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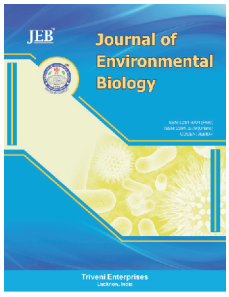

Protective effect of folic acid against arsenic induced weight loss during gestation, lactation and postnatal growth in mice

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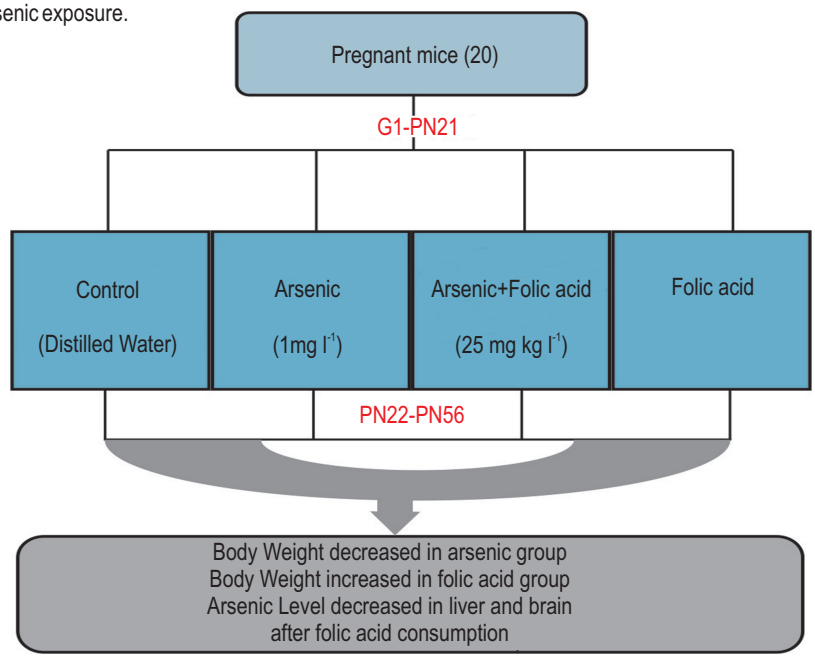
Abstract

Aim : The present study aimed to determine weight loss in sodium arsenate exposed pups during gestation, lactation and postnatal growth. Additionally, the protective role of folic acid was also assessed against impaired growth caused by arsenic during three developmental stages.

Methodology : Twenty pregnant albino mice were divided into four groups and received the following treatment in their drinking water from gestational day one (G1): control group, 1 mg l^{-1} As 1 mg l^{-1} As + 25 mg kg^{-1} FA (As + FA) by ip once a week during gestation and lactation and 25 mg kg^{-1} folic acid (FA) groups. After weaning, the litters were directly exposed to arsenic and folic acid with the same dosage.

Results : Exposure to arsenic exhibited a significant ($P<0.01$) growth impairment in the weight of offspring during lactation (1^{st}–3^{rd} week) and 22–56 ds postnatal (PN) in the As-treated group. The administration of FA through gestation and lactation by dams after 2 months could significantly ($P<0.01$) prevent As-induced weight loss in the developmental stages. Treatment with FA resulted in significantly decrease ($P<0.05$) of AS level in liver and brain.

Interpretation : These observations indicate that vitamin administration could prevent growth impairments of arsenic exposure.



Introduction

Climate Chronic arsenic exposure causes a wide range of health issues such as cancer, developmental toxicity, cardiovascular and brain dysfunction in children and young adults. This old and naturally occurring poison has a long history of toxicity in food, water, soil and air (Hughes *et al.,* 2011). It is widely present in herbicides such as organoarsenical (Chen *et al.,* 2008). Micronutrients supplements, such as folate, have protective role for decreasing some of the developmental toxicity of arsenic. Folic acid is a source for generation of methionine, facilitates the methylation process and increases arsenic secretion in urine and relieve its toxicity (Hall and Gamble, 2012). It has been reported that maternal pretreatment with folic acid can prevent fetal death and intrauterine growth restriction induced by lipopolysaccharide in mice. Protective mechanism of folic acid is related to anti-inflammatory effects and reduction of interlukins and cytokins in amniotic fluid (Zaho *et al.,* 2013). Another animal study established that folate deficiency diet during lactation resulted in decreased body weight of offspring on third week of lactation compared to normal diet consumption in rats (Berrocal-Zaragoza *et al.,* 2014).

Mice treated with 10 ppb arsenic in their drinking water, during the embryonic and postnatal period, induced significant impairment of growth in the early period of development after birth, as a result of decreased nutrients in the dam's breast milk (Kozul-Horvath *et al.,* 2012). The beneficial effects of breast feeding have been previously demonstrated, but the nutritional quality of donor breast milk is important. Other investigation has been revealed that low levels of arsenic were detected in an analysis of breast milk samples and urine from infants whose mothers lived in areas with high exposure to arsenic. Although arsenic passes through the milk, it has been indicated that breast feeding protects the infant from the hazards of arsenic (Fängström *et al.,* 2008). There are numerous contradictory results on the effects of exposure to arsenic early in life. However, it is uncertain whether breast feeding leads to reduced infant exposure to arsenic.

Recent studies have focused on the protective effects of antioxidants against As-induced organ toxicity. Folic acid is a water-soluble antioxidant that influences the metabolism of arsenic via methylation. This vitamin plays an important role in growth, development and cell function. Therefore, folic acid is required for fetal growth and contributes highly to both prenatal and postnatal development ((Liu *et al.,* 2016). In this study, for the first time, growth impairment due to arsenic exposure was examined in three developmental periods from postnatal days 1–56. The protective effect of folic acid against arsenic toxicity has also been reported.

Materials and Methods

Animal study : Twenty female BALB/C albino mice (10 weeks old, 30–70 g) were procured from the Urmia University of Medical Sciences (UMSU) Iran. The animal experiments were started after approval from Ethical committee of (UMSU) Iran. All mice were housed in cages at standard conditions (12/12 hr light and dark and temperature of 20°C and 50-60% humidity).

Experimental design : For the purpose of mating, three female mice were kept overnight with a single male. Following the detection of vaginal plugs, pregnant animals were randomly divided into four equal groups (n=5) and were marked as control, As, As + FA and FA. Dams in the control group received drinking water through gestation (G1–G21 ds) and lactation periods (PN1-PN21). The As group received drinking water treated with 1 mg l^{-1} As through gestation and lactation. One mg As was dissolved in one liter of drinking water and solution was prepared fresh each two days.

The dose of one mg was selected based on permissible limit of arsenic (0.01 mg l^{-1}) reported by EPA (EPA, 2001). The As + FA group received 1 mgl^{-1} As in their drinking water plus 25 mg kg^{-1} folic acid (Gefrides *et al.,* 2002) *via* i.p. injection on the first morning of each week through gestation and lactation. The folic acid group received drinking water plus same dose of folic acid. After weaning (PN21), the litters were directly exposed to water, arsenic and folic acid with the same dosage. The birth weight of the pups was recorded and subsequently, individual weights were taken weekly during lactation (1^{st}-3^{rd} week). The weight of the pups was then taken weekly until the eighth week of life, up to day 56 (PN56).

Estimation of arsenic content : Froze liver and brain (50 mg) were homogenized in 2.0 ml of distilled water then mixed with one ml of three normal solforic acid overnight. Tissues were digested in microwave for 10 min in a 10 ml test tube and finally mixed with sodium tetrahydroborate at pH 2 to volatile arsin generation. Arsenic content was measured by automicabsortion spectrophotometer (Perkinelmer 900H) at 193.3 nm wavelength and compared with five different concentrations of standard samples.

Statistical analysis : The experimental data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 16.0. The mean and standard error of the mean (SEM) were calculated for the weight. ANOVA was applied to assess the significance of the difference in birth weight as well as comparing the mean of arsenic level among the groups. A repeated measure ANOVA was applied to assess the difference in the mean weight through the lactation period (PN1- PN21) and after weaning (PN22-PN56). A P value of 0.05 was considered statistically significant.

Results and Discussion

The number of offspring was 6-10 per dam in the control and experimental groups. The total number of litters and average weight in the four groups were as follows: 35 litters in the control group (1.1286 ± 0.12735 g), 33 litters in the As treated group (1.0576 ± 0.11734 g), 34 in the As + FA treated group (1.6824 ± 0.12903 g) and 37 in FA treated group (1.72341 ±0.12651). In

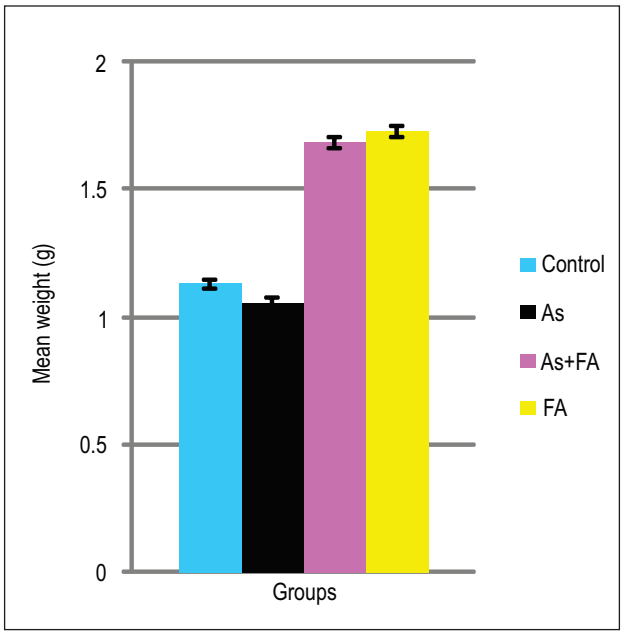


Fig. 1 : Comparison of birth weight of litter. All values are expressed as mean ± SE (SEM). The mean difference between As and control group NS-not significant at P<0.05. ANOVA showed a statistically significant increase in the mean litter weight in As+FA and FA groups compared to control and As at P<0.01

comparison with the control group, there was no significant difference in the birth weight of pups in the As group. When the mean birth weight of the As + FA and FA groups were compared with the control, the birth weight in the litter seemed to increased. In addition, the difference of the mean litter weight of the As +FA and FA groups were statistically significant when compared to control and As groups (Fig. 1).

The present finding is consistent with the previous studies Ramsey *et al.* (2017) reported that gestational arsenic exposure to 0.1 mg l^{-1} had no significant effect on offspring weight. In an another study the mean fetal body weight was not affected by As exposure (1.5, 3 and 4.5 mg kg^{-1} day^{-1}) between gestation day 8 until birth. Significant growth deficits only have been observed with a high dose of arsenic exposure during embryonic period in a mouse model (Gardner *et al.,* 2013). The administration routes of inorganic arsenic are important in determining the adverse developmental effects. Fetal malformations and growth deficiencies were reported when pregnant rats and mice were iv or ip injected with inorganic As during early gestation, as it could be taken up by the blood vessels directly (DeSesso, 2001).The filtration role of placenta is related to fetal-maternal barrier of this organ, which reduces the passage of harmful environmental contaminants (Caserta *et al.,* 2013). The offspring in the As + FA group showed a significant increase in weight compared with the As group. The mechanisms associated with the effect of FA on growth are not fully understood. Studies have suggested that hepatic insulin-like factor 2 (IGF2) expression was unregulated by injecting 150 µg FA into fertile eggs on E11, therefore, embryo

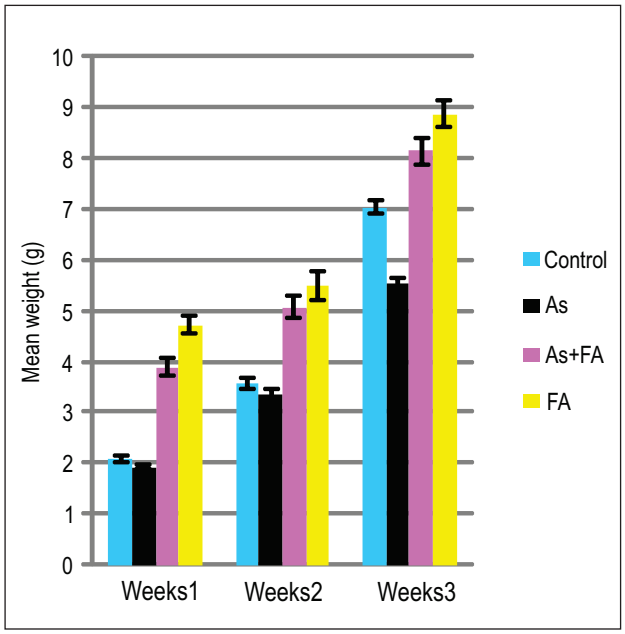


Fig. 2 : Comparison of weight of litter during the lactation period. All values are expressed as mean ± SEM. A repeated measure of ANOVA showed a statistically significant decrease in the mean litter weight in all groups at P<0.01. There was a significant increase in the body weight of litters in both As+FA and FA groups at P<0.01

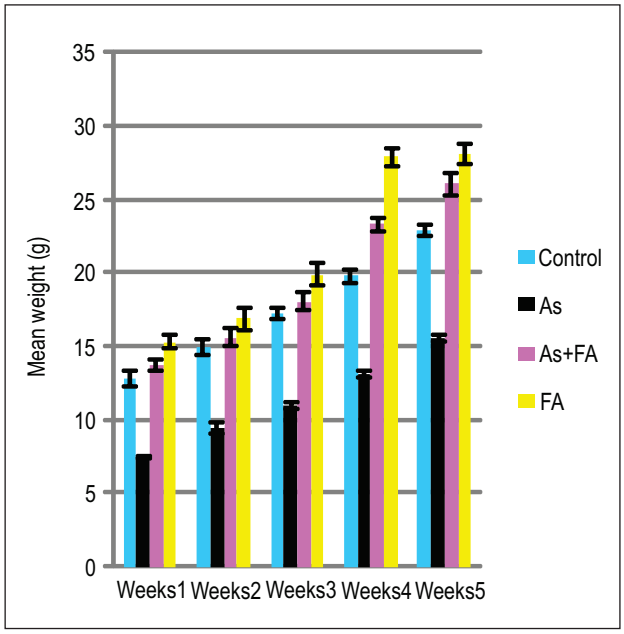


Fig. 3 : Comparison of weight of litter during postnatal days 22–56. All values are expressed as mean ± SEM. A repeated measure of ANOVA showed statistically significant decrease in the mean litter weight in all groups at P<0.01. There was a significant increase in the body weight of litters in both As+FA and FA groups at P<0.01

growth has a positive relationship with hepatic IGF2 expression, cell proliferation and DNA methylation (Liu *et al.,* 2015). One carbon transfer component like folate can transfer from mother to fetus blood circulation and cause cell proliferation, increase of protein mass and fetal growth (Kalhan, 2016).

A decrease in body weight continued throughout the lactation period until weaning at day 21 in the As group. Prominent weight loss started at the end of second week. A repeated measure of ANOVA showed a statistically significant (P<0.01) decrease in the mean litter weight compared with the control group. There was a statistically significant difference in the mean body weight during lactation between the folate groups compared to the control and As groups. There was a marked increase at the end of the lactation period in the folate groups when compared with the As group. However, weight gain in the As group was significantly lower compared with the other three groups (Fig. 2). Therefore, inorganic As is extremely toxic and has negative effects, including decreased body weight of the offspring through the lactation period (PN1-PN21). Growth impairment has been reported in a similar mouse study with lower and much higher doses of exposure (Kozul-Horvath *et al.,* 2012). Epidemiological studies have reported low concentrations of arsenic in breast milk samples of women living in areas prone to arsenic in water. A recent report also suggests that arsenic exposure via breast milk was low, because breast barrier can limit the transfer of arsenic into the mother milk (Carignan *et al.,* 2016). A review of 18 published studies since 2000 to 2016 have reported the highest concentration of arsenic (149 µg l^{-1}) in West Bengal, India, which was much lower in urine with a mean concentration of 438 µ gl^{-1} (Rebelo and Caldas, 2016). Although a low level of As is detected in breast milk, it has been reported that there was a decrease in the nutrient content of dam's breast milk, specifically in the triglycerides, which play a role in growth deficits (Kozul-Horvath *et al.,* 2012). A recent study suggests that a daily intake of folic acid was associated with breast folate concentration (Page *et al.,* 2017). The vitamins in breast milk can protect offspring against the negative potential of arsenic and likely improve growth by increased feeding and efficiency in feed utilization. Dietary folic acid intake significantly increased digestive enzyme such as lipase and tripsin activities, resulting in enhanced feed intake (Sesay *et al.,* 2016). It has been reported that folic acid supplementation increased growth markers such as weight gain, nutritional demands and survival (Barros *et al.,* 2009).

The weight gain of the litters in the folic acid group was highest. In the As treated group, the weight gain was lowest from the 4^{th}- 8^{th} week (7.44, 9.8069, 11.0793, 13.1103 and 15.6 g). The weekly i.p. in jection of folic acid (25 mg kg^{-1}) increased the weight of the litters from the 4^{th}- 8^{th} week of life (13.7437, 15.9281, 18.0781, 21.7375 and 24.1656 g). Litters in the As + FA and FA groups showed a consistent progressive increase in weight gain over the 5- week period. There was a significant increase (P<0.01) in weight gain of folate groups compared with those obtained in the As and control groups. The protective effect of folic acid against the negative effects of arsenic is given in Fig. 3.

The weight of the litter of the arsenic group decreased in the present study following a direct administration of arsenic in the drinking water five weeks after weaning. Gardner *et al.* (2013) reported severe health effects of elevated arsenic exposure in children, mainly in the form of growth. Decreased metabolic activity in the liver, muscle, kidney and lungs due to gradual deposition of As could be the main reason for decrease in weight. In present study, accumulation of arsenic was observed in liver and brain tissues. Increase of arsenic in different organs affects the metabolic and functional activities of these organs, which in turn, leads to a weight reduction (Roggeman *et al.,* 2014). It has been reported that even low doses (one µm) of arsenic can inhibit skeletal myoblast cell proliferation and decrease some protein expression and insulin-like growth factor-1 essential for growth (Liu *et al.,* 2015). In present study, the protective effect of folic acid on growth has been demonstrated. Over past decades, several experiments have been conducted to test the effects of protecting elements on As induced toxicities. The effects of barberine against As induced mitochondrial membrane rupture and decreasing reactive oxygen species (Khodayar, 2016) as well as the role of Curcuma to diminish the renal dysfunction have been reported (Saxena, 2009). The effects of folic acid administration might be due to protection from mutagenic DNA-breakage and tissue damage as well as antioxidant potential against As induced free radical products (Acharyya *et al.,* 2015). An earlier study revealed that after supplementation with folic acid for 12 weeks, there was an increase in urinary As, excreted as dimethylarsinic acid and decreased blood As concentration. Folic acid protection against As-induced apoptotic progression has been documented in a cell culture study where folic acid supplemented medium reduced As-induced toxicity and apoptosis (Xu *et al.,* 2010). The beneficial role of folic acid in reducing the growth inhibitory effects of As may be due to its antioxidant potential and ability to remove As from the blood by the acceleration of As metabolism and excretion in urine.

Level of sodium arsenate was measured in liver and brain of mice on PN56. The As level was very low in control and folic acid groups compared to As exposure groups (Table 1). This study reveals that weekly injection of folic acid could significantly

Table 1 : Sodium arsenate concentration in liver and brain in four treatment groups

Tissues	Control	As	As+FA	FA
Liver (µg g^{-1} tissue)	.00947±.0000375	.022000±.0066833	.014950±.0011000[a]	.011000±.0000816[a]
Brain (µg g^{-1} tissue)	.00768±.0000472	.016500±.0036968	.011925±.0017576[a]	.00605±.0000823[a]

All values are expressed as mean ± SEM .The mean sodium arsenate level was significantly decreased in As+FA and FA groups compared with As group at P<0.05

decrease the As levels in liver and brain tissues. After ingestion, arsenic is quickly absorbed by the gastro intestinal tract and is transferred to the liver in a short time and then immediately methylated in liver (Rodriguez *et al.,* 2004). Wang *et al.* (2013) reported the presence of As in brain and liver after subchronic exposure of sodium arsenate. Arsenic concentration in the brain of mice was lower than liver, probably due to the presence of blood-brain barrier. It is obvious that maternal diet has an important role in pre- and post-natal development. Long term exposure to arsenic through drinking water during embryonic, lactation and post-weaning periods induced growth deficits in the offsprings, as well as, increased As accumulation in liver and brain tissues. Folate administration confers significant protection probably by facilitating of As excretion and preventing its accumulation in organs.

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