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Folic Acid Intervention in Pregnancy Attenuates Arsenic-Induced Renal Toxicity: Insights from An In-Utero Exposure Animal Model

Vaishali Bhagwani, Pharmaceutical Science, Asian International University, Imphal, Manipur
Prof. (Dr.) Anup Kumar Sirbaiya, Asian International University, Imphal, Manipur

Abstract

Exposing the fetus to arsenic before birth has a tremendous implication on the development of the fetus especially those organs that are very delicate during the development period like the kidneys. In this study, maternal folic acid supplementation to mitigate neonatal arsenic-induced renal toxicity was examined. The pregnant Wistar rats were placed into control, folic acid, arsenic-exposed, and arsenic and folic acid groups and treatments were done during gestation. The biochemical analysis revealed that arsenic exposure significantly increased the urea and creatinine levels whereas Folic acid supplementation returned them to normal range. Measures of oxidative stress found that neonates exposed to arsenic had high lipid peroxidation and lower antioxidant enzyme response, and folic acid greatly enhanced oxidative balance. Pathological examination also revealed that there was severe tissue destruction of the kidney in arsenic-treated offspring and this was significantly lower in the folic acid-treated group. In general, the results suggest that folic acid provides significant biochemical, oxidative and structural protection against prenatal arsenic-induced nephrotoxicity thereby making it a possible maternal nutritional intervention in arsenic-affected areas.

Keywords: Folic acid, Arsenic toxicity, Prenatal exposure, Renal oxidative stress

1.INTRODUCTION

Exposure to environmental toxicants during pregnancy is becoming a critical issue in the world with low resource regions being the worst calamities, both in groundwater pollution and nutritional inadequacies. The formation of organs, in particular, kidney, is susceptible to even low dose toxic attacks that can interfere with the development of structural formation, as well as long-term physiological performance. Determining the low-cost and available maternal interventions that can overcome such toxicity is consequently essential in enhancing the outcome of fetus health. The application of such nutrients as folic acid in many maternal health programs can provide oxidative and developmental protective effects; nevertheless, the effects of folic acid in the prevention of arsenic-induced nephrotoxicity have not been thoroughly studied.

1.1 Background on Arsenic Exposure and Developmental Toxicity

Contamination of drinking water with arsenic is a common environmental health problem that exposes millions of people to contaminated groundwater that occurs naturally. The harm of prenatal arsenic exposure is particularly great because early filtration/metabolic regulation is a task of the kidney of the fetus, and in the context of organogenesis, it is highly sensitive. Arsenic causes oxidative stress, affects development of nephrons, disturbs glomerular morphology and predisposes to chronic renal dysfunction in adulthood.

1.2 Folic Acid as a Prenatal Protective Nutrient

Folic acid (FA) is a B-vitamin that is necessary in the synthesis of DNA, cell growth, methylation of reactions, and antioxidant defense. As much as the need to prevent neural tube defects is strongly advised, emerging evidence indicates that FA could also increase detoxification through promoting arsenic methylation pathways and decreasing oxidative stress. Although these may be the potential benefits, there is scanty research on whether maternal FA supplementation has the potential to protect fetal renal tissues against biochemical and structural changes caused by arsenic.

1.3 Research Objectives

To achieve a comprehensive understanding of the protective role of folic acid against arsenic-induced nephrotoxicity during fetal development, the study outlines the following specific research objectives:

- To evaluate the impact of prenatal arsenic exposure on renal biochemical parameters in

neonatal rats and determine whether maternal folic acid supplementation can restore these markers.

- To assess the influence of maternal folic acid administration on oxidative stress levels in the kidneys of arsenic-exposed offspring by measuring key antioxidant and lipid peroxidation indicators.
- To examine histopathological alterations in renal tissues resulting from prenatal arsenic exposure and determine the extent to which folic acid mitigates structural damage.

2. REVIEW OF LITREATURE

The previous research had already conducted a lot of research on the toxicological effects of the exposure to arsenic and protective effects of nutritional or biochemical interventions.

Chattopadhyay et al. (2019) have shown that sodium arsenite caused a considerable rise in the production of reactive oxygen species (ROS) and the activation of NF-kB-mediated inflammatory pathways in the tissues of the uterus. Their work also demonstrated that probiotics mitigated the effects of arsenic on oxidative stress through the regulation of vitamin B12, maintenance of lactate dehydrogenase, and inhibition of NF -KB activity. This research was the first indication that dietary supplements may reverse the arsenic-induced molecular damage.

Dey et al. (2021) also found that arsenic resulted in severe apoptotic degeneration in uterine tissues of Wistar rats due to the impairment in the Bcl-2/BAX pathway and the NF-KB system. They claimed that the ethyl acetate fraction of the Camellia sinensis was also effective in treatment of anti-apoptotic balance and tissue injury induced by arsenic. Their results supported the gathered opinion that natural compounds with antioxidant properties may have quantifiable therapeutic effects in response to arsenic toxicity.

In a rigorous Cochrane systematic review, Farinola et al. (2021) evaluated the effects of folic acid in mitigating arsenic toxicity in both children and adults who are exposed to the substance. They have indicated that the efficacy of folic acid supplementation was to improve the effectiveness of arsenic methylation to facilitate excretion of poisonous arsenic metabolites. Their results implied that folic acid would be a plausible type of public health intervention in the populations impacted by arsenic, especially when the nutritional deficiencies were widespread.

Albishtue (2018) **reported** that edible bird's nest possessed potent ameliorative properties that **had significantly improved** reproductive performance in female rats exposed to lead acetate. The study **demonstrated** that lead toxicity adversely **affected** ovarian function, hormonal balance, and overall fertility parameters, whereas supplementation with edible bird's nest **helped restore** reproductive physiology by enhancing antioxidant capacity and reducing oxidative tissue damage. These findings **suggested** that nutraceutical compounds with antioxidative potential **had played** a crucial role in mitigating heavy-metal–induced reproductive impairments.

In a related line of inquiry, Cheong and Nagel (2022) **examined** human variation in DNA repair pathways, immune responses, and associated cancer risk. Their research **highlighted** that interindividual differences in genomic stability and immune function **had influenced** susceptibility to environmental toxins and disease onset. The authors **emphasized** that compromised DNA repair mechanisms **had contributed** to greater genomic damage, increased inflammatory responses, and elevated cancer predisposition. This body of evidence **indicated** that both environmental exposures and biological repair capacity **had been critical** determinants of overall reproductive and systemic health.

3. Materials and Methods

The current experiment used a controlled in vivo experimental design to examine the protective effect of maternal folic acid supplementation against renal toxicity caused by arsenic to the neonatal rats. Each process was designed in such a way that it created accurate exposure conditions, dependable bio-chemical measurements and consistent histological results. The methodology then was developed in such a way that it offers a complete assessment of changes

in both the functional and structural transformation of the kidneys upon in utero exposure hence allowing the nephroprotective effects of folic acid to be accurately determined.

3.1 Experimental Animals

Wistar rats, which were adults with a weight of 180200 g, were used in the study. All the animals were kept under the usual laboratory conditions so that they would be uniform during the time of the experiment. The animals were kept in polypropylene cages with the controlled environmental conditions such as a temperature of 22 ± 2 0 C, relative humidity of 50-60 g, and a 12-hour light dark cycle. A basic pellet laboratory diet was given to them and clean drinking water was given ad libitum. All the procedures were done in compliance with the institutional ethical principles of the use and taking care of laboratory animals.

3.2 Experimental Design

Once the rats were found to be pregnant, they were randomly grouped into four which comprised of six rats per group. The control group was fed on normal diet and distilled water, and the FA group was fed on folic acid by administering 5 mg/kg/day. The third group received sodium arsenite (10 mg/kg/day), which is the state of exposure to arsenic. A combination of sodium arsenite and folic acid was given to the fourth group to determine the protective effect of folic acid in response to arsenic-induced stress. The treatments were all conducted orally on a gavage needle between gestational day (GD) 1 to GD 21, so that there would be the same exposure during the period of organogenesis and fetal development.

3.3 Sample Collection

The neonatal rats were sacrificed humanely in the first 24 hours after birth to prevent the effect of the environment after birth. After the euthanasia, the kidneys were moved meticulously, rinsed in ice-cold saline, and their weight taken. The homogenates were then subjected to biochemical measurements by means of proper buffers and processed at once. The other kidney samples were fixed separately to undergo histopathological studies to determine the structural and cellular alteration by the exposure of arsenic and the effects of folic acid supplementation.

3.4 Biochemical Analysis

Biochemical analysis was done to determine the functional status of kidney and oxidative stress. To identify the extent of renal toxism, serum and kidney tissue homogenates were studied on the markers of renal functions such as urea and creatinine. The activity of the key antioxidant enzymes including superoxide dismutase (SOD) and catalase (CAT) was measured as the index of oxidative stress, as well as the malondialdehyde (MDA) index of lipids peroxidation. All the parameters were determined by previously commercially available standard colorimetric assay kit, according to the manufacturer instructions to guarantee reliability and reproducibility.

3.5 Histopathological Examination

To analyse the kidney tissues using a microscope, kidney tissues were fixed using 10% neutral-buffered formalin, dehydrated, embedded in paraffin blocks, and sectioned at a thickness of 5 µm. The areas were stained with hematoxylin and eosin (H&E) to observe the renal structure. Microscopy aimed at the detection of structural changes such as glomerular integrity, degeneration of the tubular epithelials, inflammatory cell inflammation, vascular congestion and necrotic lesions. These observations offered morphological support of the damage induced by arsenic and the level of protection recovery after the administration of folic acid.

3.6 Statistical Analysis

All the numerical data was given in terms of mean and standard deviation (SD). To compare experimental groups statistically, one-way analysis of variance (ANOVA) was conducted and further, Tukey post hoc test was conducted to ascertain the intergroup significance. The cut-off point of 0.05 was taken to be statistically significant. The statistical methods have been used to make sure that the differences that are observed are significant and can be traced back to the experimental treatments.

4. RESULT

Evaluations of the current study were done by the use of biochemical analyses, the oxidative

stress analysis and histopathological analysis to ascertain the severity of arsenic induced renal toxicity and the protective capability of maternal folic acid supplementation. Comparative studies that were done on the four experimental groups indicated that there are strong variations between the groups in the renal function markers, antioxidant activity, and morphology of the tissues. These findings all indicate that prenatal exposure to arsenic negatively affected the kidney structure and functioning in newborns, whereas administration of folic acid afforded some significant restorative effects.

4.1 Maternal FA Attenuates Arsenic-Induced Biochemical Alterations

The presence of arsenic shows that there is a significant rise in serum urea and creatinine in the presence of arsenic than in the control group, meaning that the renal functionality is not normal. MFM folic acid supplementation significantly reduced these changes with As+FA group showing nearly the same values as the control group.

Table 1. Effect of Treatments on Renal Biochemical Parameters (Mean ± SD)

Group	Urea (mg/dL)	Creatinine (mg/dL)
Control	25.4 ± 2.1	0.42 ± 0.03
FA	24.8 ± 1.9	0.40 ± 0.02
As	42.6 ± 3.5	0.78 ± 0.05
As + FA	29.3 ± 2.4	0.48 ± 0.03

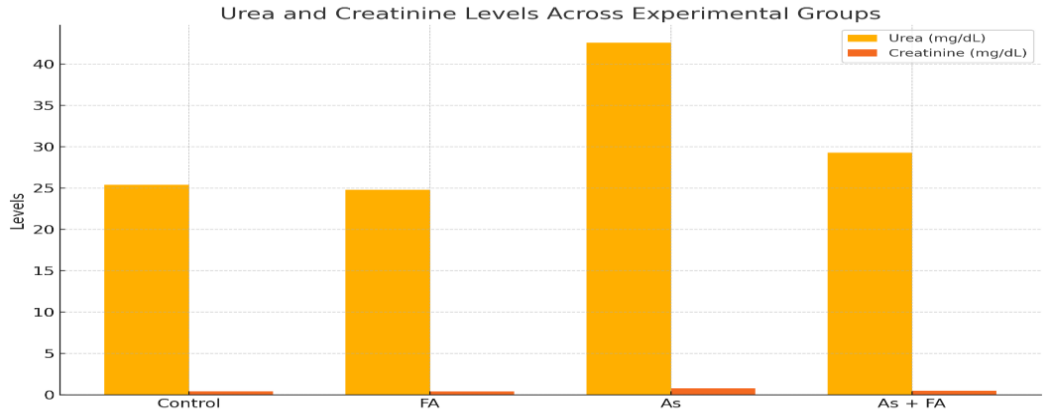

Figure 1: Graphical Representation of Urea and Creatinine Levels Across Groups

4.2 Folic Acid Reduces Oxidative Stress in Neonatal Kidneys

The prenatal exposure to arsenic led to significant increase of MDA correlating to lipid peroxidation and reduction of both the SOD and CAT enzyme activities. These oxidative changes were reversed by folic acid supplementation.

Table 2. Oxidative Stress Parameters in Renal Tissue (Mean ± SD)

Group	MDA (nmol/mg protein)	SOD (U/mg protein)	CAT (U/mg protein)
Control	1.52 ± 0.10	8.12 ± 0.45	16.4 ± 1.2
FA	1.48 ± 0.12	8.30 ± 0.50	17.0 ± 1.1
As	3.95 ± 0.20	4.15 ± 0.30	9.3 ± 0.8
As + FA	2.01 ± 0.15	7.55 ± 0.40	14.8 ± 1.0

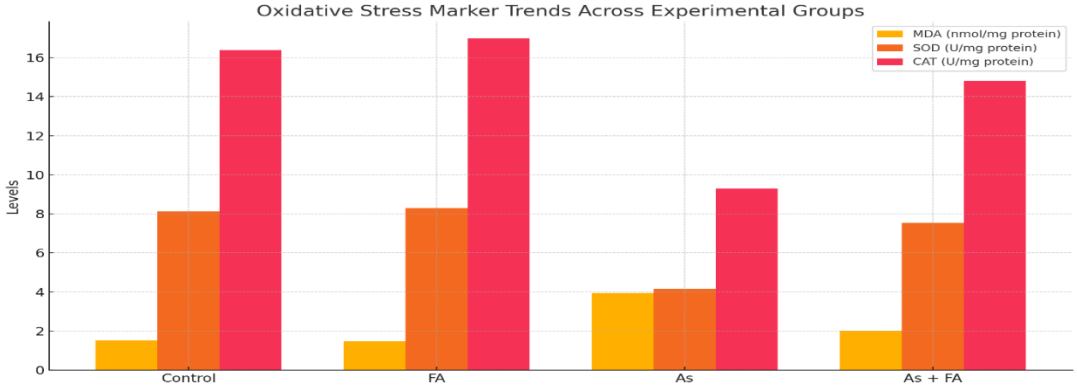

Figure 2: Oxidative Stress Marker Trends Across Experimental Groups

The graph shows that arsenic exposure was associated with a drastic rise in MDA and a fall in the levels of antioxidant enzymes, SOD and CAT; folic acid supplementation partially restored these levels to normal physiological levels.

4.3 Histopathological Restoration by Folic Acid

Most of the neonates with arsenic exposure underwent extensive renal injury as evidenced by the destruction of tubular epithelial cells, glomerular atrophy, inflammatory inflammation, and vascular congestion as observed in histological analysis. The As+FA group on the other hand had a significantly better renal architecture with little degeneration and almost normal glomerular architecture.

Table 3. Histopathological Scoring of Renal Tissue Alterations (0 = None, 1 = Mild, 2 = Moderate, 3 = Severe)

Parameter	Control	FA	As	As + FA
Tubular degeneration	0	0	3	1
Glomerular shrinkage	0	0	3	1
Inflammatory infiltration	0	0	2	1
Vascular congestion	0	0	3	1

Using microscopic analysis, it was established that folic acid co-treatment significantly decreased the arsenic-induced histopathological abnormalities and aided in the maintenance of the renal structural integrity.

5. DISCUSSION

The results of this experiment established that prenatal exposure to arsenic led to severe biochemical, oxidative, and structural disruption in the kidney of the neonatal rats whereas maternal folic acid supplementation was of significant protective value. The significant rise in the serum level of urea and creatinine in the arsenic-treated group had shown evidence of compromised renal filtration and an initial instance of nephrotoxicosis. Such a large decrease in these indicators in the folic acid co-treated group indicates that folic acid was efficient in restoring renal functional balance, presumably via the reinforcement of cellular repair systems and general metabolic homeostasis. This protective role was also supported by the oxidative stress measurement. Exposure to arsenic increased levels of MDA and lost some major antioxidant enzymes including SOD and CAT, which are indicative of increased lipid peroxidation and compromised antioxidant capabilities. Female folic acid supplementation reversed these changes because it reduced oxidative stress and induced antioxidants. It means that redox balance can be maintained with the help of folic acid in the course of fetal development, and the vulnerability of renal tissues to reactive oxygen species should be decreased.

The histopathological findings were consistent with the biochemical and oxidative results. Structural deviation was evident in arsenic exposed babies with tubular degeneration, glomerular atrophy, inflammatory infiltration, and congestion of the vessels. These were pathological characteristics of renal injury caused by arsenic. In comparison, most of the arsenic treated with folic acid group had significant saving of renal architecture, little tubular damage, and nearly normal glomerular morphology. This enhancement shows that folic acid was offering structural protection and not just biochemical and oxidative advantages. In sum, all the findings comprise the fact that folic acids supplementation during pregnancy may help to prevent the arsenic-induced renal toxicity of developing offspring. By enhancing the renal functional indicators, boosting antioxidant defenses, and preserving the tissue structure, folic acid has become a prospective nutritional supplement to mitigate fetal vulnerability to environmental toxicants. Such results emphasize the significance of maternal nutritional care in high-risk areas and provide a scientific foundation to continue the investigation of folic acid as a preventive measure of prenatal exposure to arsenic.

6.CONCLUSION

The current research shows that prenatal exposure to arsenic causes severe renal toxicity in neonatal rats, based on the evidence of altered biochemical cumulative measures, increased

oxidative stress, and severe histopathological damages. Maternal folic acid supplementation was effective to counteract the effects of such adverse outcomes, improving the markers of the renal functioning nearer to normal, decreasing lipid peroxidation, increasing the activity of antioxidant enzymes, and preserving the renal tissue architecture. These results point to the fact that folic acid offers a large amount of biochemical and structural defense against arsenic-related nephrotoxicity in fetuses. These findings demonstrate the significance of nutritional support of mothers in minimizing the effects of environmental toxicants on developing organs. As it is inexpensive, available, and already a well-advocated intervention during pregnancy, folic acid presents an opportunity to be an effective intervention in people facing the likelihood of arsenic exposure. Although the study presents solid experimental support of its protective action, there should be additional studies on the topic that could include long-term developmental appraisals and translational research in a human population to support its inclusion in the strategies of population health. On the whole, the research results indicate that folic acid supplementation may be proposed as an effective solution to protecting fetal renal health in arsenic-contaminated areas.

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