Original Article

# Sodium Arsenite Exposure during Early Postnatal Period Induces Morphological and Biochemical Changes in Rat Kidney

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## Abstract

**Introduction:** The incidence of arsenic *(As)*-induced toxicity is increasing steadily all over the globe. Consumption of *As*-contaminated water is the chief source of exposure to *As*. Kidneys are important organs involved in the excretion of the final metabolized products of inorganic *As (iAs)* and organic *As,* thus becoming highly vulnerable to *As*-induced adverse effects. The functional and morphological maturation of kidneys during the gestational period continues to a variable extent into the early postnatal period and accordingly, the vulnerability to *As* exposure is increased manifold during postnatal period. **Material and Methods:** The present study aimed to assess the function and morphology of the developing kidney of rats exposed to sodium arsenite (Na As $O_2$) (1.5 mg/kg body weight [bwt] intraperitoneally) from postnatal day 1–28. On day 29, the perfusion fixed kidney tissue was processed for paraffin embedding, whereas fresh kidney tissue was processed for biochemical estimation of reduced glutathione (GSH). Blood samples were collected intracardially for the assessment of serum urea and creatinine levels. **Results:** Functional deficits were reflected by increased levels of serum urea and creatinine levels in *iAs*-exposed animals. The GSH levels in the renal tissue of experimental animals showed a significant decrease (81.20 ± 26.79 µg/g) as against GSH levels in controls (122.45 ± 30.97 µg/g). Microscopic observations revealed obliterated Bowman's capsular space with increased cellularity in the experimental group. In addition, decrease in the number as well as size of glomeruli was noted in *iAs* alone-treated animals. **Discussion and Conclusion:** The adverse effects of *As* have been widely studied in various organ systems in adults. Our data showed a significant alteration in kidney parameters (structural and functional) of rats exposed to *Na As* $O_2$ during early postnatal period, suggesting thereby increased vulnerability of the developing kidney to *As* exposure. Postnatal exposure of neonatal rats to sodium arsenite induces adverse effects on developing kidney.

**Keywords:** *Arsenic, glutathione, kidney, postnatal period, sodium arsenite*

## Introduction

Arsenic (*As*) is a naturally occurring metalloid present abundantly in earth's crust. Because of its colorless, odorless, and tasteless properties, the presence of *As* in food, water, or air gets overlooked so that exposure to *As* becomes a threat to biological forms. Exposure to *As* classically results either from ingestion of contaminated drinking water and food, etc., or through inhalation in industrial setups. The inorganic form of *As* (*iAs*) has been reported to be more toxic than the organic form.[1,2] The two main forms of *iAs* in the drinking water are pentavalent arsenate *(iAsV)* and trivalent arsenite *(iAsIII)*; both these forms get readily absorbed through the gastrointestinal tract and metabolized by the liver. The trivalent form of *iAs* (*iAsIII)* inhibits pyruvate dehydrogenase and results in reduced citric acid cycle activity and cellular Adenosine Triphosphate (ATP) production.[3] Furthermore, *iAsIII*-induced oxidative stress inhibits the production of glutathione, which otherwise is an important cellular antioxidant.[4] *iAs*-induced toxicity has been reported to affect multiple organ systems of the body such as cardiovascular, gastrointestinal, nervous, hepatobiliary, urinary, and integumentary.[5,6] Kidney is one of the organs targeted by *As* exposure as the final metabolized products of *iAs* and organic *As* are excreted out by the kidneys.[7]

All along the developmental period, gestational and early postnatal periods across the species are considered the most vulnerable periods toward various insults.[8,9] Exposure to *iAs* in drinking water (800 ppb) during sensitive developmental periods has been reported



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to lead to increased morbidity and mortality.[10] The neonatal period or the early postnatal period is the phase of emerging metabolic and differentiation processes, which depends not only on the post conception age (gestational age + postnatal age) but also on the clinical status that can be fragile and vulnerable during this maturation process. As the kidney is a target for drug handling, better understanding of the maturation processes in the context of kidney is desirable as the main steps of drug disposition (absorption, distribution, metabolism, catabolism, and elimination/excretion) could be influenced by the impact of still ongoing developmental processes.

Recent studies suggest that *As*-induced nephrotoxicity could have its basis in disturbed antioxidant defense system, altered protein and lipid peroxidation, etc. Gestational and early postnatal periods are considered the most critical periods. However, only limited attention has been paid to the effects of exposure to toxicants (environmentally relevant levels) during these critical periods of development on various processes. Substantial effects of exposure to *As* during this sensitive developmental period have been reported earlier in various animal models, yet there is a paucity in the context of relevant data on the said subject.[10] Keeping these evidences in mind, the present work was intended to study the morphological and functional features of the kidney of rat pups, following exposure to sodium *As* during early post natal period.

## Material and Methods

The present study was carried out on pups of Wistar rats (*Rattus norvegicus*). Pregnant rats (gestational age: 18–19 days) were procured from the Central Animal Facility of the institute after approval from the institute's ethical committee (Institutional animal ethics committee (IACE) 650/11). The animals were fed standard rodent diet and drinking water *ad libitum*. The day of delivery of pups was considered as postnatal day zero (PND 0). The litters along with the dams were confined to cages, kept in temperature (20°C–26°C)- and humidity (30%–70%)-controlled environment. All procedures for the care and use of laboratory animals were carried out in accordance with the principles laid down by the Institute Ethical Committee (IEC).

### Animal groups

The mother-reared pups were randomly divided into control and experimental groups. The control group (Group I, $n$ = 12) received double-distilled (DD) pyrogen-free sterile water, whereas the animals in the experimental group (Group II, $n$ = 12) received aqueous solution of $NaAsO_2$ (1.5 mg/kg bwt). Hamilton microsyringe was used for intraperitoneal administration of DD water and $NaAsO_2$ from PND 1 to PND 28.

In the current study, the effective dose (ED) (1.5 mg/kg bwt) equivalent to 9.5% of $LD_{50}$ (15.86 mg/kg bwt) was given, with the ED being approximately $1/10^{th}$ of the $LD_{50}$.

During the treatment period (PND 1–28), the animals were weighed daily and observed constantly for general features of well-being and appearance of developmental milestones such as eye opening and development of fur.

On PND 29, the animals (Group Ia and IIa) were anesthetized and perfusion fixed transcardially. An incision was made in the midsagittal plane to expose the abdominal cavity. Both the kidneys were dissected out carefully and stored in 4% paraformaldehyde at 4°C till further processing. The animals assigned to Groups Ib and IIb were sacrificed under ether anesthesia, and the retrieved kidneys were immediately snap frozen in liquid nitrogen and transferred to −80°C.

### Kidney function test

For estimation of urea and creatinine levels in serum, blood was collected directly from the left ventricle and stored in Micro centrifuge tube (MCT). The serum was separated by centrifugation at 3000 rpm and stored at −20°C. Serum urea and creatinine were estimated by modified urease-Berthelot colorimetric method and alkaline picrate method (Jaffe's method), respectively.[11,12]

### Biochemical test (reduced glutathione assay)

Each kidney sample was weighed, sliced, and homogenized with freshly prepared sodium phosphate buffer (10% W/V). The homogenate was centrifuged (5000 rpm/min for 5 min) and to 0.2 ml of this supernatant fraction, 0.3 ml of 5% trichloroacetic acid and 4 ml of 0.3 M sodium phosphate buffer ($Na_2HPO_4$) were added to get a final volume of 4.5 ml. Finally, 0.5 ml of Ellman's reagent (5-5' dithiobis-2-nitrobenzoic acid) was added to the sample, and the absorbance was read within 15 min at 412 nm against the reagent blank.[13] A standard curve was drawn using known concentrations of reduced glutathione (GSH) solution. With the help of standard curve, GSH level was calculated, and the result was expressed as μg/g of wet tissue.[14]

### Morphology and morphometry

After trimming off the apical and basal portions of the kidneys, the middle portion was further processed for paraffin embedding. Serial sections (7 μm) were cut and stained with hematoxylin and eosin for observing under the microscope (NIKON E600) mounted with DS cooled camera (M/S. Nikon Corp., Minato-ku, Tokyo, JAPAN) and fitted with image analysis system. For morphometric analysis, low- (×10) and high-power (×40) digital photomicrographs were captured and studied for glomerular numbers along with diameter measurements of glomeruli. For this purpose, every tenth section in the series was chosen, with the sections being incorporated from the hilar region of the kidney.[15] In each section, ten randomly chosen fields were considered for counting the glomerular number (within a standard rectangular grid [500 μm × 500 μm] placed on

the section) and determining their diameter. The mean number of glomeruli per mm² was calculated and to overcome the bias, the glomeruli touching the left and the lower margins of the grid were excluded from quantitative analysis. For the glomerular perimeter measurements, the outline of the glomerulus was drawn by manually outlining the Bowman's capsule on the screen by the cursor after selecting the tool AREA, and the software generated the area and their equivalent diameters.

### Statistical analysis

The mean values for various parameters among the control and experimental animals were compared using Mann–Whitney U and Kruskal–Wallis tests. GSH levels (mean ± standard deviation [SD]) for each group were treated as clustered data, and their differences were compared. SPSS software version 17 (SPSS Inc., Chicago, IL, USA) was used for the analysis. $P \leq 0.05$ was considered statistically significant in all the tests.

## Results

### Gross features, body weight, and kidney weight

The general somatic developmental features such as ear unfolding (PND 4), development of fur (PND 6), and eye opening (PND 14) occurred on scheduled time in both the control and experimental animals. The gross features (shape and size) of the kidneys at the time of sacrifice did not show any significant difference in the control and experimental groups.

The mean body weight (bwt) of the control and experimental animals at PND 1 was 5.33 ± 0.49 and 5.25 ± 0.45 g, respectively. On PND 29 (the day of sacrifice), approximately ninefold increase in the body weight, i.e. 44.33 ± 2.49 and 43.08 ± 2.31 g, was observed in both the control and experimental groups, respectively, with the control group showing marginally higher weight gain [Figure 1].

At the end of the experimental period, the average weight of the right and left kidneys was comparable in the control and experimental animals, with 296.3 ± 26.7 (right) mg and 286.3 ± 36.2 mg (left) in the former group as against 289.4 ± 30.1 mg (right) and 271.1 ± 28.4 mg (left) in the latter group [Table 1].

### Kidney function tests

The mean value of serum urea and creatinine levels determined on PND 29 was 65 ± 12.104 and 0.54 ± 0.11 in the experimental animals, while for the control group, the corresponding values were 48.33 ± 12.67 and 0.35 ± 0.10, respectively [Table 1], thus suggesting a statistically significant ($P \leq 0.05$) increase in the levels of experimental animals.

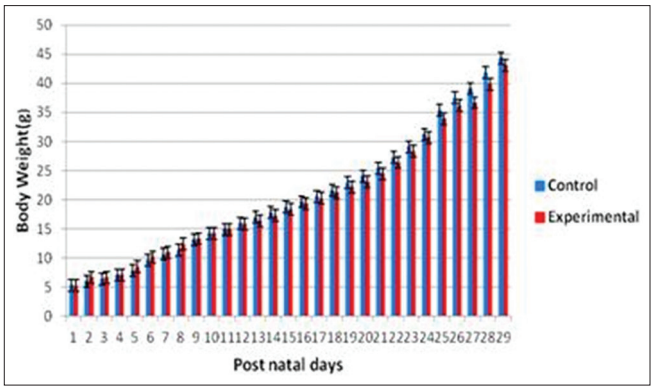


**Figure 1: Bar diagram showing the gain in body weight of control and experimental animals during the experimental period**

### Reduced glutathione levels

The mean value of GSH in the renal tissue of experimental animals was 81.20 ± 26.79 μg/g as against 122.45 ± 30.97 μg/g in the control group, thereby indicative of statistically significant ($P < 0.05$) decrease in GSH level/g of renal tissue in the experimental group [Table 1 and Figure 2].

### Morphology and morphometry

Microscopic observations revealed a well-defined corticomedullary demarcation with maintained cytoarchitecture in kidney sections of both the control and experimental groups. The renal cortex presented abundance of renal corpuscles and tubular profiles, with the medulla showing majorly of tubular profiles arranged as gentle curves in the outer part. The proximal and distal convoluted tubules were arranged compactly around the glomerulus in both the groups. Bowman's capsular spaces were seen to be obliterated in the experimental group [Figure 3]. Glomerulus in the control animals showed normal profile, while increased glomerular cellularity was evident in the experimental group.

The number of glomeruli (mean ± SD) in the control group was 12.1 ± 0.4 per mm², whereas the corresponding value for the experimental group was 9.8 ± 0.4 per mm². The glomerular diameter (mean ± SD) in the control animals was 60.25 ± 0.2 μm as against 57.3 ± 0.8 μm in the experimental group, whereas the corresponding values for glomerular perimeter (mean ± SD) in the control and experimental groups were 187.21 ± 8 μm and 184.21 ± 8 μm, respectively, pointing toward a significant decrease in all the parameters in the experimental animals.

## Discussion

The present study focused on the possible adverse effects of sodium *As* exposure during postnatal period (PND 1–PND 28) on the elementary parameters of renal maturation in rats. Nephrogenesis in rats extends till 11th–15th days of postnatal life.[16] Accordingly, the physiological maturation during postnatal period progresses up to variable time points in different species. While

**Table 1: Kidney weight (mg) and levels of urea, creatinine (serum), and glutathione (renal tissue) of control and experimental groups**

| Group | Weight of kidney (right) (mg) | Weight of kidney (left) (mg) | Serum urea | Serum creatinine | GSH (µg/g) |
|---|---|---|---|---|---|
| Control | 296.3±26.7 | 286.3±36.2 | 48.33±12.67 | 0.356±0.10 | 122.45±30.97 |
| Experimental | 289.4±30.1 | 271.1±28.4 | 65.00±12.10 | 0.54±0.11 | 81.20±26.79 |

GSH=Glutathione

the immature rat kidneys attain maturity on weanling, in humans, it takes 2–3 years for the same. During this developmental period, considerable differentiation of functional units of the kidney takes place.

The comparable gain in bwt of both the groups could be attributed to the shorter period of exposure, which might not have been sufficient to alter the gross development significantly, as substantiated by previous studies.[17,18] However, Nandi *et al*. observed poor gain in body weight of animals exposed to *As* (10 ppm) for an extended period of 12 weeks as compared to animals exposed for a period of 4 and 8 weeks.[19] Chinoy and Shah administered 0.15, 0.30, 1.5, and 3 mg/kg bwt of $As_2O_3$ to adult rats for a period of 3 weeks (5 days/week) but did not observe any noticeable weight gain in animals.[20] Another report showed a dose-dependent (0.3, 3, and 10 mg/kg) decrease in weight gain when exposure to $As_2O_3$ was carried out by inhalation.[21] This decrease in bwt was explained by the investigators as an outcome of decrease in food consumption by the experimental animals. In the present study, the shorter duration of exposure with a relatively low level of test substance (1.5 mg/kg bwt $As_2O_3$) might have failed to bring any significant change in bwt gain.

*As* is reported to bind with sulfhydryl groups of proteins and enzymes, thereby interfering with their metabolism.[22] In addition, *iAs*-induced nephrotoxicity has been attributed to *iAs*-induced generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), in turn altering the cellular antioxidant defense system.[23] *iAs* has been reported as an inhibitor of several antioxidant substances in the body such as glutathione, glutathione peroxidase, thioredoxin reductase, and superoxide dismutase.[24,25] The GSH present in the majority of cells serves as the chief cellular antioxidant.[26,27] In the present study, the GSH in the kidney tissue was found to be 122.45 ± 30.97 and 81.2 ± 26.79 µg/g in the control and experimental groups, respectively; the significant decrease in GSH level in the experimental group could be suggestive of *iAs*-induced alteration in oxidative stress status. Gopalkrishnan and Rao observed significant decrease in GSH levels in mouse kidney receiving *As* trioxide (0.5 mg/kg bwt) over a period of 45 days.[28] Patel and Kalia also reported a significant decrease in GSH in kidney tissue of adult Albino Wistar rats following subchronic exposure to sodium *As* at a dose of 5.5 mg/kg bwt/day orally for 30 days.[29]

The renal function status was evaluated by measuring the serum urea and creatinine levels. A significant increase in

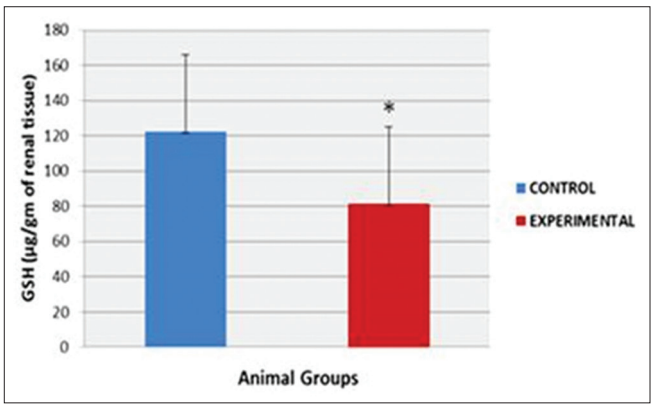


**Figure 2: Levels of reduced glutathione in renal tissue of control and experimental animals (significant decrease in glutathione level in experimental vs. control animals)**

serum urea and creatinine levels in the experimental animals did suggest *iAs*-induced derangement in kidney function. These observations are in line with earlier reports (Patel and Kalia, 2010). Patel and Kalia observed a significant increase in serum creatinine level in adult Wistar rats treated with 5.5 mg/kg bwt of sodium *As* orally.[29] Saxena *et al*. subjected Albino rats to *iAs* exposure (1.5 mg/kg bwt) for 1 day (acute) and 7 days (0.2 mg/kg bwt) (subacute) and observed a significant increase in serum urea and creatinine level.[30] In the kidney, *As* combines with sulfhydryl group of proteins present in glomerular filtration membrane, leading to oxidative stress and generation of ROS.[2,31,32] *As* also attaches to lipid, thereby resulting in lipid peroxidation and deposition of lipid droplets in the slit pores of glomerular filtration membrane.[33] Both the abovementioned mechanisms could result in decreased glomerular filtration rate, thereby leading to retention of nitrogenous waste products in the blood and causing elevation of serum creatinine. *As*-mediated increase in the production of ROS could enhance lipid peroxidation and cellular damage in renal tissue, thereby resulting in chronic renal damage as suggested by Kokilavani *et al*. and Kaneko.[34,35]

Morphological observations of glomerular hypercellularity could be indicative of the ongoing inflammatory process. Singhs and Rana (2007) reported glomerulonephritis, proximal tubular necrosis, and epithelial damage in *As* trioxide-treated rats at the sublethal dose of 4 mg/g bwt through gavages for a period of 30 days.[36] Focal obliteration of Bowman's spaces along with tubular alterations and mononuclear inflammatory cell infiltrate was reported by Rubatto Birri *et al*. in the kidney of adult

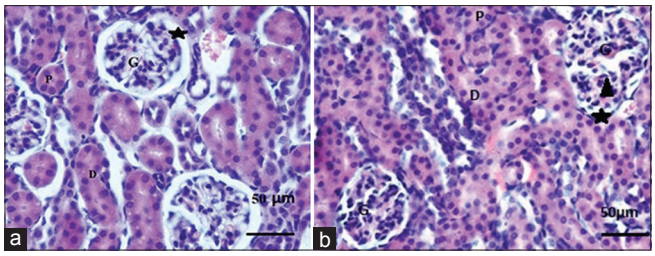


**Figure 3: Photomicrographs of hematoxylin and eosin-stained sections of kidney from control group (a). (A) Well-defined glomerulus (G), Bowman's space (★), proximal convoluted tubule (P), and distal convoluted tubule (D). Photomicrographs of *iAs*-treated group (b). (B) Obliteration of Bowman's space (★), increased glomerular hypercellularity (▲), and indistinct outline of proximal convoluted tubules (P) and distal convoluted tubules**

Wistar rats treated with 100 ppm of sodium *As* in drinking water for a period of 60 and 120 days.[37] This might be suggestive of increased mesangial cell and matrix reactivity. Our observations pointing toward increased effect on cortex of the kidney could partly be due to increased blood flow to this region as compared to medulla.

According to Brenner and Mackenzie and Luyckx and Brenner, determination of glomerular mass could be an important correlating factor in evaluating the pathophysiology of renal diseases.[38,39] Based on these suggestions, it is hypothesized that studies designed to evaluate the number and size of the glomeruli in physiological and pathological conditions could provide a relevant clue toward the status of renal function. In the current study, the mean glomerular number per mm² presented a significant decrease in the experimental group. The mean glomerular diameter also showed a significant decrease in the experimental group as compared to the control group. The diameter of glomeruli in rats during suckling has been reported to be 60 µm in a previous study by Arataki *et al*., and our findings of glomeruli diameter in controls are in agreement with this report.[40] Chinoy and Shah in 2004 reported a significant decrease in glomerular diameter in adult male mouse kidney following treatment with *As* trioxide (0.5 mg/kg bwt) for 30 days.[20] The significant decrease in glomerular diameter in the experimental group could be explained on the basis of shrinkage of glomeruli.

## Conclusion

In the present study, the altered morphological and morphometric parameters of the kidney in the experimental animals pointed toward *As*-induced changes at the structural level, whereas the increased levels of serum and urea and the decreased GSH levels in the renal tissue of experimental animals were suggestive of *As*-induced functional deficit and oxidative stress. These observations hence provide the preliminary biochemical and the morphological evidence of *As*-induced nephrotoxicity in rat pups subjected to sodium *As* exposure during early postnatal period. However, understanding the exact mechanism of *As*-induced nephrotoxicity needs much more elaborate experimentation with determination of many more parameters at morphological, behavioral, and ultra-structural levels.

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Department of Anatomy, AIIMS, New Delhi, 29, India.

### Conflicts of interest

There are no conflicts of interest.

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