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Original Article

Embryotoxic effects of sodium arsenate in *Mus musculus*

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Abstract

Arsenic level is rising day by day in various parts of the world including Pakistan as a result of zinc and copper smelting and pesticide usage. Inorganic arsenic (sodium arsenate) other than general lethality additionally can be a teratogenic agent. Present research work is focused on the teratogenic impacts of sodium arsenate given to gravid mice (*Mus musculus*). Different doses of sodium arsenate 18.25, 37.5 and 75mg/kg B.W. were administered orally at day 6 of gestation (single exposure group) to 15 females/dose group along with one control group having 15 females, which was given only distilled water. Pups were recovered on 18th day of gestation and fixed for morphometric morphological and histological analysis. Morphometric observations indicated significant ($P \leq 0.05$) variations among groups in certain parameters (body weights, crown rump length, head and eye circumferences) against control. Microcephaly, hygroma, skin hemorrhages, scoliosis and appendage deformities among arsenic treated groups were observed. Histological studies revealed abnormalities like spina bifida, cardiac malformations, miss-happened cochlea and oropharynx. It is concluded that sodium arsenate causes teratogenicity in developing mice.

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INTRODUCTION

Presently concomitant environment and professional exposure of individuals, subject them to heavy metals in different ways (Flora, 2011). However some heavy metals like iron copper etc. is also required in trace amount to perform normal cellular functions. But on the other hand, if the exposure becomes extensive, it can cause cellular damage, inflammation or even cancer (Leonard *et al*., 2004). In nature arsenic (As) is one of the most widely distributed metalloid, which is present in both organic and inorganic forms (Messarah *et al*., 2012). Human beings are exposed to mostly inorganic forms of arsenic *i.e.,* pentavalent As^{5+} and trivalent As^{3+} via drinking water, industrial pollution, mining and food (Tseng, 2009).

In developing countries like Bangladesh, India, Vietnam, Nepal, China, Myanmar and Pakistan arsenic is documented as a big hazard to general public health. In many regions of Pakistan arsenic concentration in drinking water is very high than standard value of 10 ppb (µg/L) set by WHO. In 1990s, high concentration of arsenic was reported in Pakistan's huge water basins e.g. arsenic concentration in Tarbela was reported (620 µg/L), Chashma (750 µg/L) and in Lloyd (620 µg/L) by Ashraf *et al.* (1991). Groundwater of Karachi contained 80 µg/L of arsenic (Rahman *et al*., 1997). In east Punjab, arsenic contamination in groundwater reached upto 1900 µg/L to 2400 µg/L and 91% of trials were observed beyond the standard limits of WHO *i.e.,* 10 µg/L (Farooqi *et al*., 2007a). Literature cited above states that, drinking water with this high contamination of arsenic pose a great risk for human as well as wild life.

Vulnerability to arsenic for human can be either by oral ingestion or by inhalation (Singh *et. al*., 2011). Inorganic soluble forms are being easily absorbed by the gastrointestinal tract from where it can be delivered to blood stream and through blood distributed to body organs and tissues by passing through liver. It commonly accumulates in liver, kidney, central nervous system (Vahidnia *et al*., 2008).Liver is a major site for detoxification of arsenate to metabolize into arsenite and then reduced or

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oxidized to methylated arsenicals in hepatocytes which are excreted through urine (Styblo *et al*., 2002). Arsenate poisioning mainly target liver causing malfunctioning of liver, symptoms include abdominal pain and reduced appetite (Mazumder, 2005). Chronic exposure to inorganic arsenate As^{5+} can produce a number of changes in hepatic morphology like inflammation, apoptosis and necrosis (Xie *et al*., 2004). Human and rodents are at high risk to chemical toxicity during gestation (Anderson *et al*., 2000). Arsenate can cross human and animal placental barrier easily and can also pass through uterine fetal system (Chattopadhyay *et al*., 2002). So animals and human are at potential risk to develop congenital abnormalities when exposed to arsenate (Xie *et al*., 2004).

Due to certain deficiencies in literature regarding arsenate route of exposure, lack of analysis for maternal toxicity, insufficient number of animals in treated groups and scarce results. It is unavoidable to explore its toxicity profile, especially from teratogenic point of view.

MATERIALS AND METHODS

Swiss Webster strain of albino laboratory mice, *Mus musculus,* was used during this research work. These animals was kept under standard protocol of 12-hour day night (circadian) cycles, in 13□× 18□ steel cages. The room temperature was maintained at 27±2°C. They were provided with commercially prepared food pellets, chick feed No.13 chick feed. Familiar males were caged with estrus females overnight. A vaginal plug and/or presence of sperms in the vaginal smear confirm mating and that was considered as day 0 of gestation. Sodium arsenate ($Na_2HAs_4O.7H_2O$) was obtained from BDH laboratories supplies poole, Bh15 1 TD, England. Different concentration of sodium arsenate was prepared in such a way that each 0.1ml of the solution will contain the desired concentration of sodium arsenate. Appropriate doses were prepared and administered that were75mg/kg, 37.5mg/kg and 18.25mg/kg to 15 females. These concentrations of sodium arsenate were administered orally to different groups of pregnant mice at day 6 of gestation (single exposure group). Following this exposure period, the pregnant mothers were anesthetized with anesthetic Ether and fetuses were recovered on day 18 of gestation. Along with dose groups, there was a control group (given distilled water only). The pregnant uteri can be easily dissected out. These were photographed *in-situ* for gross morphological observations. These embryos were weighed and then fixed in Bouin's fixative for 48h. The fixed embryos were transferred to 70% ethanol for further examination. The morphological studies include careful examination of each fetus under stereoscopic binocular microscope so as to record different types of morphological abnormalities of caranio-facial, trunk, limb, tail and the axis of fetuses.

The morphometric studies involved fetal weight, crown rump length, head circumference, eye circumference, length of fore- and hind limbs and tail length. For histological study, fetuses were fixed and processed for sectioning (Ahmad *et al*., 2005), serial paraffin sections were stained by Hematoxylin and Eosin (H&E) using Bancroft ant Layton (2013) protocol. The 3µm sections were studied under a stereoscopic research microscope (LABOMED Luxeo 4D) for their in detail description. Special attention was paid to histopathological signs within the component tissue. These findings were recorded in the form of microphotographs. All quantitative differences between the control and experimental embryos were subjected to statistical analyses on SPSS software version 16.0.

RESULTS

During this study, 15 females were used in each dose group. No. of fetuses recovered from control, 18.25, 37.5 and 75mg/kg were 123, 99, 90 and 85 respectively. Percentage of malformations was 0.00, 21.21, 31.11 and 35.29% in control and different dose groups (Table I).

Morphological studies:

Morphological observations in control group showed normal development of organs with normal size (Fig.1A). Deformities observed in dose group 18.75mg/g B.W. were distorted axis 20.02%, hind limb deformities15.15%, forelimb deformities 18.18%, hemorrhages 10.10%, and microcephaly 2.02% (Fig. 1B-C). Indose group 37.5mg/g B.W. distorted axis 21.11%, fore limbs deformities 17.77%, hemorrhages 27.77%, hind limb deformities 23.33% and runt fetus 3.33% was observed (Fig.1, D-E). In dose group 75mg/g B.W. distorted axis 21.17%, fore limbs deformities

23.52%, hemorrhages 21.17%, hygromas18.82%, runt size 16.47%, hind limb deformities14.11%, scoliosis 2.35%, open eyelid 11.76%, apedia 1.17%, microphthalmia 4.70%, and kinky tail occurred at 12.94% (Fig.1G-H).

Morphometric studies

Morphometric studies showed that crown rump length, eye circumference, brain circumference, forelimb size, hind limb size body weight decreased significantly (P<0.001) (Table II) as compared to control group.

Histological studies:

Histological sections selected from control group exhibited normal development of spinal cord, oropharynx, cochlea, right, left ventricles and atrium of heart, lungs, liver and stomach (Fig. 2A,C,E). While selected histological sections from dose group 75mg/g B.W. revealed spina bifida, degeneration in ventricle of heart, degeneration in stomach, miss happen oropharynx, cochlea and superior vena cava (Fig. 2, B,D,F).

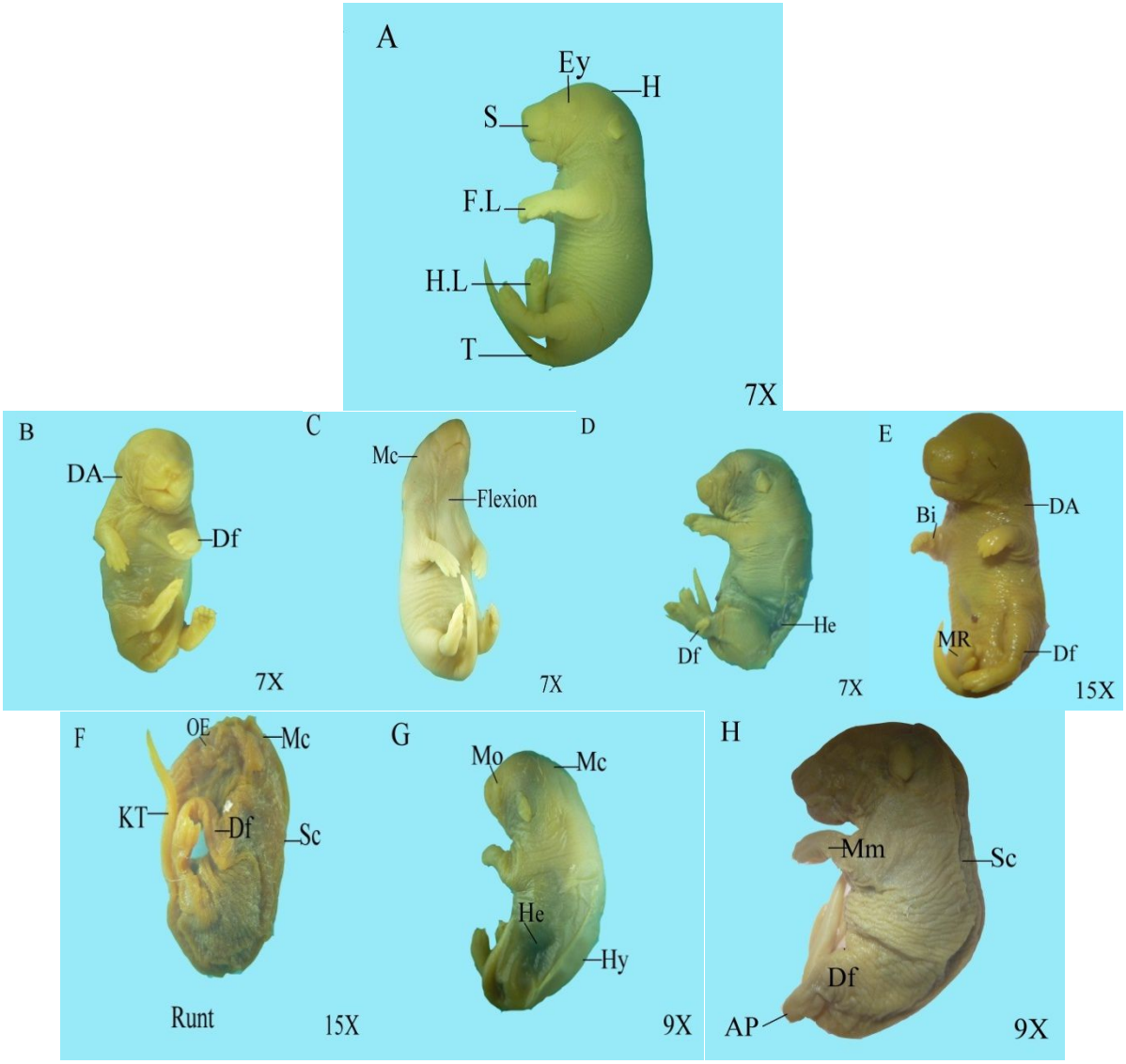

Figure1:A: **Macrophotographs of 18 days old mice fetus recovered from Control group**.
B,C: Macrophotographs of 18 days old mice fetuses recovered from 18.75mg/kg B.W.
D,E: Macrophotographs of 18 days old mice fetuses recovered from 37.5mg/kg B.W.
F,G,H: Macrophotographs of 18 days old mice fetuses recovered from 75.00mg/kg B.W.
H: head, Ey: eye. F.L: forelimb, H.L: hind limb, S: snout, T: tail, DA: distorted axis, Df: deformed, Mc: microcephaly, He: hemorrhage, Bi: bifurcation, MR: malrotation, KT: kinky tail, Sc: Scoliosis, OE: open eyelid, Hy: hygroma, Mo: microphthalmia, Mm: micromelia, AP: A-pedia

Table I: Developmental defects of sodium arsenate on development of 18-days old fetuses recovered from pregnant mice, administered orally with different concentrations on 6th day of gestation

Dose Groups	No. of females used	No of fetuses recovered	% of malformed fetuses
Control	15	123	0.00
18.25mg/kg	15	99	35.29
35.5 mg/kg	15	90	31.11
75 mg/kg	15	85	21.21

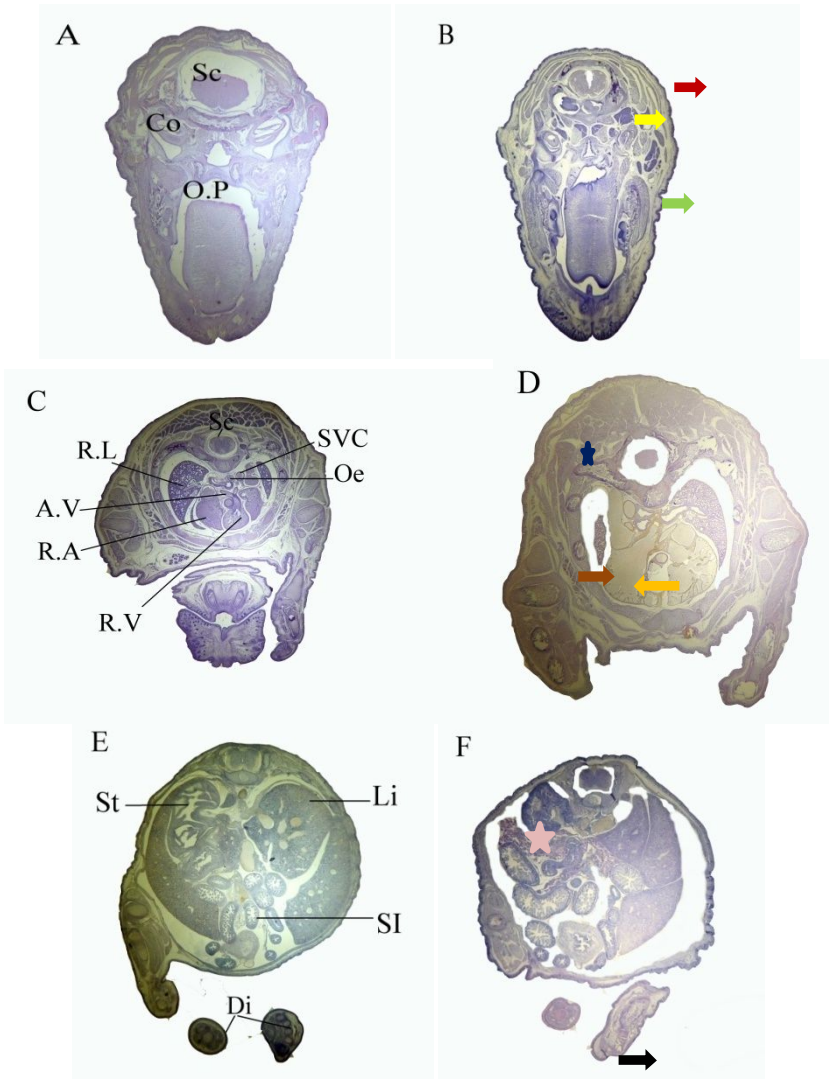

Figure 2: A,C,E:selected sections of 18 days old mice fetuses recovered from control group. Sc: spinal cord, Co: cochlea, O.P: oropharynx, SVC: superior vena cava, R.L: right lung, A.V: atrio-ventricle valve, R.A: right atrium, R.V: right ventricle, Oe: oesophagus, St: stomach, Li: liver, SI: small intestine, Di: Digits. **B,D,F: selected sections of 18 days old mice fetuses recovered from Dose group 75mg/kg B.W**. **Red Arrow**: Spina Bifida, **Yellow Arrow**: Miss happen Cochlea, **Green Arrow**: Miss happen oropharynx, **Blue star**: Miss happen spinal cord, **Brown Arrow**: Miss happen superior vena cava, **Orange Arrow**: degeneration in ventricle, **Pink Star**: Degenerated Stomach, **Black Arrow**: Deformed digits

Table II: Morphometric observations of sodium arsenate on development of 18-days old fetuses recovered from pregnant mice, administered orally with different concentrations on 6th day of gestation

Dose groups	Weight± S.E (mg±S.E)	C.R Length mm± S.E	Fore limb mm± S.E	Hind limb mm± S.E	Head Circum-ference mm2± S.E	Eye Circum-ference mm2± S.E	Tail length mm± S.E
Control	1998±113.28	24.28±1.07	9.23±.31	8.21±.84	21.75±.81	4.68±027	10.88±.28
18.25 mg/kg	1511±66.54*	16.11±.89***	6.88±.36**	6.98±.46	17.19±.86*	2.97±.28***	8.91±.25*
35.5 mg/kg	1304±90.24***	14.02±.82***	6.52±.50***	6.88±.56	14.98±1.11***	2.86±.26***	8.48±.58**
75 mg/kg	1055±160.48***	12.42±1.14***	5.80±.53***	6.19±.52*	14.62±1.49***	2.81±.31***	8.22±.51***

Asterisks show significant difference against controls; ***= P < 0.001, **= P < 0.01 and * = P < 0.05.

DISCUSSION

Exposure to high levels of arsenic (As) causes carcinogenicity, mutagenicity and teratogenicity (Shi *et al.,* 2004). Usually, arsenic in trivalent form cause greater toxicity than pentavalent arsenate. But both trivalent and pentavalent compounds are thought to contribute to both arsenite and arsenate exposure because pentavalent arsenate is metabolized into trivalent arsenite (Hill *et al*., 2008). Birth defects are the major cause of deaths in new borns (Ahir *et al*., 2013). According to some researches from the past sodium arsenate is teratogenic (Leonard and Leuwerys, 1980), but the data about to arsenate teratogenicity is not well established only few studies regarding its potential to produce birth defects are not conclusive. The outcomes of the present studies showed that sodium arsenate when given orally to pregnant mothers, the size of the pups was decreased and the rate ofresorptions were increased with the increase in dose concentration and exposure time. These results are in accordance with Hopenhayn *et al*. (2003)**.** They performed a cohort study in cities where arsenic contamination in water, was very high and the birth weights of children reduced significantly. Exencephaly and microcephaly observed in developing mice during this study was relatively in high ratios is compliance with DeSesso *et al.* (2001) who studied neural tube defects anencephaly in hamsters when treated with sodium arsenate. Deformities in skeleton, and vertebral defects such as kyphosis and distorted axis were noticed during current studies, which are in conformity with Beaudoin (1974). Histopathologically, neural tube defects such as spina bifida observed which are in accordance with Ahir *et al*. (2013) who reported craniofacial abnormalities like microcephaly as well as neural tube defects like spina bifida and anencephaly in chick embryos. During present research, cardiac malformations like degeneration in ventricle of heart were perceived which is in agreement with Spiegelstein *et al*. (2005). They studied that by exposure of sodium arsenate to pregnant mice, folatetransport deficiencies take place which cause cardiac and neural tube malformations. Human presentation to arsenic amid pregnancy shows up ready to increase. Arsenic is at present a critical part of industries worldwide. Taken together, the universal way of arsenic, the affectability of people to arsenic poisonous quality, and the teratogenicity of maternal oral arsenic introduction ponder a potential linkage between human arsenic introduction and hoisted dangers for neural tube and other inborn imperfections. This gives sound reason to perform more complex human epidemiological studies to figure out whether ecological arsenic introduction represents a teratogenic risk to uncovered human populations.

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