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The effects of arsenate on wild-type L1 stage larvae of *Caenorhabditis elegans*

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Abstract: ***Arsenic toxicity is a global problem and its exposure causes several diseases. Arsenic exists in less toxic pentavalent form and more toxic trivalent form. Arsenic-induced oxidative stress is well known and it even causes the death of adult Caenorhabditis elegans (C. elegans). In the present study, we have investigated the effects of less toxic pentavalent arsenate on the first development stage i.e. L1 larva of wild-type C. elegans. To see the toxicity of arsenate, synchronized L1 larvae were exposed to different concentrations of arsenate i.e. 0.75 mg/L, 1.5 mg/L, 3 mg/L and 6 mg/L at 170C for 24 hours. Further, live larvae were counted. Our results showed the survival percentage of arsenate treated L1 larvae at 0.75 mg/L and 1.5 mg/L were 53.85 ± 8.52 and 4.67 ± 1.83 respectively. However, at higher concentrations (3 mg/L and 6 mg/L) all L1 stage larvae died. In the control study 94.18 ± 2.06 percentage, L1 larvae were survived. They appeared to be more sensitive to arsenate toxicity than that of the adult worms. Moreover, the mechanism/s behind it is not well understood. Thus to comment on the sensitivity and mechanism/s of arsenic at different developmental stages furthermore investigations are required.***

Key words: ***Arsenic-induced, death, survival, toxicity.***

I. INTRODUCTION

Arsenic (ARs) contamination has been reported around the globe [1]. However, its contamination levels differed from 1 to 3050 µg/L in the rural ground water samples [2]. Lower than 10 µg/L ARs contaminant in the drinking water is safe for human [3] as per World health organization (WHO) guidelines. If it's exposure crosses the critical barrier of 10 µg/L and is used as drinking water, it produces adverse effects on numerous body organs, systems and eventually death [3,4,5].

C. elegans used as an excellent model organism for understanding the effects of metal and non-metal in the various cellular processes [6]. ARs exists in trivalent and pentavalent forms and trivalent form appears most toxic [7]. The report suggested arsenic-induced oxidative stress affected the life of adult worms and died at higher exposure [8]. However, ARs toxicity on the L1 larvae was not explored. In the present study, we exposed synchronized L1 stage larvae with different concentrations (0.75 mg/L, 1.5 mg/L, 3.00 mg/L, and 6.00 mg/L) of ARs (v) to elucidate its toxicity. We tried to understand the effect of less toxic pentavalent form on the first developmental stage i.e. L1 larval stage of C. elegans. Data indicates that L1 larvae showed more sensitivity than adult worms and mortality increased with increase in the concentration of ARs (v) solution.

II. MATERIALS AND METHODS

Wild-type *C. elegans* N2 (var. Bristol) were cultured and maintained in our laboratory. Further, worms were transferred to new petri dishes on nematode growth medium (NGM) seeded with OP50 strain *Escherichia coli* [9]. All plates were incubated at 17^0C for proper growth. After 5 days NGM plates having maximum gravid worms were selected for synchronization. Worm cultures were synchronized as described in the protocol [10]. In this experiment, alkaline hypochlorite solution was used for bleaching the worms and collected eggs were incubated overnight in the M9 buffer at 17^0C. Thus, all *C. elegans* were arrested at the L1 larval stage. 12 mg of $Na_2HAsO_4.7H_2O$ (Himedia, India) also called ARs (v) was dissolved in 1 L of distilled water and thus 12 mg/L stock solution was prepared. Four working solutions (1.5 mg/L, 3 mg/L, 6 mg/L and 12 mg/L) were prepared by serial dilution of stock solution.

The L1 stage larvae were exposed to different concentrations of ARs (v). Enzyme-linked immunosorbent assay (ELISA) 48 wells plate was used for this purpose. 150 µl of distilled water and 150 µl of a solution having L1 stage

larvae were mixed in the first well of ELISA plate and treated as control. In next four wells, we added 150 µl of four different ARs (v) solutions (1.5 mg/L, 3 mg/L, 6 mg/L and 12 mg/L) and in each well 150 µl of a solution having L1 stage larvae were mixed. Thus, the final exposure of ARs (v) treated *C. elegans* became 0.75 mg/L, 1.5 mg/L, 3 mg/L and 6 mg/L respectively. The ELISA plate was covered with aluminum foil and incubated at 17^0 C for 24 hours. The numbers of live and dead worms were determined by manual counting. The larvae showed slight movement were treated as live, however; the larvae showed no movement after 10-12 seconds of continuous observation were considered as dead in this experiment.

After 24 hours of incubation, approximately 50 µl of solution from wells of ELISA plate were taken by using micropipette and dispensed on the glass slide to determine the number of live and dead worms. The glass slide was visualized under a microscope (Olympus, CX21, LED) at 4X objective and live and dead worms were counted. The counting was repeated for five times for each concentration and number of live and dead worms of control and four ARs (v) treated groups were calculated (Table 1). Finally, the data obtained from control and experimental groups were converted into the percentage of live and dead worms (Table 1) for analysis. We conducted four separate sets of experiments and the percentage data from different groups were pooled for further inter-group statistical analyses using Holm-Sidak test.

Statistical analysis: The percentage data has been expressed as mean ± SEM. Data from control and 0.75 mg/L, 1.5 mg/L, 3 mg/L, 6 mg/L ARs (v) treated larvae were statistically analyzed applying a one-way analysis of variance (ANOVA) using Sigma Stat software (Jandel Scientific, USA). Holm-Sidak test was used for determining the significance level and at least p ≤0.05 was considered significant.

III. RESULTS

In the present study toxicity of ARs (v) was tested on synchronized L1 stage larvae of *C. elegans.* All live and dead larvae were calculated and all data have been converted into percentage of live worms (Table 1)

Effects of 0.75 mg/L and 1.5 mg/L ARs (v) treatments:

The mean (±SEM) survival percentage of L1 larvae decreased from 94.18 ± 2.06 in control to 53.85 ± 8.52 in 0.75 mg/L ARs (v) treated worms, a decrease of 57.17% [F(1,7)= 21.17, P=0.004] as compared to the control (Figure 1 and Table 1). Further, as the concentration of ARs (v) increased to 1.5 mg/L the survival percentage decreased from 94.18 ± 2.06 in control to 4.67 ± 1.83, a decrease of 4.96% [F(1,7)=1055.26, P≤ 0.001] as compared to the control (Figure 1 and Table 1).

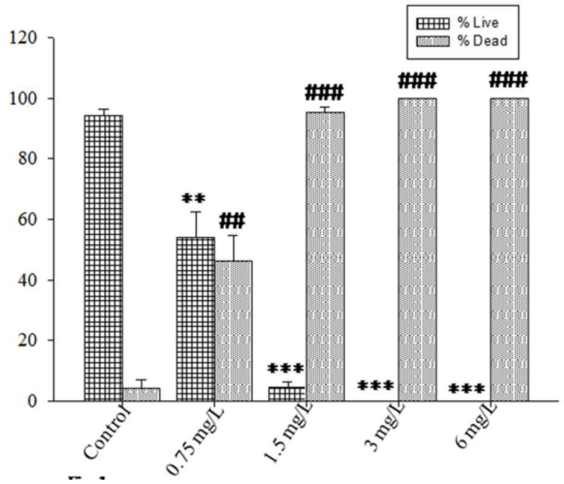

Figure 1: Histogram represents mean percentage ± SEM of Live and dead L1 larvae after 24 hrs exposure at the different concentration of ARs (v). ***/### P ≤ 0.001; **/## P ≤ 0.01; significantly different from control.

Effects of arsenate toxicity on synchronized L1 stage larva of *C. elegans*

	Control		0.75 mg/L		1.5 mg/L		3.0 mg/L		6 mg/L	
	Li/To	Li%	Li/To	Li%	Li/To	Li%	Li/To	Li %	Li/To	Li %
E-1	98/104	94.23	151/373	40.48	8/150	5.33	0/49	0	0/86	0
E-2	309/339	91.15	191/499	38.27	15/336	4.46	0/538	0	0/183	0
E-3	85/85	100	85/132	64.39	9/101	8.91	0/120	0	0/317	0
E-4	116/127	91.33	60/83	72.28	0/57	0	0/290	0	0/518	0
%M ± S	94.18 ± 2.06		53.85 ± 8.52		4.67 ± 1.83		0 ± 0		0 ± 0	

Table 1: The number of live and total L1 larvae after ARs (v) exposure were presented. The data has been converted into the percentage. Abbreviations: L: Litre; Li: Live; mg: milligram; M: mean; S: SEM; To: Total.

Effects of 3 mg/L and 6 mg/L ARs (v) treatments:

The toxicity effect on the higher concentrations was remarkable. We observed that not a single worm sustained the toxicity effect of ARs (v) till 24 hrs. All animals died at concentrations of ARs (v) at 3 mg/L and 6 mg/L (Figure 1 and Table 1).

IV. DISCUSSION

In the present study, we observed that ARs (v) is toxic for *C. elegans* even in the developmental L1 stage of larvae. L1 stage larvae were exposed at 0.75 mg/L, 1.5 mg/L, 3.00 mg/L, and 6.00 mg/L of ARs (v). At doses 0.75 mg/L and 1.5 mg/L, the survival percentage was 53.85 ± 8.52 and 4.67 ± 1.83 respectively. However, no L1 stage larvae were survived at higher concentrations (3 mg/L and 6 mg/L) of ARs (v). In our study the observed effects were specific to ARs (v) and not

due to other factors because a) control experiments showed the survival percentage 94.18 ± 2.06 (Figure 1 and Table 1); b) survival percentages are not uniform and they are decreasing with the increase in ARs (v) concentrations.

To investigate the ability of L1 larvae for withstanding the ARs (v) induced toxicity, they were exposed to different concentrations of ARs (v) solution and incubated at 17^0C for 24 hours. We have not checked the survival of larvae in less than 24 hrs of exposure. Therefore, we cannot comment the effects in below time. ARs is a well known carcinogenic agent and observed in food, water, soil and air [11]. It also creates oxidative stress and ARs-induced oxidative stress affects the life of L1 larvae and causes death within 24 hrs of its exposure.

ARs exists in two different oxidative states, i.e. ARs (iii) and ARs (v) and available in organic and inorganic forms. ARs (v) is less toxic as compared to ARs (iii) [7]. Liao and Yu in 2005 showed ARs (iii) and ARs (v) toxicity on adult *C. elegans* and observed the death of worms at different concentrations. Comparing the data from present study and previous study [8], the L1 larvae appeared to be more sensitive to ARs (v) cytotoxicity than that of the adult worms. The mechanism(s) for ARs cytotoxicity is not known and effects of ARs at different stages of *C. elegans* are also not clear. Present work shows the more cytotoxicity in an initial developmental stage of *C. elegans*, however to comment on the mechanism further more investigations are required.

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