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HISTOLOGICAL ASSAYS ON IMPACT OF ARSENIC ON THE BRAIN AND ARSENIC-INDUCED MORTALITY IN *LUCILIA CUPRINA* (WIEDEMANN, 1830) (DIPTERA: CALLIPHORIDAE)

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ABSTRACT: The study investigated the effect of various arsenic concentrations on the brain tissue of *L. cuprina* employing histological slide preparations via feeding and injection treatments. Arsenic-induced mortality was observed across concentrations of 0.01 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, and 1 ppm over a 72-hour period. Total mortality within this timeframe was corrected by using Abbott's correction formula.The mean mortality resulting from exposure to various concentrations of As in both cases was analyzed using Levene's test followed by one-way ANOVA by SPSS (v. 26) and the posthoc analysis was conducted by Duncan's Multiple Range Test. The highest mortality recorded at 1 ppm for both feeding (9.33±0.88) and injection (10.67±0.33) treatments. Conversely, the lowest mortality occurred at 0.01 ppm for both fed (1.67±0.33) and injected *L. cuprina* (2.33±0.33), indicating a positive correlation between mortality and arsenic concentration. Comparison of feeding and injection methods revealed consistently higher mortality with injection. Histological slide preparations revealed varying degrees of brain tissue degradation, categorized into four groups (A=0-30%, B=31-60%, C=61-80%, and D=81-100%). The highest mean of maximum affected brains (Category D) was observed at 1 ppm for both feeding (14.67±1.53) and injection (16.33±1.53), while the minimum affected brains (Category A) occurred at 0.01 ppm for feeding (17.67±0.58) and injection (17.00±1.00). The results highlight the importance of conducting thorough toxicological investigations that cover a range of metal contaminants, including arsenic, and involve diverse insect species.
Key words: *Lucilia cuprina*, blowfly, arsenic, mortality, ecotoxicology, histology

INTRODUCTION

Arsenic, with the symbol as and atomic number 33, is a Group 15 element on the periodic table, ranking as the 20th most abundant element globally, with a molecular weight of 74.9216 atomic mass units (Engwa et al. 2019). It is included in the metalloid groups and may be found in all environmental

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matrices in a variety of forms such as oxides, sulfides, and salts of iron, sodium, calcium, copper, and other metals (Singh et al. 2007). It is extremely toxic and oncogenic. Toxicity of As was detected as early as 2000 B.C. (Zaman et al. 1995). It predominantly affects the sulfhydryl group of cells, causing anomalies in cell respiration and cell enzymes, as well as mitotic dysfunction (Tchounwou et al. 2003).

The impact of arsenic on biological systems has long been a subject of scientific inquiry. Numerous studies have explored the detrimental effects of arsenic on various organisms, with a growing emphasis on its impact at the molecular and cellular levels. Arsenic has an adverse impact on insects' fecundity, as reported by Pickett and Patterson (1963), where they reported the exposure of four species of adult dipterans (*Rhagoletis pomonella, Drosophila melanogaster, Drosophila hydei,* and *Musca domestica*) to sub-lethal concentrations of arsenates (lead arsenate ($PbHAsO_4$) and calcium arsenate ($Ca(AsO_4)_2$) suppressed egg production which resulted in various degrees of fecundity reduction.

In the realm of insect toxicology, Australian sheep blowfly, *Lucilia cuprina*, has gained attention as a model organism due to its relevance in forensic entomology. It is a significant pest causing myiasis in humans and flystrike (cutaneous myiasis) in sheep. The presence and abundance of *Lucilia cuprina* can also serve as indicators of environmental conditions, including the availability of suitable breeding sites and the overall health of ecosystems. Insects are employed as both active and passive bioindicators to discover and assess the degree of pollution in a specific environment.

Therefore, the research aims to investigate the impact of arsenic on the brain tissue of *Lucilia cuprina* and assess its mortality.

MATERIAL AND METHODS

The present study was done in the Entomology Laboratory of the Department of Zoology of the University of Dhaka from November 2021 to February 2022. Collection and rearing of files: Around 200-300 pupae of three days old fly were collected from laboratory colonies at the Blowfly Rearing Plant of the Institution of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment, Savar, Dhaka. These pupae were placed in a plastic container covered with sterilized net fabric for aeration and incubated at a controlled temperature of 28±2°C and a relative humidity of 68±5% to facilitate the emergence of adult flies.

Treatment with Arsenic (As): Treatment was done by feeding and injecting of flies. To obtain a series of arsenic concentrations (0.01 ppm, 0.05 ppm, 0.1 ppm,

0.5 ppm, 1 ppm), employed 0.0416 mg, 0.208 mg, 0.416 mg, 2.08 mg, and 4.16 mg of $NaHAsO_4 \cdot 7H_2O$ per liter of water correspondingly. In the control groups, 20 mg of finely ground bovine liver and cotton balls soaked in 20 ml of tap water were given as diets. Upon emergence from pupae, flies were fed with water-soaked in cotton balls and sugar. In the treatment groups, 20 ml standard solution of arsenic of 0.01 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, 1 ppm were used respectively to soak in cotton balls for each treatment. Upon emergence, the zero-day old flies were injected with serial doses of 0.01,0.05, 0.1 ,0.5 and 1ppm. Same flies were injected at ages two days and four days. Flies were injected into the abdominal tip while still alive. Each group (both control group and treatment group) was kept under close observation for 72 hours and data on blowfly mortality was collected at 24 hours intervals.

Dissection and preservation: The brain of the insect were dissected after 72 hours and washed in a physiological solution made with 0.9g of NaCI in 100 ml of distilled water. The tissues were then preserved in 1.5 ml microcentrifuge tubes in 70% alcohol which were later washed in 90% and 100% alcohol respectively to dehydrate the tissue completely.

Histological procedure: Tissues were initially placed in xylene, followed by embedding in paraffin wax at 80°C. After cooling, trimming and section cutting of wax containing tissue, the trimmed ribbons of tissue wax block were attached to the slides by Mayer's Albumin and subjected to a xylene treatment. Gradual alcohol dehydration (100%, 90%, 70%, 50%) and distilled water wash were performed for hydration. The staining was done by dipping Hematoxylin and Eosin. Dehydration in ascending alcohol concentrations (70%, 90%, 100%) was followed by xylene clearing. Finally, tissues were mounted with Canada balsam-covered slides and examined under a microscope at 20x and 40x magnification for identification and damage assessment.

Statistical analyses: Data obtained for the mortality test were corrected with Abbott's formula (Abbott, 1925):

(T-C/100-C) ×100 Where T= Mortality treatment= Mortality in control.

Levene's test was conducted to assess the homogeneity of variances among groups for the total mortality of blowflies, followed by a one-way ANOVA to compare means across groups, and post hoc comparisons between groups were carried out using Duncan's Multiple Range Test (DMRTs) to evaluate individual differences. The statistical analyses were performed using IBM SPSS Statistics (v. 26.0) software.

RESULTS AND DISCUSSION

Mortality of treated *Lucilia cuprina*: Blowfly mortality over a 72-hour period, following exposure to arsenic at concentrations of 0.01 ppm, 0.05 ppm, 0.1 ppm, 0.5 ppm, and 1 ppm through both feeding and injection methods were presented in figure 1.

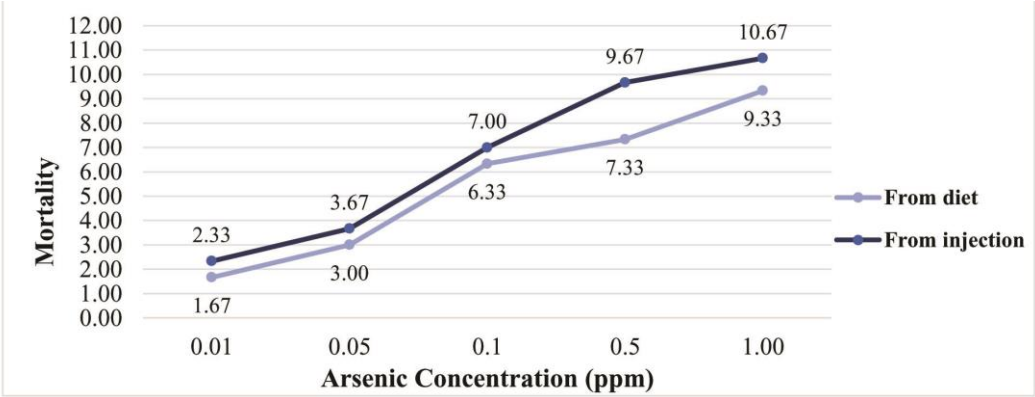

Fig. 1. Dose vs response curve of mean mortality from 72 hours of observation.

The results of the mortality test from the figure 1 depicted a positive correlation between mortality rates and escalating concentrations of arsenic in both of the treatment methods (diet and injection). As the arsenic concentration escalated, the mean mortality rates showed a consistent upward trend in both types of treatments. Notably, mortality due to injection was relatively higher compared to feeding. Specifically, the highest mean mortality rates were recorded at the highest arsenic dose of 1 ppm, with values of 9.33 ± 0.88 for the feeding method and 10.67 ± 0.33 for the injection method. Conversely, the lowest mean mortality rates were observed at the lowest arsenic concentration of 0.01 ppm, with values of 1.67 ± 0.33 for fed specimens and 2.33 ± 0.33 for injected specimens. This pattern suggests a dose-dependent relationship between arsenic exposure and mortality, with both treatment methods exhibiting similar trends but injection inducing higher mortality rates across all concentrations.

Levene's test was employed to assess the homogeneity of variances among groups. For the Diet group, the Levene statistic was 1.125 with a significance level of .398. In the Injection group, the Levene statistic was .303 with a significance level of .869. Following the assessment of homogeneity of variances, one-way analysis of variance (ANOVA) was performed to compare the means across the groups, revealing significant differences in the means of blowfly mortality for both the Diet ($F(4,10) = 27.906$, $p < .001$) and Injection groups ($F(4,10) = 85.000$, $p < .001$). Pairwise multiple comparisons with DMRTs from diet revealed no significant differences between the mortality rates at 0.01 ppm and 0.05 ppm, as well as between 0.1 ppm and 0.5 ppm. Likewise, in the injection group, pairwise comparisons indicated no significant differences between the mortality rates at 0.5 ppm and 1.00 ppm.

Table 1. Levene's test and ANOVA test results regarding blowflies mortality in response to various doses of Arsenic in diet & injection

Test of Homogeneity of Variance			One-way ANOVA	
Mortality	Levene Statistic	Sig.	F	Sig.
From diet	1.125	.398	27.906	0.000
From Injection	.303	.869	85.000	0.000

Histological changes in brain of L. cuprina: Histological brain samples were categorized into four groups assuming on extent of arsenic damage and comparison with control or unaffected brains using criteria such as brain degeneration, gland conditions, optic lobe status, and presence or absence of different cells in the nervous system (i.e., Category A = 0-30% BA, Category B = 31-60% BA, Category C = 61-80% BA, Category D = 81-100% BA, where BA represents Brain affected).

In response to varying doses of arsenic (As) administered through feeding, the number of affected brains across different categories is illustrated in Figure 2. In the ascending doses of 0.01 ppm,

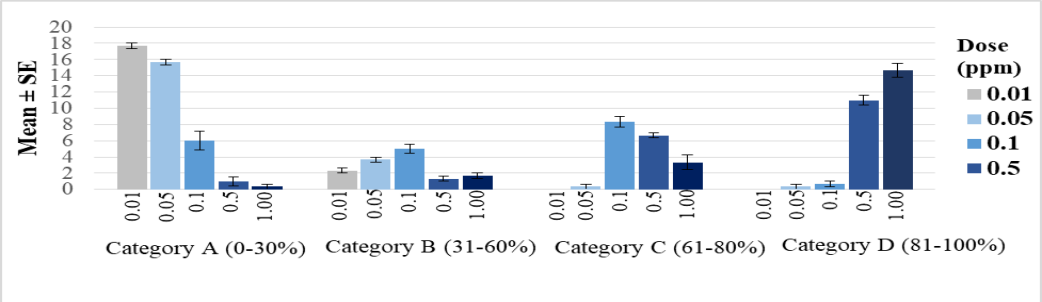

Fig. 2. Categories showing the number of brains affected in response to different doses of as in diet.

0.05 ppm, 0.1 ppm, 0.5 pm, and 1 ppm, the highest mean of the maximum affected brain (Category D) was achieved at 1 ppm for feeding (14.67±1.53) and the highest mean of the minimum affected brain (Category A) was achieved at the dose of 0.01 ppm for feeding (17.67±0.58).

Furthermore, the graphical representations in Figures 3 to 8 vividly depict the comprehensive influence of feeding diverse doses of arsenic on brain tissues. These figures serve as visual aids, offering a clear depiction of how different doses of arsenic exert influence on or affect the brain.

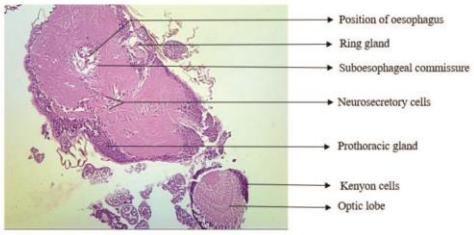

Fig. 3. Transverse Section (T.S.) of the brain of Blowfly (Control)

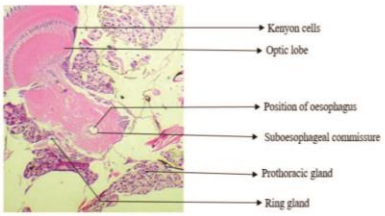

Fig. 4. T.S of the brain of Blowfly fed with 0.01 ppm arsenic (as).

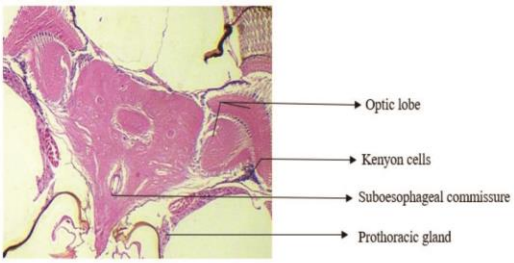

Fig. 5. T.S. of the brain of Blowfly fed with 0.05 ppm as.

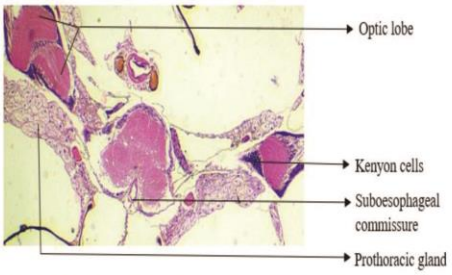

Fig. 6. T.S. of the brain of Blowfly fed with 0.1 ppm as.

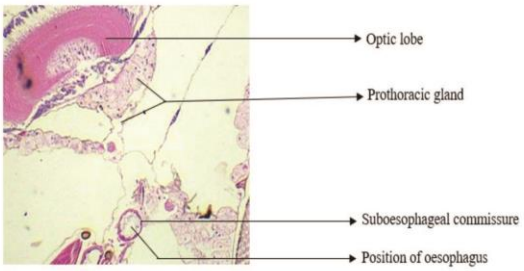

Fig. 7. T.S. of the brain of Blowfly fed with 0.5 ppm as.

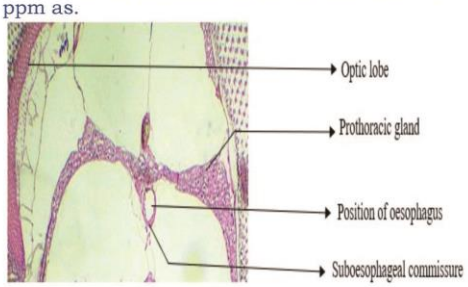

Fig. 8. T.S. of the brain of Blowfly fed with 1 ppm as.

The homogeneity of variances across the four categories (A, B, C, and D) in response to various doses of as in the diet was assessed using Levene's test. The specific Levene statistic for each category is presented in Table 2. Subsequently, a one-way ANOVA was conducted to compare the means of affected brains across these categories. The corresponding F-statistics for each category are detailed in Table 2. Post hoc pairwise multiple comparisons using DMRTs were conducted to explore specific group differences across these categories. In Category A, no significant differences emerged between affected brains at 1.00 ppm and 0.5 ppm. For Category B, no differences were detected among 1.00 ppm and 0.5 ppm, 0.01 ppm and 0.05 ppm, and 0.05 ppm and 0.1 ppm. Category C exhibited no differences between 0.01 ppm and 0.05 ppm, and 0.05 ppm and 1.00 ppm. In Category D, no differences surfaced among 0.01 ppm, 0.05 ppm, and 0.1 ppm, with the highest affected brains at 1.00 ppm.

Table 2. Levene's test and ANOVA test results across the four categories (A, B, C, and D) in response to various doses of As in the diet

Test of Homogeneity of Variance			One-way ANOVA	
Affected brain	Levene Statistic	Sig.	F	Sig.
Cat. A	1.249	.351	164.944	0.000
Cat. B	.303	.869	14.857	0.000
Cat. C	3.571	.067	47.962	0.000
Cat. D	2.616	.099	182.292	0.000

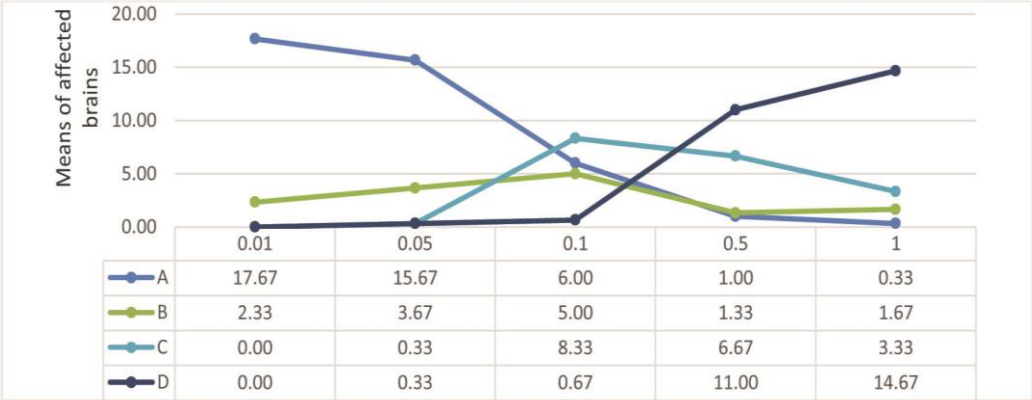

Fig. 9. Dose-response curves of the means of the affected brains in different categories for various doses of as in the diet.

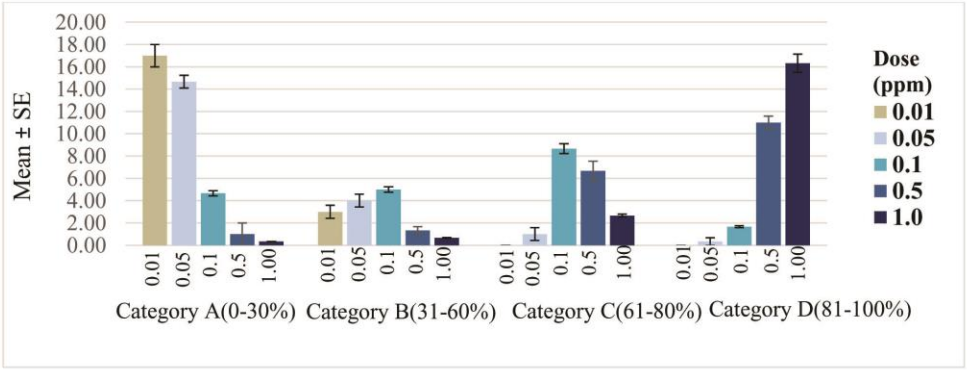

Fig.10. further elaborates, the number of affected brains in different categories resulting from injecting various doses of as escalate from 0.01 ppm to 1 ppm. A parallel pattern is observed in

Dose-response curves in figure 9 illustrating the means of affected brains across different categories in response to various doses of as in the diet. The curves provide a visual representation of how the response varies with increasing doses for each category. The lowest mean of the maximum affected brain (Category D), recorded at 1 ppm for injection (16.33±1.53), mirroring the results obtained from feeding. Conversely, the highest mean of the minimum affected brain (Category

A) is attained at the dose of 0.01 ppm for injection (17.00±1.00), emphasizing the consistency of this trend across administration methods. The consequences of injecting different doses of arsenic into brain tissues of *L. cuprina* are visually conveyed through Figures 11 to 15. The damages are notably characterized by the degeneration of nerve cells, gland conditions, optic lobe status. These visual representations contribute to a nuanced understanding of the dose-dependent relationship between arsenic exposure and its effects on brain, offering valuable insights into the nuances of arsenic toxicity and its implications for brain tissues across specified categories at varying dosage levels.

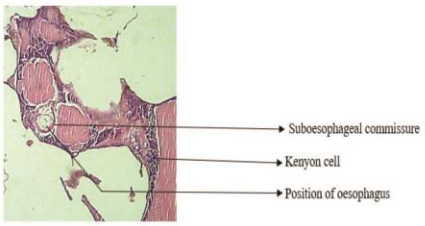

Fig. 11. Transverse Section (T.S.) of the brain of Blowfly injected with 0.01 ppm arsenic (as).

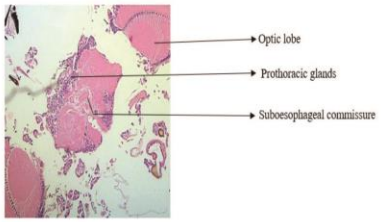

Fig.12. T.S of the brain of Blowfly injected with 0.05 ppm as.

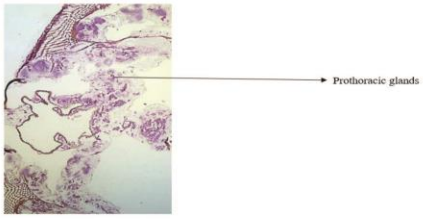

Fig. 13. T.S. of the brain of Blowfly injected with 0.1 ppm as.

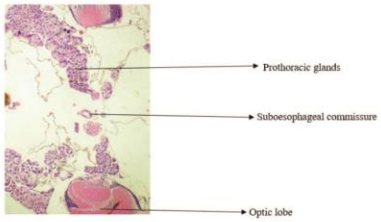

Fig. 14. T.S. of the brain of Blowfly injected with 0.5 ppm as.

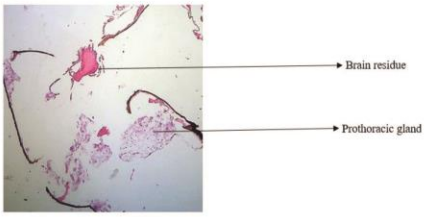

Fig. 15. T.S. of the brain of Blowfly injected with 0.1ppm as.

Levene's test and one-way ANOVA were also performed across categories (A, B, C, and D) in response to injecting various doses of As. The corresponding Levene statistic and F-statistics for each category are presented in the table 3. Post-hoc DMRTs comparisons were also performed across categories in response to injecting as in cat. A, no significant differences were found between 1.00 ppm and 0.5 ppm, with the highest impact at 0.01 ppm. For Cat. B, no differences

were detected among 1.00 ppm and 0.5 ppm, 0.01 ppm and 0.05 ppm, and 0.05 ppm and 0.1 ppm, with the peak impact at 0.1 ppm. Cat. C showed no significant differences between 0.01 ppm and 0.05 ppm, as well as 0.05 ppm and 1.00 ppm, with the highest impact at 0.1 ppm. In Cat. D, no significant differences surfaced among 0.01 ppm, 0.05 ppm, and 0.1 ppm, with the most affected brains observed at 1.00 ppm.

Table 3. Levene's test and ANOVA test results across the four categories (A, B, C, and D) in response to various doses of As in the injection

	Test of Homogeneity of Variance		One-way ANOVA	
Affected brain	Levene Statistic	Sig.	F	Sig.
Cat. A	.882	.509	182.433	0.000
Cat. B	.215	.924	13.318	0.001
Cat. C	2.386	.121	34.667	0.000
Cat. D	2.538	.106	163.933	0.000

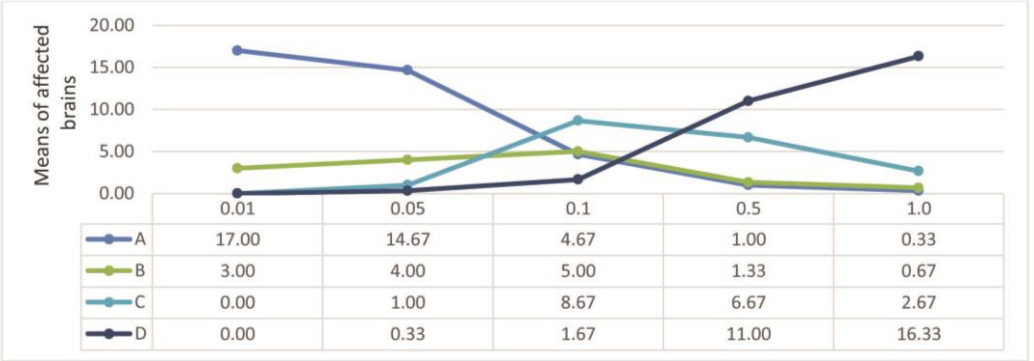

Fig. 16. Dose-response curves of the means of the affected brains in different categories from injecting various doses of as.

In Figure 16, the dose-response curves illustrate the means of affected brains across different categories from injecting various doses of As. Each curve represents a distinct category (A, B, C, and D), providing a visual representation of how the response changes with different levels of arsenic exposure. The curves showcase the trends and variations in the means, offering insights into the impact of varying doses on the affected brains in each category.

CONCLUTION

The brain tissue damage, as well as mortality found in the Australian Sheep Blowfly *Lucilia cuprina*, suggests that this insect is extremely sensitive to metal pollution. Understanding the effects of arsenic on the brain of this insect not only sheds light on the toxicological implications for *L. cuprina* but also contributes to broader discussions about environmental arsenic contamination and its potential consequences on insect populations. Therefore, there is a need

for integrative toxicological studies encompassing a broader range of metal pollutants, including arsenic, and insect species, to understand the intricate interplay between environmental contaminants and insect physiology.

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