Indian J. Applied & Pure Bio. Vol. 38(2), 469-476 (2023). **ISSN: 0970-2091**
A web of Science Journal

The effects of inorganic Arsenic dosage on *Drosophila melanogaster* third instar larvae motility

Anushree, Md Zeeshan Ali and *Jawaid Ahsan

[a]Drosophila Behavioral Laboratory
Department of Biotechnology, Central University of South Bihar (CUSB)
Gaya- 824 236 (India)
Anushree (0000-0002-7420-0019)
Md Zeeshan Ali (0000-0002-2101-1695)
***Corresponding author:** Jawaid Ahsan (0000-0002-2977-9922)
Phone: +91- 8521627799
E-mail: **jahsan@cub.ac.in**

Abstract

Arsenic is a primary environmental toxin that has a considerable detrimental influence on 300 million people and poses a major concern for people worldwide. The fruit fly *Drosophila melanogaster* as a model system is used to explore arsenic response pathways due to available robust behavioral assays and similarity to humans at different levels. The fruit fly larvae are even simpler and could be used a good model for toxicity assays. In this study, the toxic effects of inorganic arsenic on the locomotory ability of *Drosophila* third-instar larvae were investigated. The study was divided into two groups; the first batch of larvae served as the arsenic treated group and received exposure at doses of 1 mM and 1.5 mM arsenic and the second group of larvae were untreated which served as control group. The locomotory activity in both treated and untreated larvae was monitored in a time dependant manner. A significant reduction in the locomotory ability of the treated third instar larvae was observed with increasing concentration of arsenic. The findings of this study reveal the toxic effect of inorganic arsenic on larval motility.

Key words : third instar larvae, *Drosophila melanogaster*, inorganic arsenic, trivalent, locomotion, crawling.

Arsenic is considered to be a metalloid based on its chemical composition. It belongs to the Group 15 of the periodic table. It is found in a wide variety of minerals across the crust of the Earth, frequently in association with other metals and sulphur. China, Russia, and Morocco are the leading manufacturers of ascorbic acid (As) in recent years[27]. According

to reports, including those from West Bengal and Bangladesh, the presence of arsenic in groundwater has led to its substantial exposure and widespread poisoning[5]. In these areas, more than 50 million people consume groundwater with arsenic concentration higher than 50 g/L [16]. Almost six continents of the world are reported to have high concentrations of arsenic in drinking water[1,19]. There are potential health risks associated with arsenic poisoning, which affects an estimated 300 million individual globally[10].

High levels of arsenic are reportedly building up in the food chain, including in cereals and vegetables from arsenic-contaminated soil or arsenic-contaminated irrigation water, as well as in shellfish, fish, chicken, and milk[11,16,20]. Exposure to arsenic is caused by a number of anthropogenic activities, including mining and smelting operations, excessive groundwater withdrawal and pumping, the use of phosphate fertilisers, coal combustion, and industrial usage in the production of glass, semiconductors, pharmaceuticals, wood preservatives, and pesticides.[2,23,34] The toxicity of arsenic has the ability to cause harm to a diverse range of creatures, including humans[3,19].

Arsenic exposure has been linked to the development of a variety of adult-onset disorders, including cancer, diabetes, skin blemishes, and cardiovascular disease[15,22,28]. Arsenic exposure during embryogenesis has been linked to a shorter body length in zebrafish[14], slower growth in tilapia[30], and an increased risk of malformations in killifish[9]. Studies show that arsenic can negatively impact muscular development in addition to lowering birth weight. There are reports of impairments in walking ability in Japanese newborns who unintentionally ingested formula tainted with arsenic[8]. In rodent and fish muscle development is altered due to arsenic exposure[13,32]. Arsenic poisoning causes headaches, mental disorientation, and motor weakness[7]. Extreme Arsenic levels in drinking water in Bangladesh have been linked to motor abnormalities in children[21].

Drosophila melanogaster is an excellent model organism due to its shorter reproduction cycle, distinct developmental stages, known genome sequence, and human-like physiology. To our knowledge, there are no studies on the toxic effect of arsenic on the motor activity of fruit flies. Thus, the goal of the present study was to examine the effects on motor behavior due to arsenic exposure modelled in *Drosophila* third instar larvae.

Fly care and husbandry :

Wild-type flies of the Oregon R+ strain were reared on cornmeal media and maintained at 25ºC with 12 hour light/dark cycle in a BOD incubator. The media comprised high-grade polenta (corn), glucose, sugar, agar, yeast powder, and antifungal and antibacterial agents such as propionic acid and orthophosphoric acid, respectively, obtained from HiMedia (Mumbai, India). The flies were periodically transferred into fresh media bottles for proper breeding, growth, and health maintenance.

Chemicals :

Sodium (meta) Arsenite ($NaAsO_2$) with ≥ 90 percent purity (MW 129.91 g/mol)

was used for larval treatment. Different concentration of sodium arsenite were made in 5% sucrose solution as solvent. Polyethylene glycol – 6000 (PEG 6000), sodium chloride (NaCl), potassium chloride (KCl), calcium chloride ($CaCl_2$), disodium phosphate (Na_2HPO_4) and monopotassium phosphate (KH_2PO_4) were used for larvae isolation. All the chemicals used were of high grade and obtained from Himedia. The odorants ethyl acetate (EA) used as reinforcers were obtained from Sigma-Aldrich and were of high grade.

Materials :

Glass Petridishes of diameter 90 mm (3160065) were used for treatment and behavioral assays were obtained from Borosil, India. Paint brushes having soft bristles from Faber-Castell were used. Strainer having fine net (500 μm) were used for larvae isolation were purchased from the local market.

Larvae isolation and sodium arsenite treatment :

About an average of 150-200 random fruit flies were transferred into the fresh media bottles and placed into the incubator (25°C) for mating and egg laying. After 20 hours of egg laying, the bottles were made fly free and kept the bottle for further development at controlled temperature of 25°C for 3 days. Third instar larvae develop from eggs in about 72 h and they actively dig the corn media. The third instar larvae are used for experiments for neurobehavioral analysis. These larvae were isolated by collecting upper layer of corn media gently and smoothly in a strainer with the help of a paintbrush such that larvae do not get harmed. With the help of paintbrush, the coarse media having larvae were transferred into the vial containing 30 percent PEG-6000 solution (300 gm of PEG 6000 dissolved in 1000 mL distilled water) for separation of larvae from media. The larvae float at the top while the media settle down at bottom of the vial due to the difference in their relative density. The top layer of the vial is poured into the strainer and rinsed thoroughly twice under running distilled water to wash out the PEG solution adhered to the larval body. The larvae free from media and PEG solution is then collected into a Petri plate containing 0.5 mL of Ringer's solution[12,26] to maintain osmotic balance and hence prevent desiccation within larvae till the experiments are performed. Ringer's solution is comprised of two different solutions (Solution A and Solution B) mixed in 1:1 ratio. Ringer's solution comprised of 128 mM NaCl, 4.7 mM KCl, 1.8 mM $CaCl_2$, 0.9 mM Na_2HPO_4, and 0.37 mM KH_2PO_4.

After harvesting third instar larvae from media bottle, larvae were treated at specific arsenic concentration. About 20 mL of 1 percent Agar solution was poured into a Petri plate. Three concentrations of sodium arsenite used for the treatment of larvae were 1mM and 1.5mM. These concentrations selected were based on the arsenic effect study by Rizki *et al*.,[25] on *Drosophila*. In the Agar Petri plate, 2.5 mL of sodium arsenite solution was added and harvested larvae were transferred into it for treatment for 17 h. These treated larvae were further used in various experimental assays.

Statistics : Statistical analysis was performed using Graph Pad Prism (Graph Pad

Software, San Diego, CA, US; version 8.0.2). One-way student t-test was applied to determine the significant difference between differently treated fly samples. Significant p values were indicated as *** (≤0.0001).

Behavioral experiments :

Larval crawling assay: Agar petri plate was prepared by pouring 20 mL of 1% agar solution. A thin layer of agar was made on Petri plate to help the larva in crawling. The agar Petri plate was placed on a standard graph paper. Both treated and untreated larvae were tested for their locomotory ability. A larva treated at a specific arsenic concentration was placed at the center within the square box marked in the graph of the Petri plate (**Fig. 1**). A resting period of 3 seconds was given and then recorded larva locomotion for 15 sec. The lines which they traverse within 15 sec recorded were determined. Total number of smaller lines (0.5 cm) each larva crawled was summed up to determine the distance in centimetres (cm) they travelled within 15 seconds.

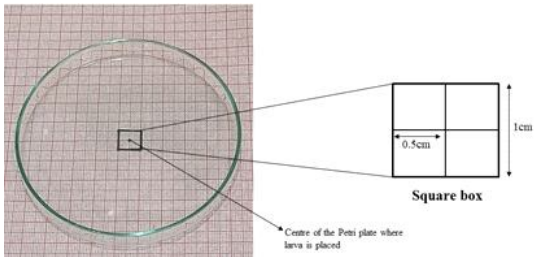

Fig. 1. Schematic representation of larval crawling assay. The number of smaller lines crawled by third instar larvae in 15 seconds are estimated to determine larva motility.

More than 75 percent of the untreated larvae were able to crawl an average distance of 2.84 cm within 15 sec. 1 mM arsenic treated larvae crawled an average distance of 2.57 cm within the duration of 15 sec (Fig. 2).

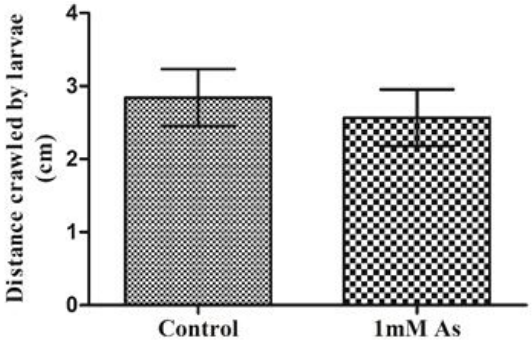

Fig. 2. The bar graph represents the crawling ability of untreated and treated (1 mM As) third instar larva. Error bar represents mean ± S.D. Student *t*- test analyzed the statistically significant difference (*p =0.02) between the different sample mean with 95% confidence interval and R squared value= 0.11.

There was significant decrease in the locomotory activity of larvae due to the arsenic feeding. The maximum and minimum distance crawled by 1.5 mM arsenic treated larvae were 1.5 cm and 0.5 cm respectively within 15 seconds (Fig. 3).

There was a decrease of 16.37 percent in crawling ability of 1 mM arsenic treated larva in comparison to untreated larva. As the concentration of arsenic was increased the crawling ability of third instar larvae was observed to be decreasing in a time dependent manner. There was about 60.79 percent decrease in crawling ability of 1.5 mM arsenic treated larva relative to control within the duration of 15 seconds (Fig. 4).

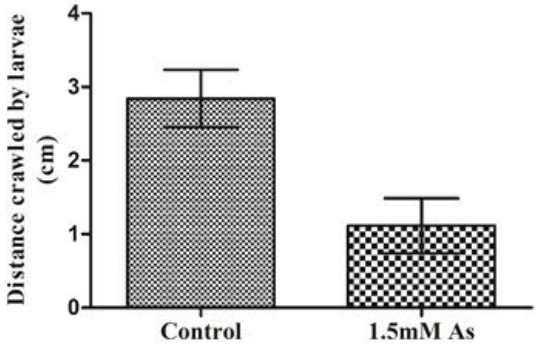

Fig. 3. The bar graph represents the crawling ability of untreated and treated (1.5 mM As) third instar larva. Error bar represents mean ± S.D. Student *t*- test analyzed the statistically significant difference (***p <0.0001) between the different sample mean with 95% confidence interval and R squared value = 0.84.

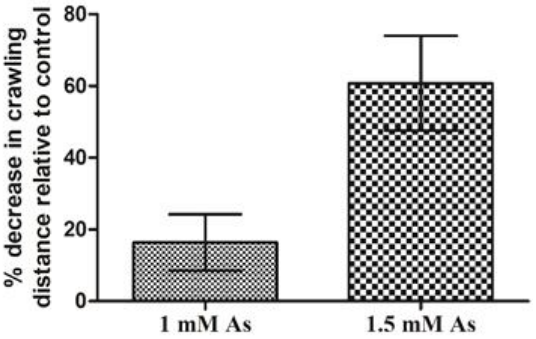

Fig. 4. The bar graph represents the relative percentage decrease in crawling activity of arsenic treated third instar larvae at different concentrations with respect to untreated larvae. Student *t*- test analysed the significant difference between the arsenic treated larvae motility at different concentrations (***P>0.0001).

Although there was a small difference in crawling of larva between control and 1 mM arsenic treated, but was statistically significant. But when the arsenic concentration was increased to 1.5 mM for treating third instar larvae, the crawling ability of ability drastically decreased. There was a huge percentage difference of 44.42 percent in the locomotory activity between 1 mM and 1.5 mM arsenic treated larvae. The bar graph represents the average distance crawled by larva in 15 seconds for both the treated and untreated larva (Fig. 2 and Fig. 3). The crawling of larva at higher concentration of arsenic was highly affected due to its toxic effect.

Locomotion is necessary for most animal behavior, including food-seeking, mating, territorial defense, and escape from predators or adverse environments. Age, sex, genetic background, and environmental conditions including food, temperature, humidity, and light can all influence locomotor activity in *Drosophila*[34]. The locomotory activity of fruit flies are an important behavioral phenomenon in several *Drosophila* models of human neurological disorders, including tauopathy, Huntington's disease[6], Parkinson's disease[24], and spinocerebellar ataxia[5], it has been discovered to have an impairment in its function.

In the present study, the crawling distance travelled by untreated third instar larvae was higher than the arsenic treated fruit fly larvae. It clearly indicates that exposure to inorganic arsenic led to the larval locomotory impairment. As the concentrations of arsenic was increased the crawling ability of larvae decreased significantly. The findings of present study corroborate with the previous study on rodent, exposure to arsenic either delayed or

prevented muscle formation affecting their locomotion[29,32]. Both the 1 mM and 1.5 mM arsenic-treated *Drosophila* larvae demonstrated considerably reduced motility in a concentration-dependent manner when compared to the untreated control group, a symptom of neurological disease. Studies using rat models have shown that exposure to arsenic affects a number of behaviors and systems connected to aspects of memory, learning, motor function, and locomotion. Similar findings were obtained in this study of *Drosophila* larvae locomotory ability post-arsenic exposure providing an insight into the behavioral impairment due to arsenic.

We acknowledge the financial support from the University Grants Commission (New Delhi, India) and the Central University of South Bihar (Gaya, Bihar, India) for this research.

References :

1. Ayotte J.D., D.L. Montgomery, S.M. Flanagan, and K.W. Robinson (2003) *Environmental science & technology*, *37*(10): 2075–2083. https://doi.org/10.1021/es026211g.
2. Bjørklund G., P. Oliinyk, R. Lysiuk, M.S. Rahaman, H. Antonyak, I. Lozynska, L. Lenchyk, and M. Peana (2020) *Archives of toxicology*, *94*(6): 1879–1897. https://doi.org/10.1007/s00204-020-02739-w.
3. Cervantes C., G. Ji, J.L. Ramírez, and S. Silver (1994) *FEMS microbiology reviews*, *15*(4): 355–367. https://doi.org/10.1111/j.1574-6976.1994.tb00145.x.
4. Chakraborti D., M.M. Rahman, A. Mukherjee, M. Alauddin, M. Hassan, R.N. Dutta, S. Pati, S.C. Mukherjee, S. Roy, Q. Quamruzzman, M. Rahman, S. Morshed, T. Islam, S. Sorif, M. Selim, M.R. Islam, and M.M. Hossain (2015) *Journal of trace elements in medicine and biology: organ of the Society for Minerals and Trace Elements (GMS)*, *31*: 237–248. https://doi.org/10.1016/j.jtemb.2015.01.003.
5. Chakravorty A., A. Sharma, V. Sheeba, and R. Manjithaya (2022) *Frontiers in molecular neuroscience*, *15*, 842772. https://doi.org/10.3389/fnmol.2022.842772.
6. Chongtham A., B. Barbaro, T. Filip, A. Syed, W. Huang, M. R. Smith, and J. L. Marsh (2018) *Methods in molecular biology (Clifton, N.J.)*, *1780*: 75–96. https://doi.org/10.1007/978-1-4939-7825-0_5.
7. Chouhan S., and S.J. Flora (2010) *Indian journal of experimental biology*, *48*(7): 666–678.
8. Dakeishi M., K. Murata, and P. Grandjean (2006) *Environmental health : a global access science source*, *5*, 31. https://doi.org/10.1186/1476-069X-5-31.
9. Gonzalez H.O., J. Hu, K.M. Gaworecki, J.A. Roling, W.S. Baldwin, J.L. Gardea-Torresdey, and L.J. Bain (2010) *Marine environmental research*, *70*(2), 133–141. https://doi.org/10.1016/j.marenvres.2010.04.003.
10. Hassan, Manzurul. (2018) Arsenic in Groundwater: Poisoning and Risk Assessment. 10.1201/9781315117034.
11. Ivy N., T. Mukherjee, S. Bhattacharya, A. Ghosh, and P. Sharma (2022) *Environmental geochemistry and health*, 10.1007/s10653-022-01330-9. Advance online publication. https://doi.org/10.1007/s10653-022-01330-9.

12. Khurana S., W.K. Li, and N.S. Atkinson (2010) *PloS One*, *5*(12): e15259. https://doi.org/10.1371/journal.pone.0015259
13. Lantz R.C., B. Chau, P. Sarihan, M.L. Witten, V.I. Pivniouk and G.J. Chen (2009) *Toxicology and applied pharmacology*, *235*(1): 105–113. https://doi.org/10.1016/j.taap.2008.11.012.
14. Li D., C. Lu, J. Wang, W. Hu, Z. Cao, D. Sun, H. Xia, and X. Ma (2009) *Aquatic toxicology (Amsterdam, Netherlands)*, *91*(3): 229–237. https://doi.org/10.1016/j.aquatox.2008.11.007.
15. Mink P.J., D.D. Alexander, L.M. Barraj, M.A. Kelsh and J.S. Tsuji (2008) *Regulatory toxicology and pharmacology: RTP*, *52*(3): 299–310. https://doi.org/10.1016/j.yrtph.2008.08.010.
16. Mondal D., M.M. Rahman, S. Suman, P. Sharma, A.B. Siddique, M.A. Rahman, A. Bari, R. Kumar, N. Bose, S.K. Singh, A. Ghosh and D.A. Polya (2021) *The Science of the total environment*, *754*: 142082. https://doi.org/10.1016/j.scitotenv.2020.142082.
17. Mondal P., C.B. Majumder and B. Mohanty (2006) *Journal of hazardous materials*, *137*(1): 464–479. https://doi.org/10.1016/j.jhazmat.2006.02.023.
18. National Research Council (NRC). (2001) *Arsenic in Drinking Water: 2001 Update*. National Academies Press (US).
19. Naujokas M.F., B. Anderson, H. Ahsan, H.V. Aposhian, J.H. Graziano, C. Thompson, and W. A. Suk (2013) *Environmental health perspectives*, *121*(3): 295–302. https://doi.org/10.1289/ehp.1205875.
20. Nigra A.E., K.E. Nachman, D.C. Love, M. Grau-Perez and A. Navas-Acien (2017) *Environmental health perspectives*, *125*(3): 370–377. https://doi.org/10.1289/EHP351.
21. Parvez F., G.A. Wasserman, P. Factor-Litvak, X. Liu, V. Slavkovich, A.B. Siddique, R. Sultana, R. Sultana, T. Islam, D. Levy, J.L. Mey, A. Van Geen, K. Khan, J. Kline, H. Ahsan, and J.H. Graziano, (2011) *Environmental health perspectives*, *119*(11): 1665–1670. https://doi.org/10.1289/ehp.1103548.
22. Platanias L.C. (2009) *The Journal of biological chemistry*, *284*(28): 18583–18587. https://doi.org/10.1074/jbc.R900003200.
23. Rahaman M.S., M. Akter, M.M. Rahman, M.T. Sikder, T. Hosokawa, T. Saito and M. Kurasaki (2020) *Environmental toxicology and pharmacology*, *74*: 103302. https://doi.org/10.1016/j.etap.2019.103302.
24. Riemensperger T., A.R. Issa, U. Pech, H. Coulom, M.V. Nguyễn, M. Cassar, M. Jacquet, A. Fiala and S. Birman (2013) *Cell Rep.* *5*(4): 952—960.
25. Rizki M., E. Kossatz, A. Velázquez, A. Creus, M. Farina, S. Fortaner, E. Sabbioni, and R. Marcos (2006) *Environ Mol Mutagen*, *47*(3): 162-168. doi:10.1002/em.20178.
26. Robb J.A. (1969) *The Journal of Cell Biology*, *41*(3): 876-885. https://doi.org/10.1083/jcb.41.3.876
27. Sackett Penny. (2016) Elemental cycles in the Anthropocene: Mining aboveground. 10.1130/2016.2520(11).
28. Schuhmacher-Wolz U., H.H. Dieter, D. Klein, and K. Schneider (2009) *Critical reviews in toxicology*, *39*(4): 271–298. https://doi.org/10.1080/10408440802291505.
29. Steffens A.A., G.M. Hong, and L.J. Bain

(2011) *Toxicology and applied pharmacology*, *250*(2): 154–161. https://doi.org/10.1016/j.taap.2010.10.006.

30. Tsai J.W., and C.M. Liao (2006) *Archives of environmental contamination and toxicology*, *50*(1): 144–152. https://doi.org/10.1007/s00244-005-1054-z.
31. Woods J.K., S. Kowalski, and B. Rogina (2014) *Journal of visualized experiments: JoVE*, (86), 51449. https://doi.org/10.3791/51449.
32. Yen Y.P., K.S. Tsai, Y.W. Chen, C.F. Huang, R.S. Yang, and S.H. Liu (2010) *Environmental health perspectives*, *118*(7): 949–956. https://doi.org/10.1289/ehp.0901525.
33. Yousuf O.A., R. Kai Ruan, and Grace Zhai. (2012) *Human Molecular Genetics*, *21*(2): Pages 237–250, https://doi.org/10.1093/hmg/ddr449.
34. Zinke L. (2020) Groundwater Arsenic. *Nat Rev Earth Environ 1:* 558. https://doi.org/10.1038/s43017-020-00110-2.