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Effect of Arsenic on Early Chick Development

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Abstract

Exposure to arsenic through drinking water is associated with the number of diseases including cancer, Increasing concentrations of toxic metals and chemicals, and their intake by humans and animals have become a major global concern of public health. Presence of these substances in drinking water is the major source of ingestion. Recent studies on pregnant females show the detrimental effects of arsenic ingestion through water. Arsenic causes fetal loss, low birth weight and miscarriages. However, the effects of arsenic using model organism is still understudied. In order to study the effects of arsenic on embryo, we have used chick embryo as model organism. Chick eggs were incubated in incubator at 37 centigrade, after 36 hours of incubation these eggs were taken out to determine the stage of chick embryo by Hamburger-Hamilton stages (HH). The embryos were treated with sodium arsenate dissolved in Phosphate Buffer Saline (PBS-invitrogen) solution. After incubating the treated eggs for 24 hours, the embryos were taken out to observe the effects. Images were taken with Digital camera. The treatment of chick embryos with arsenic caused reduction in the weight and size of embryos treated with sodium arsenate than control (BSS treated) embryos. In addition, the survival rate of chick embryos treated with sodium arsenate was significantly lower. Moreover, defects in angiogenesis were also observed in sodium arsenate treated embryos. This suggests that arsenic might cause the defects of chick embryos at early stages.

Keywords: Audio description, accessibility, character, memory, reception study.

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1. Introduction

Arsenic has been reported to human health hazards [1, 2]. The main source of arsenic poisoning is drinking water, mainly ground water that contains high concentrations of arsenic. A 2007 study found that over 137 million people in more than 70 countries are probably affected by arsenic poisoning of drinking water [3].

Long term exposure to arsenic is related to vitamin A deficiency, which is related to heart disease and night blindness [4]. Arsenic has similarly toxic effects ranging from cancers and nerve damage in humans [5]. Arsenic has been known for same time to have a deleterious effect on developing vertebrate embryo [6]. Although both man and domestic animals may be exposed to a variety of arsenic animal may be exposed to a variety of arsenic compounds only a few such compounds have been investigated with regard to possible prenatal effect [5].

Arsenic can pass placenta and can severely affect embryo. Many reports have been documented to indicate the effects of arsenic on development of embryo. Recent studies on pregnant females show the detrimental effects of arsenic ingestion through water [7]. Many studies have also examined arsenic effect on embryological development to determine the signaling pathways that the toxin impacts. Studies have suggested that arsenic is teratogenic, and it has been shown to cross the mammalian placenta, affecting developing embryos whose mothers were scarified on gestation day 18. Observations were then made of prenatal mortality, fetal malformations and fetal weights. One third of each litter was cleaved and stained for skeletal observations [8]. Exposure to arsenic – contaminated drinking water during pregnancy is associated with low birth weight and fetal loss, and there is concern that the infant's development may be affected [9]. Arsenic causes fetal loss and miscarriages [9].

Chick embryo has been used as a model organism to study angiogenesis either at the stage, when chorioallantoic membrane (CAM) is formed by the fusion of the chorion and allantoic membrane or at the earlier stages from day 3 to day 5, when bleed vessels are formed on yolk sac. CAM is formed on day 7 of incubation. However, yolk sac blood vessels system, which surrounds the early stage embryo, has also been studied to understand angiogenesis. Arsenic has been reported to affect development of embryo in human; however, the mechanism of development has not been studied. The main purpose of this research is to investigate the role of arsenic on development of chick embryo at early stages, particularly focusing on survival rate of arsenic treated embryos and effects of arsenic on angiogenesis

2. Methodology

Chick eggs were obtained from poultry institute Karachi. These eggs were incubated at 37 centigrade in the humidified incubator. After 72 hours of incubation, 3 ml of fluid was taken out of egg and a window was made on the top of egg to observe the development of embryo. Normally developed embryos were used for experiments and all malformed embryos were excluded. The stages of chick embryo development were determined using Hamburger-Hamilton stages (HH). Chick embryos were treated with 100nm of sodium arsenate dissolved in BSS (invitrogen). Control embryos were treated with BSS only (invitrogen). The window of these treated eggs was sealed with transparent tap and the eggs were incubated for 48 hours.

The sealed window was open after 48 hours of treatment (5th day of chick development) and heart beat of embryos were observed to assess the survival rate. Vessels formation was also observed under microscope and photos were also taken using Digital Camera. Embryos were dissected out and washed with Phosphate buffer saline solution (PBS) for weight and height measurements. Observations were also made for any noticeable morphological abnormalities of treated and untreated embryos. Statistical analysis was carried out suing SPSS version 16

3. Results

Treatment with sodium arsenate severely affected the survival rate of embryos. Figure 1 shows the survival rate with control was 88% and with BSS only the survival rate was 72% and the treatment of chick embryos with 200nm sodium arsenate markedly reduced the survival rate to 56%. This might be due to harsh treatment with sodium arsenate. Apart from reduction in survival rate we have also found an obvious reduction in the size and weight of embryo.

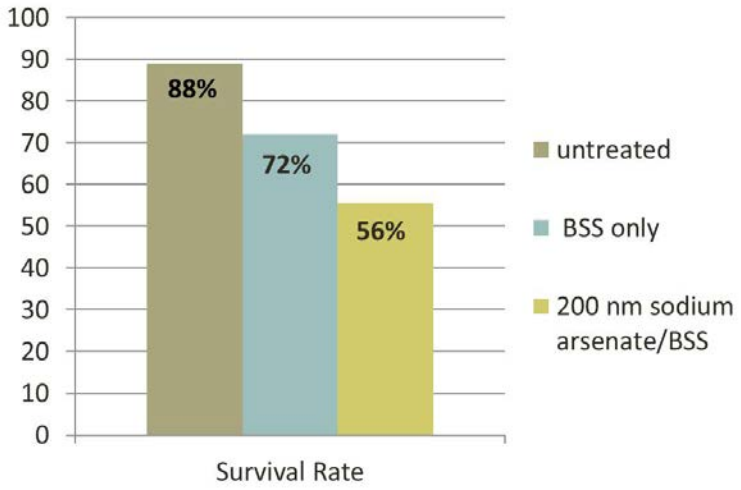

Figure 1.Graphical representation of the survival rate of chick embryos

Figure 2 shows normal angiogenesis in chick embryo treated with BSS only, when these embryos were treated with 100 nm sodium arsenate, we observed defects in angiogenesis (Fig 2 B). There was reduction in number of vessels formed compared with embryos treated with BSS only (Figure 2 A). In addition, there was also difference in the size of vessels, however, we could not determine the size and it needs to be studied in the future. These results suggest that sodium arsenate affects the formation of vessels and this also indicates that decrease in size of embryos might be due to defects in extra-embryonic vessels formation.

4. Discussion

The data we collect shows that arsenic is detrimental for the developing embryo [10, 11]. Number of

epidemiological studies have been carried which indicate that drinking arsenic contaminated water can cause the miscarriages and deaths of human embryo [12, 13], the data we present here confirm that arsenic causes reduction in the size of embryo. Some other studies also have been carried out in other model organisms which indicate the similar results [7, 14]; however our study was focused only on early chick embryo.

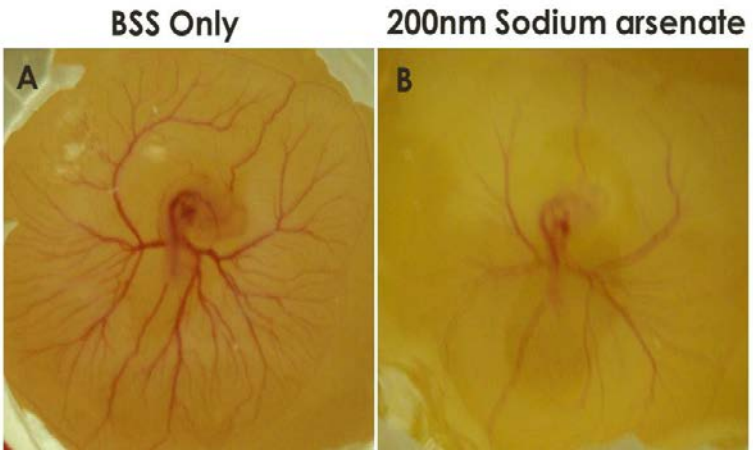

Figure 2. Blood vessels formation in BSS treated and sodium arsenate treated embryos.

We have also found decrease in survival rate this might indicate that embryos exposed to arsenic have increased risk of deaths and many epidemiological studies also are in conformity with these results [15]. This has also been shown that arsenic contamination can cause under growth of embryos [7, 16]. We also have shown that embryos, which were treated with sodium arsenate, have stunted growth and this might be due to defects in the extra-embryonic tissue formation. Chick model is useful for studying the angiogenesis [16], we have shown in our data that arsenic can severely affect angiogenesis, another study have also been conducted which indicate the defects in angiogenesis [16]. These results indicate that arsenic can cause deaths of chick embryo and it can also cause reduction of embryo weight and size.

5. Conclusion

Collectively, our results show that arsenic has harmful effects on developing embryos. In short, we have presented that data which further confirm the epidemiological studies which indicate that arsenic is detrimental for developing embryo. In addition, we have found that arsenic damages the early vasculature of chick embryo.

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