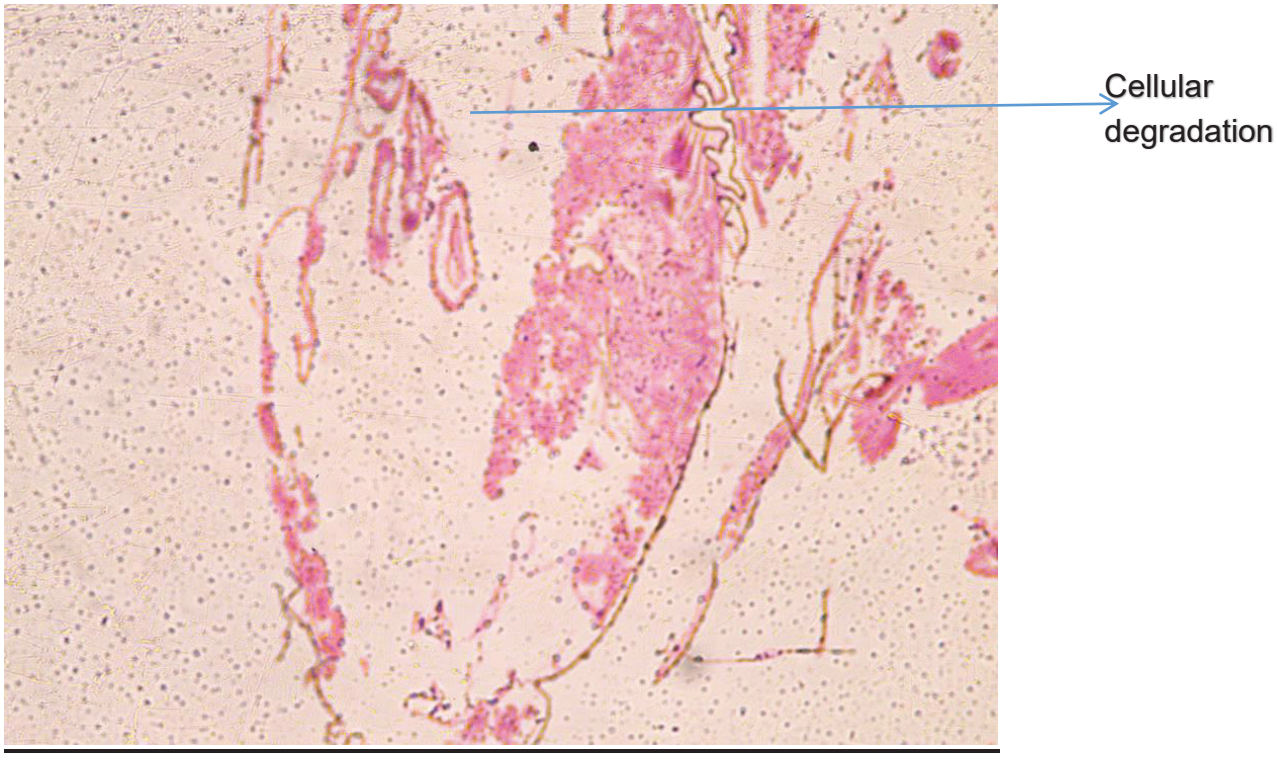

Figure 41. L.S. of Adult of *C. megacephala* 0.2(10X).

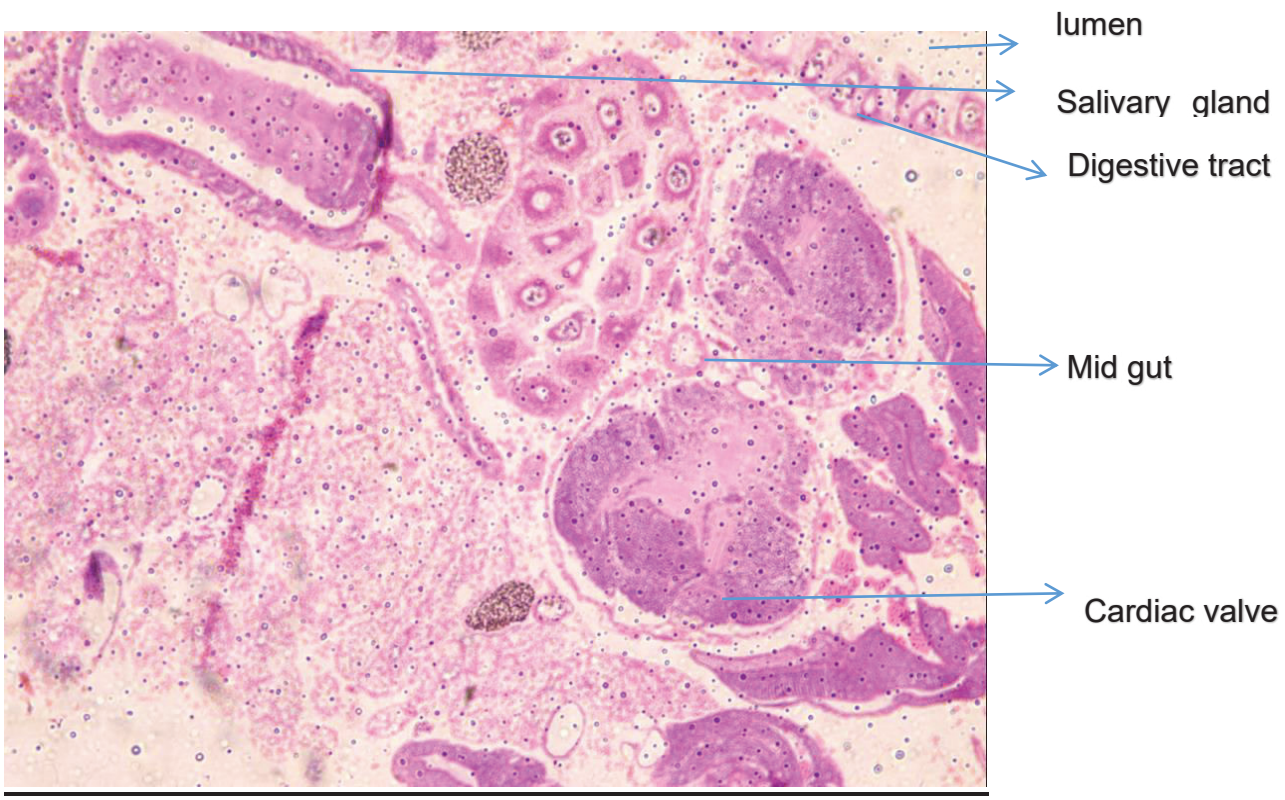

Figure 42. L.S. of pupa of *C. megacephala* 0.1 (10X).

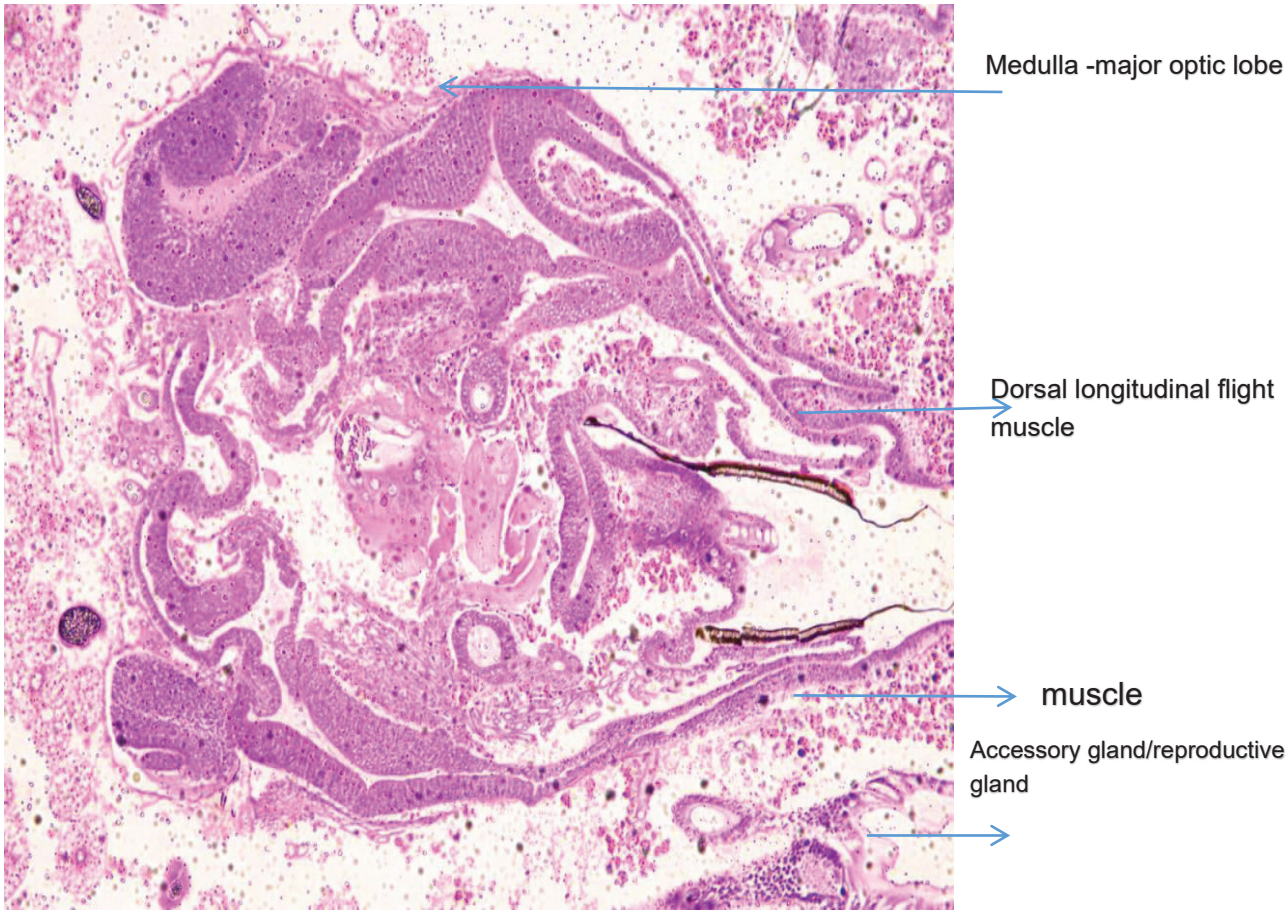

Figure 43.L.S. of adult C. megacephala 0.1 (10X).

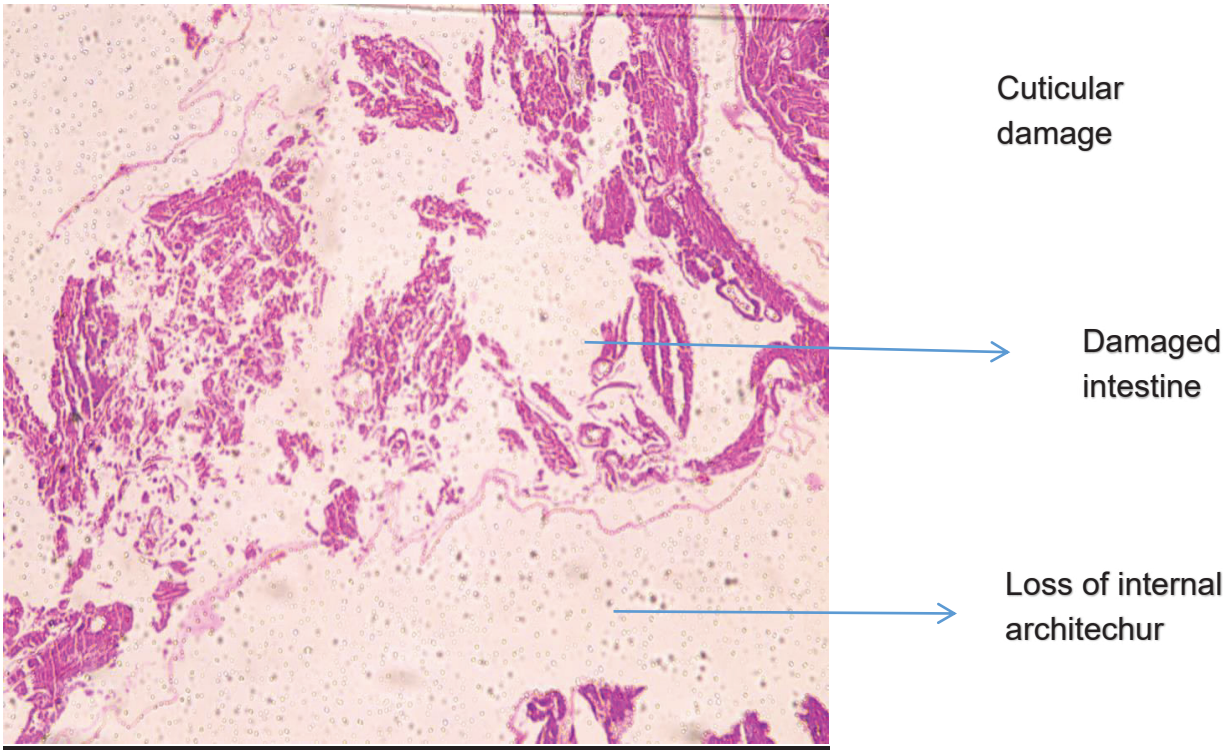

Figure 44. L.S. of larvae of *C. megacephala* at a dose of 0.3 ml/g.(10X)

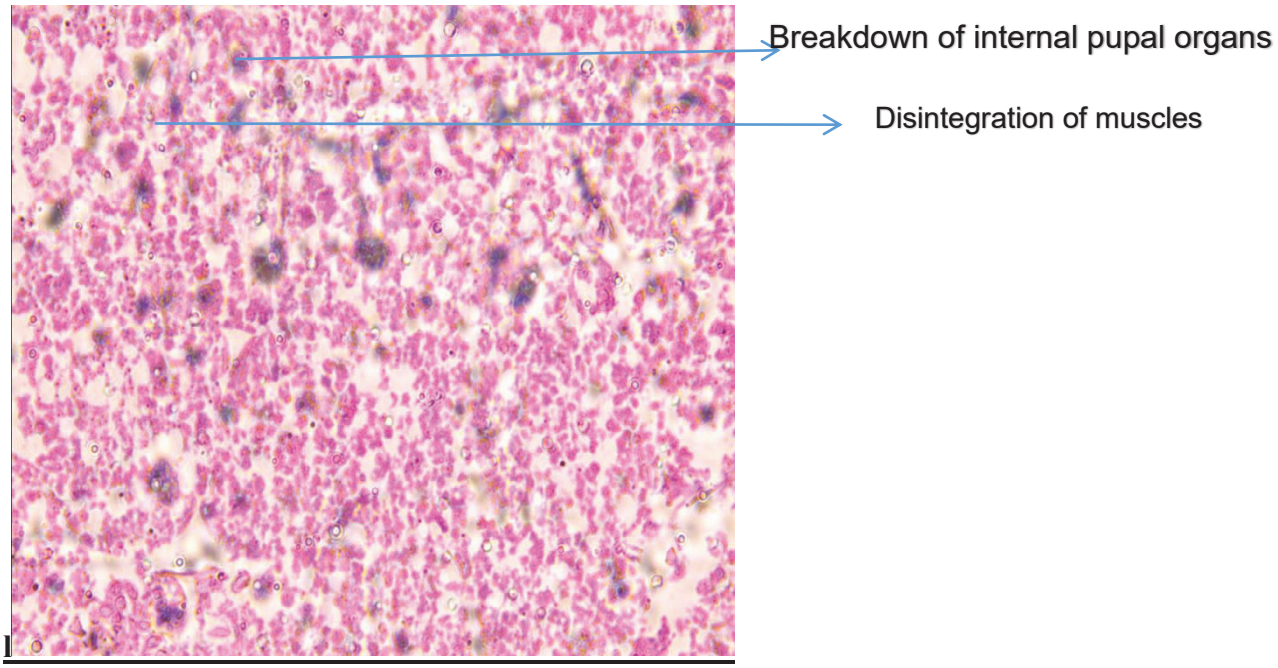

Figure 45. L.S. of pupa of C. megacephala at a dose of 0.3 ml/g. (40X)

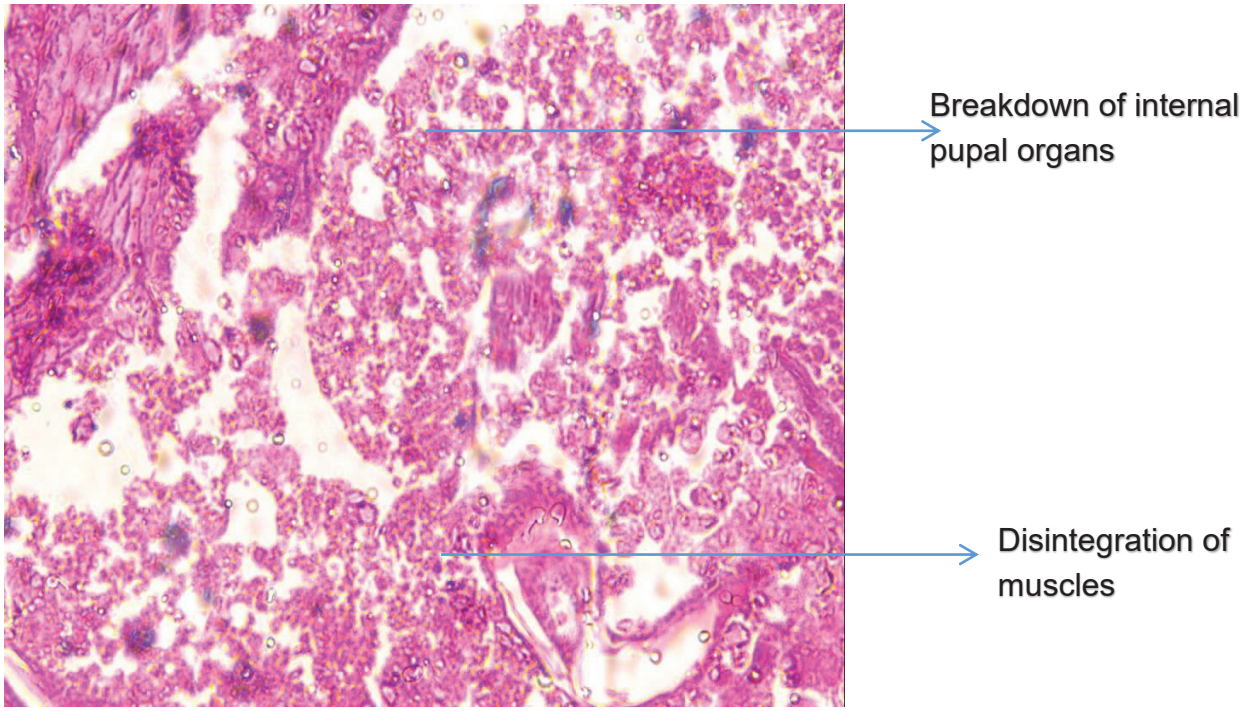

Figure 46. L. S. of pupa of *C. megacephala* at a dose of 0.3 ml/g.(40X)

The histological evaluation of *C. megacephala* following formalin exposure demonstrated a progressive, dose-dependent deterioration of tissues, reflecting the toxic impact of this chemical on insect development. In the control group, the pupae and adults exhibited compact, well-organized architecture with clearly defined epithelial and muscular layers, intact nuclei, and distinct differentiation of imaginal tissues, indicating normal development.At the lowest tested dose (0.1 ml/g), only mild alterations were observed. The tissue architecture remained largely intact, but early signs of cytotoxicity appeared in the form of slight vacuolization in some cells and the onset of larval tissue breakdown. Imaginal discs and epithelial boundaries were still distinguishable, suggesting that at this concentration, the organism was able to tolerate and partially compensate for the toxic insult.With increasing exposure (0.2 ml/g), histological damage became more pronounced. Tissue architecture displayed

moderate disorganization, with reduced compactness and increased intercellular spaces. Cellular degeneration was evident, as shown by clear cytoplasmic vacuolization and irregularly shaped nuclei, indicating the onset of necrotic and apoptotic processes. Importantly, imaginal tissues appeared poorly defined, suggesting that formalin interfered with normal differentiation pathways essential for successful metamorphosis. Larval remnants also showed signs of moderate histolysis, with scattered cell debris in the background, highlighting impaired tissue remodeling.

At the highest concentration tested (0.3 ml/g), the effects were catastrophic. Severe cytotoxicity was evident with a complete loss of organized tissue architecture. Muscle fibers and epithelial layers were disintegrated, the cuticle was irregular and thinned, and organ boundaries were indistinguishable. Extensive cytoplasmic vacuolization created large empty spaces within the tissues, reflecting edema and collapse of cellular components. Nuclear degeneration was widespread, with many nuclei appearing pyknotic, lysed, or faded, consistent with advanced necrosis. The entire section appeared fragmented and amorphous, with massive debris accumulation, indicating near-complete failure of proper tissue differentiation and development.

Taken together, these findings highlight that formalin exposure disrupts the normal histological integrity of *C. megacephala* in a clear dose-dependent manner. While low concentrations cause only mild and potentially reversible alterations, moderate to high doses severely compromise cellular structures, impair metamorphosis, and ultimately lead to tissue collapse. This evidence strongly suggests that formalin exerts profound cytotoxic effects on blow fly development, interfering with both larval histolysis and adult tissue differentiation, and thereby threatening the survival and normal biological functioning of the organism.

CHAPTER-05
DISCUSSION

Discussion

The results of this study reveal that exposure to accelerate concentrations of formalin exerts a profound, dose-dependent impact on *C. megacephala*, affecting not only larval mortality but also adult morphology, coloration, and behavior. The united evidence from descriptive statistics, inferential analysis, and direct observations demonstrates that formalin toxicity manifests as both lethal and sublethal effects, each enhancive with higher concentrations.

1. Dose–dependent Relationship and Mortality Trends

Mortality data revealed a progressive increase in response to higher doses, with mean values of 1.788 ± 0.223% at 0.001 ml, 2.400 ± 0.731% at 0.010 ml, 6.611 ± 0.418% at 0.100 ml, 7.179 ± 0.336% at 0.200 ml, and 9.478 ± 0.977% at 0.300 ml. One-way ANOVA confirmed highly significant differences among dose groups ($F = 236.925$, $p < 0.001$), and Duncan's Multiple Range Test (DMRT) revealed stepwise separation of mortality rates, indicating strong dose-dependent toxicity. This pattern suggests a toxicity threshold between 0.010 and 0.100 ml/g, beyond which physiological compensation fails and lethal effects escalate rapidly.

2. Structural Changes: Reduction in Size

Surviving adults from higher-dose treatments disclose a noticeable decrease in body size compared to controls. The stunting effect was most pronounced at 0.200 and 0.300 ml/g, indicating that formalin impairs normal growth and development.The dose- and volume-dependent variations in all four morphometric parameters. The graph clearly illustrates that increasing formalin concentrations and exposure volumes are associated with reduced body size and appendage development, although some fluctuations occur at lower doses.

3. Colouration Alterations

Colour changes were another consistent sublethal effect. While untreated flies displayed the characteristic metallic green-blue sheen, those from higher formalin concentrations often appeared dull, faded, or patterned, with some showing brownish or blackish patches. The metallic sheen in blow *C. megacephala* flies is essentially a product of cuticle microstructure and pigment deposition. Formalin exposure may disrupt the biosynthesis of pigments such as pteridines and ommochromes or alter cuticle integrity, while oxidative stress may accelerate pigment degradation. Such

changes not only reduce visual vitality but may also influence ecological interactions, including mate recognition and predator deterrence.

4. Behavioral Impairments: Flight and Mobility

The present research revealed that high dose survivor adult blow flies were very dull with reduced flight ability as well as some cases totally flight loss. The suvivor adults displayed unorganized walking, weakened wing vibration, and extended resting periods. These damage may result from neuromuscular damage, wing malformation, or reduced energy reserves stemming from larval-stage stress. Sublethal neurotoxic effects have been documented in other insect species exposed to chemical stressors, supporting the representation that formalin impacts motor control as well as general vigour.

5. Linking Mortality and Sublethal Effects

The observed morphological and behavioral abnormalities are not independent of the mortality data; instead, they likely represent different stages of the same toxicological continuum. At lower doses, most individuals survive but show subtle physiological stress (e.g., dull coloration, mild size reduction). At intermediate doses, mortality increases sharply, and surviving flies exhibit more severe morphological defects and impaired mobility. At the highest doses, mortality predominates, and the few survivors are severely stunted, discolored, and incapable of sustained flight.

6. Ecological and Forensic Implications

These changes are very significant-according to ecological consequences. *C. megacephala* plays a crucial role in carrion decomposition and nutrient recycling. Impaired mobility and altered coloration could reduce foraging efficiency, competitive ability, and reproductive success, potentially affecting ecosystem functioning. From a forensic viewpoint, altered growth rates and reduced size could lead to computation in post-mortem interval (PMI) estimations if formalin contamination of the environment or remains is not recognized .

7. Comparative Discourse

The patterns observed here are consistent with previous reports of environmental pollutants causing both lethal and sublethal effects in insects. For example, exposure to heavy metals and certain pesticides has been shown to produce similar outcomes, including reduced adult size, pigment loss, and locomotor deficits. The strong dose–response relationship observed in this study reinforces the broader principle that

increasing contaminant exposure often triggers both quantitative (mortality) and qualitative (morphological/behavioral) changes.

Finally, Formalin exposure imposes a multifaceted toxic burden on *C. megacephala*, combining high mortality at elevated doses with marked sublethal effects in survivors. These outcomes are tightly dose-dependent and reflect formalin's capacity to disrupt fundamental biological processes during development. Considering the species' ecological and forensic importance, the findings emphasize the need to evaluate and regulate formalin contamination in environments where these insects occur.

CHAPTER-06
CONCLUSION

Conclusion

The recent study highlighted ,mortality,reduced size and development and tissue damage of blow fly which indicates the insect is highly toxic effect of formaldehyde and formalin even in very low concentration.

Moreover,it is highly recommended to regulate comparative longitudinal section studies of the samples the chronic exposer of formalin and formaldehyde at different stages of blow fly is significantly associated with cellular degenaration,clear cytoplasmic vacuolation and histologic damage.

Formaldehyde and formalin known as a versatile chemical ,which have a wide range of applications in different industries globally.Though its versalite use,it is seen that ,the chemicals are very harmfull to many tissues ,cells and organs as well as it can cause chronic damage and severe poisoning to different organ systems.It is requred further studies to know the unknown mechanisms through which formaldehyde exerts its toxicity.

Even if it is not possible to reduce the use of formaldehyde and formalin,it is obvious to ensure the policies to reduce the use of formalin and formaldehyde to protect the food chain,safe guarding the biodiversity and minimize its harmful effect on human.

CHAPTER-7

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LITERATURE CITED:

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CHAPTER-8
APPENDIX

Appendix

Trait evaluated: abdomen length (mm)

Table 10. Descriptive Statistics, and Test for abdomen length (mm) of Blowflies in Response to Different Treatment Doses

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
.001	12	2.2792	1.91352	.55239	1.0634	3.4950	.40	5.25
.010	12	2.8000	2.41473	.69707	1.2658	4.3342	.50	6.50
.100	12	1.2917	.91000	.26270	.7135	1.8699	.50	3.00
.200	12	1.9967	1.80740	.52175	.8483	3.1450	.40	6.00
.300	12	1.4750	.73870	.21325	1.0057	1.9443	.60	3.00
Total	60	1.9685	1.71408	.22129	1.5257	2.4113	.40	6.50

a. Trait = Abdomen Length (mm)

ANOVA[a]							
Value							
	Sum of Squares	df	Mean Square	F	Sig.		
Between Groups	17.884	4	4.471	1.582	.192		
Within Groups	155.462	55	2.827				
Total	173.347	59					
a. Trait = Abdomen Length (mm)							
Post Hoc Tests							
Description of different parameters of different size of Blowfly abdomen length and analysis by using **Multiple Comparisons[a]** in response to various doses of formalin.							
Dependent Variable: Value							

(I) dos_code		(J) dos_code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.001	.010	-.52083	.68637	.451	-1.8963	.8547
		.100	.98750	.68637	.156	-.3880	2.3630
		.200	.28250	.68637	.682	-1.0930	1.6580
		.300	.80417	.68637	.246	-.5713	2.1797
	.010	.001	.52083	.68637	.451	-.8547	1.8963
		.100	1.50833*	.68637	.032	.1328	2.8838
		.200	.80333	.68637	.247	-.5722	2.1788
		.300	1.32500	.68637	.059	-.0505	2.7005
	.100	.001	-.98750	.68637	.156	-2.3630	.3880
		.010	-1.50833*	.68637	.032	-2.8838	-.1328
		.200	-.70500	.68637	.309	-2.0805	.6705
		.300	-.18333	.68637	.790	-1.5588	1.1922
	.200	.001	-.28250	.68637	.682	-1.6580	1.0930
		.010	-.80333	.68637	.247	-2.1788	.5722
		.100	.70500	.68637	.309	-.6705	2.0805
		.300	.52167	.68637	.450	-.8538	1.8972
	.300	.001	-.80417	.68637	.246	-2.1797	.5713
		.010	-1.32500	.68637	.059	-2.7005	.0505
		.100	.18333	.68637	.790	-1.1922	1.5588
		.200	-.52167	.68637	.450	-1.8972	.8538

*. The mean difference is significant at the 0.05 level.

a. Trait = Abdomen Length (mm)

Homogeneous Subsets

Value[a]

	dos_code	N	Subset for alpha = 0.05
			1
Duncan[b]	.100	12	1.2917
	.300	12	1.4750
	.200	12	1.9967
	.001	12	2.2792
	.010	12	2.8000
	Sig.		.053

Means for groups in homogeneous subsets are displayed.

a. Trait = Abdomen Length (mm)

b. Uses Harmonic Mean Sample Size = 12.000.				

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum
					Lower Bound	Upper Bound	
.001	12	1.5042	1.38965	.40116	.6212	2.3871	.20
.010	12	1.4125	1.10168	.31803	.7125	2.1125	.30
.100	12	1.1000	.76752	.22156	.6123	1.5877	.30
.200	12	1.2500	1.01646	.29343	.6042	1.8958	.40
.300	12	1.0000	.60453	.17451	.6159	1.3841	.40
Total	60	1.2533	.99634	.12863	.9960	1.5107	.20

ANOVA[a]							
Value							
	Sum of Squares	df	Mean Square	F	Sig.		
Between Groups	2.111	4	.528	.514	.726		
Within Groups	56.458	55	1.027				
Total	58.569	59					
a. Trait = Thorax Length (mm)							
Post Hoc Tests							

Trait evaluated: Thorax Length (mm)

Table 11. Descriptive Statistics, and Test for Thorax Length (mm) of Blowflies in Response to Different Treatment Doses

Dependent Variable: Value							
(I) dos_code			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.001	.010	.09167	.41362	.825	-.7373	.9206
		.100	.40417	.41362	.333	-.4248	1.2331
		.200	.25417	.41362	.541	-.5748	1.0831
		.300	.50417	.41362	.228	-.3248	1.3331
	.010	.001	-.09167	.41362	.825	-.9206	.7373
		.100	.31250	.41362	.453	-.5164	1.1414
		.200	.16250	.41362	.696	-.6664	.9914
		.300	.41250	.41362	.323	-.4164	1.2414
	.100	.001	-.40417	.41362	.333	-1.2331	.4248
		.010	-.31250	.41362	.453	-1.1414	.5164
		.200	-.15000	.41362	.718	-.9789	.6789
		.300	.10000	.41362	.810	-.7289	.9289
	.200	.001	-.25417	.41362	.541	-1.0831	.5748
		.010	-.16250	.41362	.696	-.9914	.6664
		.100	.15000	.41362	.718	-.6789	.9789
		.300	.25000	.41362	.548	-.5789	1.0789
	.300	.001	-.50417	.41362	.228	-1.3331	.3248
		.010	-.41250	.41362	.323	-1.2414	.4164
		.100	-.10000	.41362	.810	-.9289	.7289
		.200	-.25000	.41362	.548	-1.0789	.5789
a. Trait = Thorax Length (mm)							

Homogeneous Subsets							
Value[a]							
dos_code		N	Subset for alpha = 0.05				
			1				
Duncan[b]	.300	12	1.0000				
	.100	12	1.1000				
	.200	12	1.2500				
	.010	12	1.4125				
	.001	12	1.5042				
	Sig.		.286				
Means for groups in homogeneous subsets are displayed.							
a. Trait = Thorax Length (mm)							
b. Uses Harmonic Mean Sample Size = 12.000.							

Trait evaluated: Wing Length (mm)

Table 12. Descriptive Statistics, and Test for Wing Length (mm) of Blowflies in Response to Different Treatment Doses

Trait = Wing Length (mm)								
Description of different parameters of different size of Blowfly Wing Length and analysis by using **Descriptives**[a] in response to various doses of formalin.								
Value								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
.001	12	3.0083	1.81081	.52273	1.8578	4.1589	.30	5.30
.010	12	3.4250	1.97628	.57050	2.1693	4.6807	.30	5.50
.100	12	2.6167	1.46028	.42155	1.6888	3.5445	.30	4.10

.200	12	3.0167	1.65135	.47670	1.9674	4.0659	.40	5.00
.300	12	2.1917	1.82879	.52793	1.0297	3.3536	.50	5.20
Total	60	2.8517	1.74536	.22532	2.4008	3.3025	.30	5.50
a. Trait = Wing Length (mm)								
ANOVA[a]								
Value								
	Sum of Squares	df	Mean Square	F	Sig.			
Between Groups	10.456	4	2.614	.849	.500			
Within Groups	169.274	55	3.078					
Total	179.730	59						
a. Trait = Wing Length (mm)								
Post Hoc Tests								

Multiple Comparisons[a]							
Dependent Variable: Value							
(I) dos_code			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.001	.010	-.41667	.71621	.563	-1.8520	1.0186
		.100	.39167	.71621	.587	-1.0436	1.8270
		.200	-.00833	.71621	.991	-1.4436	1.4270
		.300	.81667	.71621	.259	-.6186	2.2520
	.010	.001	.41667	.71621	.563	-1.0186	1.8520
		.100	.80833	.71621	.264	-.6270	2.2436
		.200	.40833	.71621	.571	-1.0270	1.8436
		.300	1.23333	.71621	.091	-.2020	2.6686
	.100	.001	-.39167	.71621	.587	-1.8270	1.0436
		.010	-.80833	.71621	.264	-2.2436	.6270
		.200	-.40000	.71621	.579	-1.8353	1.0353
		.300	.42500	.71621	.555	-1.0103	1.8603
	.200	.001	.00833	.71621	.991	-1.4270	1.4436
		.010	-.40833	.71621	.571	-1.8436	1.0270
		.100	.40000	.71621	.579	-1.0353	1.8353
		.300	.82500	.71621	.254	-.6103	2.2603
	.300	.001	-.81667	.71621	.259	-2.2520	.6186
		.010	-1.23333	.71621	.091	-2.6686	.2020
		.100	-.42500	.71621	.555	-1.8603	1.0103
		.200	-.82500	.71621	.254	-2.2603	.6103
a. Trait = Wing Length (mm)							

Homogeneous Subsets							
Value[a]							
dos_code		N	Subset for alpha = 0.05				

			1				
Duncan[b]	.300	12	2.1917				
	.100	12	2.6167				
	.001	12	3.0083				
	.200	12	3.0167				
	.010	12	3.4250				
	Sig.		.131				
Means for groups in homogeneous subsets are displayed.							
a. Trait = Wing Length (mm)							
b. Uses Harmonic Mean Sample Size = 12.000.							

Multiple Comparisons[a]

Dependent Variable: Value

(I) dos_code		(J) dos_code	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.001	.010	-.52083	.68637	.451	-1.8963	.8547
		.100	.98750	.68637	.156	-.3880	2.3630
		.200	.28250	.68637	.682	-1.0930	1.6580
		.300	.80417	.68637	.246	-.5713	2.1797
	.010	.001	.52083	.68637	.451	-.8547	1.8963
		.100	1.50833*	.68637	.032	.1328	2.8838
		.200	.80333	.68637	.247	-.5722	2.1788
		.300	1.32500	.68637	.059	-.0505	2.7005
	.100	.001	-.98750	.68637	.156	-2.3630	.3880
		.010	-1.50833*	.68637	.032	-2.8838	-.1328
		.200	-.70500	.68637	.309	-2.0805	.6705
		.300	-.18333	.68637	.790	-1.5588	1.1922
	.200	.001	-.28250	.68637	.682	-1.6580	1.0930
		.010	-.80333	.68637	.247	-2.1788	.5722
		.100	.70500	.68637	.309	-.6705	2.0805
		.300	.52167	.68637	.450	-.8538	1.8972
	.300	.001	-.80417	.68637	.246	-2.1797	.5713
		.010	-1.32500	.68637	.059	-2.7005	.0505
		.100	.18333	.68637	.790	-1.1922	1.5588
		.200	-.52167	.68637	.450	-1.8972	.8538

Trait evaluated: Total Body Length (mm)

Table 13. Descriptive Statistics, and Duncan's Multiple Range Test for Total Body Length (mm) of Blowflies in Response to Different Treatment Doses

(a) Descriptive Statistics

Trait = Total Body Length (mm)								
Descriptives[a]								
Value								
	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
.001	12	4.7083	3.93826	1.13688	2.2061	7.2106	1.00	11.00
.010	12	5.5333	4.34309	1.25374	2.7739	8.2928	1.10	11.00
.100	12	3.4250	2.16254	.62427	2.0510	4.7990	1.10	7.50
.200	12	4.2417	3.14743	.90858	2.2419	6.2414	1.30	10.00
.300	12	3.0750	1.38703	.40040	2.1937	3.9563	1.50	5.00
Total	60	4.1967	3.20624	.41392	3.3684	5.0249	1.00	11.00
a. Trait = Total Body Length (mm)								
ANOVA[a]								
Value								
	Sum of Squares	df	Mean Square	F	Sig.			
Between Groups	46.849	4	11.712	1.151	.342			
Within Groups	559.670	55	10.176					
Total	606.519	59						
a. Trait = Total Body Length (mm)								
Post Hoc Tests								
Multiple Comparisons[a]								
Dependent Variable: Value								

(I) dos_code			Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
LSD	.001	.010	-.82500	1.30229	.529	-3.4349	1.7849
		.100	1.28333	1.30229	.329	-1.3265	3.8932
		.200	.46667	1.30229	.721	-2.1432	3.0765
		.300	1.63333	1.30229	.215	-.9765	4.2432
	.010	.001	.82500	1.30229	.529	-1.7849	3.4349
		.100	2.10833	1.30229	.111	-.5015	4.7182
		.200	1.29167	1.30229	.326	-1.3182	3.9015
		.300	2.45833	1.30229	.064	-.1515	5.0682
	.100	.001	-1.28333	1.30229	.329	-3.8932	1.3265
		.010	-2.10833	1.30229	.111	-4.7182	.5015
		.200	-.81667	1.30229	.533	-3.4265	1.7932
		.300	.35000	1.30229	.789	-2.2599	2.9599
	.200	.001	-.46667	1.30229	.721	-3.0765	2.1432
		.010	-1.29167	1.30229	.326	-3.9015	1.3182
		.100	.81667	1.30229	.533	-1.7932	3.4265
		.300	1.16667	1.30229	.374	-1.4432	3.7765
	.300	.001	-1.63333	1.30229	.215	-4.2432	.9765
		.010	-2.45833	1.30229	.064	-5.0682	.1515
		.100	-.35000	1.30229	.789	-2.9599	2.2599
		.200	-1.16667	1.30229	.374	-3.7765	1.4432

a. Trait = Total Body Length (mm)

Homogeneous Subsets

Value[a]

dos_code		N	Subset for alpha = 0.05
			1
Duncan[b]	.300	12	3.0750
	.100	12	3.4250
	.200	12	4.2417
	.001	12	4.7083

	.010	12	5.5333					
	Sig.		.097					
Means for groups in homogeneous subsets are displayed.								
a. Trait = Total Body Length (mm)								
b. Uses Harmonic Mean Sample Size = 12.000.								

Trait evaluated: Larval Length (mm)

Table 14. Descriptive Statistics, ANOVA, and Duncan's Multiple Range Test for Larva Length (mm) of Blowflies in Response to Different Treatment Doses

(a) Descriptive Statistics

Dose (ml)	N	Mean	Std. Deviation	Std. Error	95% CI Lower	95% CI Upper	Minimum	Maximum
0.001	12	3.8333	3.07522	.88774	1.8794	5.7872	1.20	10.00
0.010	12	4.8167	3.54909	1.02453	2.5617	7.0717	1.50	10.10
0.100	12	3.5917	2.31102	.66714	2.1233	5.0600	1.20	8.00
0.200	12	3.6083	2.13731	.61699	2.2503	4.9663	1.50	7.00
0.300	12	3.9833	3.54653	1.02380	1.7300	6.2367	.20	10.10
Total	60	3.9667	2.91708	.37659	3.2131	4.7202	.20	10.10

(b) One-Way ANOVA

Source	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	12.115	4	3.029	.340	.850

Within Groups	489.938	55	8.908		
Total	502.053	59			

(c) Duncan's Multiple Range Test (DMRT)

Dependent Variable: Larva Length (mm)

Dose (ml)	N	Subset for α = 0.05
.100	12	3.5917
.200	12	3.6083
.001	12	3.8333
.300	12	3.9833
.010	12	4.8167

Sig.: 0.380

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 12.000

Trait evaluated: Pupal Length (mm)

Table 15. Descriptive Statistics, ANOVA, and Duncan's Multiple Range Test for Pupal Length (mm) of Blowflies in Response to Different Treatment Doses

(a) Descriptive Statistics

Dose (ml)	N	Mean	Std. Deviation	Std. Error	95% CI Lower	95% CI Upper	Minimum	Maximum
0.001	12	4.5000	3.52884	1.01869	2.2579	6.7421	1.00	10.00
0.010	12	4.4250	3.01093	.86918	2.5119	6.3381	1.30	8.10
0.100	12	3.3333	2.71204	.78290	1.6102	5.0565	.50	8.00

0.200	12	3.4583	2.07910	.60018	2.1373	4.7793	1.20	7.00
0.300	12	4.0667	3.47938	1.00441	1.8560	6.2774	1.20	10.20
Total	60	3.9567	2.94667	.38041	3.1955	4.7179	.50	10.20

(b) One-Way ANOVA

Source	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	13.962	4	3.491	.385	.818
Within Groups	498.325	55	9.060		
Total	512.287	59			

(c) Duncan's Multiple Range Test (DMRT)

Dependent Variable: Total Body Length (mm)

Dose (ml)	N	Subset for α = 0.05
.100	12	3.3333
.200	12	3.4583
.300	12	4.0667
.010	12	4.4250
.001	12	4.5000

Sig.: 0.407

Means for groups in homogeneous subsets are displayed.

Harmonic Mean Sample Size = 12.000