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Acute Exposure of Arsenic Affects Fecundity and Reproduction in *Drosophila melanogaster*

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ABSTRACT Arsenic-contaminated groundwater has been a matter of concern worldwide, especially in developing countries like India. High levels of exposure to arsenic have been linked to a wide range of neuronal, cardiac, dermatologic, and malignant disorders. However, few studies have focused on examining the relationship between arsenic exposure and reproductive health outcomes. The aim of this study is to examine the effects of inorganic arsenic on female fruit flies' reproductive ability. The *Drosophila melanogaster* could be used as a good model for studying the effects of Arsenic on female fecundity because of its advantages like short life cycle, high fecundity (egg-laying), genome similarity with humans, and ease of maintenance in the lab. Results of the study document the decline of young female flies' fertility and fecundity in a dose-dependent manner. The findings from this investigation may support the role of arsenic exposure in increasing the risk of infertility in females.

INTRODUCTION

The widespread presence of Arsenic and its adverse effects on human health make it a significant cause of concern on a global scale. Leaching, erosion, and mining can all contribute to the contamination of drinking water sources with Arsenic, a naturally occurring element in the crust of the Earth. Countries like Bangladesh, China, India, Bangladesh, and Taiwan on the Asian continent, Hungary in Europe, Argentina, Mexico, the northeast, and the western U.S. on the American continent have all been found to have high levels of Arsenic (Hashim et al. 2019; McArthur 2019; Dhillon 2020; Shaji et al. 2021). Also, the US Agency for Toxic Substances and Disease Registry (ATSDR) ranked arsenic first in the toxic substance priority list (ATSDR 2013). Elevated Arsenic concentration is reported to be accumulating in the food chain, such that in grains and vegetables through Arsenic-contaminated soil or water used for irrigation; seafood, fish, poultry, and milk (Bundschuh et al. 2012; Molin et al. 2015; Nigra et al. 2017; Mondal et al. 2021; Ivy et al. 2022). The usage of Arsenic for industrial purposes, such as in the manufacturing of glass, semiconductors, pharmaceuticals, wood preservatives, pesticides, and herbicides, has reportedly increased (Rahaman et al. 2020). Human activities, such as excessive groundwater pumping and withdrawal, the use of phosphate fertilizers, Arsenic-enriched pesticides, and Arsenic-contaminated irrigation water for agricultural practices, as well as mining and smelting operations, coal combustion, industrial processes, and the use of timber preservatives, contribute to Arsenic contamination (Punshon et al. 2017; Bjørklund et al. 2020; Zinke 2020). Arsenic toxicity has the potential to harm a wide range of organisms, including humans (Cervantes et al. 1994). The teratogenic potential of inorganic Arsenic is more remarkable for the trivalent rather than the pentavalent state (Hunter 2000; Lammon et al. 2003; Peng et al. 2022). Epidemiological studies have indicated that exposure to inorganic Arsenic is associated with increased risks of vascular and cardiovascular disease, diabetes, reproductive and developmental problems, neurologic and cognitive disorders, and various cancers, including those of the skin, lung, bladder, kidney, and (NRC 2001; Wasserman et al. 2004; Bhowmick et al. 2018; Chakraborti et al. 2018; Saha and Ray 2019; Sun et al. 2019; Karagas et al. 2019). Consequently, Arsenic and "poison" have become interchangeable in the mind of public. An estimated 50 million people in Bangladesh are at risk for severe health effects due

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to Arsenic poisoning, and the administration of current arsenicosis victims has already stressed the fragile public health system (Chaudhuri 2004; Rahman et al. 2018).

Infertility has become a global concern, particularly for women (Escada-Rebelo et al. 2022). Despite advancements in the field of female reproductive health, the treatment of female infertility remains difficult because the underlying causes in a substantial number of cases remain unknown (Wang et al. 2019; Carson et al. 2021). Arsenic exposure has been shown to significantly cause infertility, low sperm quality, decreased gonadotropins level, and erectile dysfunction in men, according to a few recent epidemiologic studies (Nie et al. 2006; Hsieh et al. 2008; Meeker et al. 2010; Renu et al. 2018). Additionally, exposure to hazardous metals in animals results in male infertility, impairs spermatogenesis, lower testosterone level, oxidative damage, necrosis, bleeding, or losses of germ cells in cattle (Guvvala et al. 2020). Arsenic can potentially cause toxicity in the ovaries and the uterus and interfere with the neuroendocrine system responsible for regulating female sex hormones (Chattopadhyay et al. 2001). However, the effects of Arsenic exposure on reproduction and development are not well understood, and it is still unknown how Arsenic causes reproductive toxicity.

The *Drosophila* genome was sequenced in 2000 (Adams et al. 2000), and its database is maintained at FlyBase, a valuable resource for retrieving biological information about *Drosophila* species (Marygold et al. 2016). Furthermore, *Drosophila* is an excellent *in vivo* model organism for studying toxicity because it has a short life cycle, clear developmental stages, a known genome sequence, and similar physiology to humans (Calap-Quintana et al. 2017). Hence, it makes fruit flies promising arthropods to rapidly test toxicity in whole organisms and determine the molecular mechanisms behind it.

Objectives

Although there are a few studies to find the toxic effect of Arsenic on adult flies, not much is known about the Arsenic effects on fecundity, reproduction, development, and the eggs in the fruit fly. In the present study, the researchers studied the toxic effect of Arsenic on female fly fecundity and thus its reproductive ability in time and dose dependant manner. The researchers have further discussed a few possible mechanisms underlying Arsenic-induced reproductive toxicity.

MATERIAL AND METHODS

Fly Care and Husbandry

Wild-type flies of the Oregon R+ strain were reared on cornmeal media and maintained at 25^0C on a 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. The last pupal stage bottles were made fly-free and kept in the incubator. Then, the flies were transferred to fresh media vials from the bottles after 18h -20h. When the flies aged 5-days old, they were used further for experiments to monitor reproductive health of flies.

Chemicals

Sodium (meta) arsenite ($NaAsO_2$) with ≥90 per cent purity (MW 129.91 g/mol) was used for treatment. The attractant odorant, ethyl acetate (EA) was used in the assays, and mineral oil was used as diluent (solvent) to decrease the odor's volatility. All these chemicals were of the highest purity and were purchased from Sigma-Aldrich, St. Gallon, SG.

Flies Fecundity

The polyethylene terephthalate (PET) bottles with a capacity of 100ml were obtained from the local market and were used for culturing the flies. The fecundity of five-days-old virgin female flies was estimated in the cut bottles by transferring five female and five male flies together in each bottle for mating. These bottles were cut into two halves to count the number of eggs laid properly. Firstly, normal corn meal media containing specific Arsenic concentration was poured into the bottom half of the cut bottles. After the media was cooled down, the two halves were joined with cello tape. In the control cut bottle, media with no Sodium arsenite was poured, and in the treatment cut bottle,

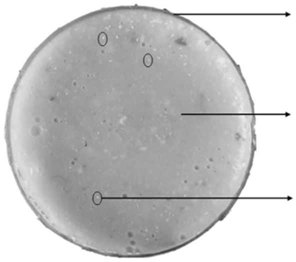

Fig. 1. Bottom half of cut-bottle containing cornmeal media for counting eggs

media with different concentrations (1mM,1.5mM, and 2mM) of Sodium arsenite were used for the experiments. These concentrations of Arsenic were chosen based on the studies conducted by Rizki et al. (2006) on *Drosophila*. For the experiments, five-days-old virgin female flies and male flies were transferred into each cut bottle containing control and Arsenite containing media. After 18 hours, the number of eggs laid was counted by removing the tape and counting the eggs under a stereo-microscope (Fig. 1). After estimating the egg count, the two halves of the cut bottle were fixed again using tape, and placed into the BOD incubator at 25° C for monitoring the later developmental stages.

RESULTS

In population biology, fecundity is comparable to fertility, which is the natural ability to have offspring and is assessed by the number of gametes or eggs laid by females. The five female untreated flies laid an average of 36 eggs within 18 h of mating on the media surface of the cut bottles. While the average number of eggs laid by five female arsenic-treated flies fed with 1mM Sodium arsenite dissolved media in 18 h was obtained as 22. The difference between eggs laid in 1mM Arsenite-treated media by female flies and in untreated media by female flies was 14. This experimental assay was repeated (n) eight times to avoid any error in counting the eggs and for the data to be statistically significant. The number of eggs laid in each trial is shown in Table 1. The average number of eggs laid by five female flies fed with 1.5mM Sodium arsenite treated media in 18 h was obtained as 16. The difference between eggs laid in 1.5mM Arsenite-media fed female flies and control media female flies was 20. The difference between eggs laid in control and 1.5mM Arsenite-treated media was analyzed to be statistically significant. Similarly, by increasing the concentration of Sodium arsenite in media to 2mM the eggs laid by female flies in 18 h decreased further to an average number of 14. The difference between eggs laid in 1mM Arsenite-media-fed female flies and control media female flies was 22. Statistically, a significant difference was obtained between the eggs laid by female flies fed with 2mM Arsenic media and control untreated media. As the concentration of Sodium arsenite in the media was increased for flies to feed, the number of eggs laid by female flies decreased compared to the control untreated media-fed flies, such that 1mM Sodium arsenite media had the maximum number of eggs laid compared to 1.5mM and 2mM Arsenite treated media. The arsenic-treated and untreated media containing eggs are shown in Figure 2. All the treated as well untreated experimental trials were repeated eight times and the eggs laid in each trial are shown in Table 1. The significant difference between the fecundity of treated female flies at different Arsenite concentrations was analyzed statistically using an unpaired student *t*-test. There was a decrease in fecundity percentage of Arsenite-fed flies relative to control untreated media-fed flies, obtained as 38.98 percent, 60.46 percent, and 66.17 percent approximately for

Table 1: The number of eggs laid by female flies fed with arsenic (As) treated and untreated media within 18 h. The number of repeated trials for each treated and untreated is eight times (n = 8)

	Number of eggs laid in 18 h								
Samples ↓ / Repeated trials (*n*) →	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*Mean values* ± SD
Control (n=8)	40	38	30	35	37	36	40	39	36.9±3.3
1mM As (n=8)	17	25	23	20	24	19	22	25	21.9±2.9
1.5mMAs (n=8)	15	12	10	15	17	19	20	17	15.6±3.4
2mM As (n=8)	15	16	15	13	11	17	13	11	13.9±2.2

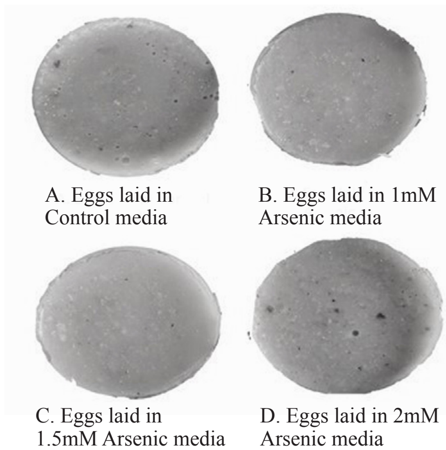

Fig. 2. Eggs laid in media containing different Arsenic concentrations (B-D) and in control media (A). The maximum number of eggs are laid by 1mM Arsenic containing media while the minimum number of eggs are laid by 2mM Arsenic containg media

1mM, 1.5mM, and 2mM, respectively. Table 2 represents the relative percentage decrease of flies' fecundity at various concentrations of Sodium arsenite dissolved media with respect to control media flies for each experimental trial (n).

First-instar larvae hatched from eggs in all the media, whether having dissolved Sodium arsenite at different concentrations or control untreated media. The larvae developed in Arsenic media were sedentary in comparison to untreated media. There was a clear distinction of later stages as pupa could be observed to develop only in 1mM Sodium arsenite treated media on the fifth day from the day of eggs were laid. At higher concentrations of Arsenic, no further stages of development were observed from day two.

Table 2: The percentage decrease in fecundity of female flies fed with arsenic (As) treated media relative to untreated (Control) media is represented

Repeated trials (n=8)	*Relative % decrease in fecundity of female flies*		
	1mM As	*1.5mM As*	*2mM As*
1	45	62.5	68.4
2	34.2	68.4	71
3	37.8	56.7	59.4
4	42.8	57.1	62.8
5	35.1	54	70.2
6	36.1	58.3	58.3
7	45	57.5	67.5
8	35.9	69.2	71.8

DISCUSSION

Arsenic has been widely known as a teratogen and could affect a foetus's growth and development. The two parameters, concentration and duration of Arsenic, play a significant role in influencing growth retardation and death of the foetus (Golub et al. 1998; Abdul et al. 2015). Changes in the level of catecholamines in the brain caused by Arsenic exposure could cause inorganic Arsenic toxic effects in the female and male reproductive system (Ali et al. 2019). The upregulation of serotonin and downregulation of norepinephrine in the midbrain and diencephalon leads to lower gonadotrophin synthesis and secretion (Kaminski et al. 1997). The toxic effect of Arsenic in a time-dependent manner on flies' development had been reported earlier by (Beamish et al. 2021), but there has been no mention of the Arsenic effect on fecundity of female flies. The present study revealed that female flies' fecundity decreased by increasing the Arsenite concentration in media, which corroborated with the findings of Quansah et al. (2015). Similarly, reproductive toxicity was shown in inorganic Arsenic-treated female mice on their their oocyte meiosis, decreased corpus luteum number, and their preimplantation development (Navarro et al. 2004; Nath et al. 2020). Several studies have found that an increase in foetal and infant mortality or impaired foetal growth might be due to Arsenic exposure during development since Arsenic has the potential to move across membranes quickly (Vahter 2009). An average difference of 21 percent and 27 percent was obtained in the fecundity of arenite-treated flies at 1.5mM and 2mM, respectively, relative to 1mM Arsenite-treated flies. None of the eggs were hatched into pupae at 1.5mM and 2mM Arsenite media bottles compared to either control media containing no Arsenite or 1mM Arsenite media bottles. Even the eggs hatched in 1mM media into larvae and pupae did not develop into adults, unlike eggs in the control media. Arsenic metal ingestion possibly impairs reproductive health by causing oxidative stress which further leads to massive germ cell destruction and leutinizing hormone (LH), follicle-stimulating hormone (FSH), increase of cortisol, and testosterone changes (Zubair et al. 2017; Rahman et al. 2019; Guvvala et al. 2020; Das et al. 2021) These toxic effects on fecundity significantly depend on the dose, route, and gestation periods of Arsenic exposure. Arsenic crosses the placenta with relative ease, and a few studies on human suggests it may moderately increase the risk of stunted foetal growth and infant mortality (Vahter 2009; Guan et al. 2012), which was found similar to the researchers findings in fruit flies. The larvae developed in the 1mM Arsenite media bottle could not climb the wall of the bottles compared control media bottles. As a result, larvae developed in 1mM Arsenite medium clearly exhibited a deficit in movement. The growth of *Drosophila* larvae was significantly found to be impaired in the presence of Arsenic.

CONCLUSION

The findings in this study reveal that Arsenic exposure significantly reduces the reproduction rate and fecundity of female flies in a time and dose-dependent manner. To the researchers knowledge, this is the first study of the toxic effect of arsenic on the reproduction and fecundity of *D. melanogaster.* To comprehend the fundamental mechanism of action of arsenic, further research is required.

RECOMMENDATIONS

Although there is some evidence that arsenic may be detrimental to fetus development, the literature at this time cannot conclusively link environmental arsenic exposure to problems with reproductive health. Although not conclusive, the facts reported in this research support arsenic toxic effects on female fertility and fecundity. The present arsenic global concern requires more studies like these.

CREDIT AUTHORSHIP CONTRIBUTION STATEMENT

Anushree: Conceptualization, Writing - original draft, Methodology, Data curation, Software. Md Zeeshan Ali: Software. Jawaid Ahsan: Validation, Supervision.

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DECLARATION

The authors declare that this manuscript has not been published or submitted for publication elsewhere.

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