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Arsenic induced renal toxicity and its correlation with high lipid peroxidation in swiss albino mice

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Abstract- Around the world, millions of people get exposed to a high level of heavy metals through their drinking water. Among these metals, Arsenic is one of the most potent toxic agent and prevalent in the environment in its inorganic form i.e. Sodium Arsenite ($NaAsO_2$). Sodium Arsenite is observed to possess high reactivity with thiol groups. The present study was conducted to investigate the effects of low doses of Sodium Arsenite on kidney and its correlations with oxidative stress in Swiss albino mice. Mice were dissected for histological & lipid per oxidation evaluation after 4th, 6th and 8th weeks. Significant rise in lipid per oxidation was observed in Sodium Arsenite dosed Swiss albino mice. Mild to severe types of necrosis and degenerative changes in the kidney via histological studies were noticed. Mononuclear cell infiltration and vacuolization in the tubules resulted in reduced glomerulus space. It could be safely concluded that degenerative changes in kidney tubular epithelium may be attributed to Arsenic. Metabolites of Arsenic which are excreted from kidneys may also cause cellular damage leading to kidney dysfunction. These findings in mice model might be useful for a better understanding of the toxic effects of Arsenic, to develop an effective ameliorative treatment against Arsenic mediated effects on human health.

Key words: Arsenic, Swiss Albino Mice, Kidney, histology and LPO.

INTRODUCTION

Heavy metals are hazardous compounds which a major concern to human health alleviating the quality of life. These heavy metals are potentially toxic to the multicellular life due to high accumulation in the body.[1] Many studies have shown that heavy metals are widely present in the environment and cause deleterious effects on human health.[2-8] Among these heavy metals, Arsenic is one of the most 20 hazardous substances, is known to cause several diseases like carcinogenicity, diabetes, genotoxicity, hypertension, peripheral neuropathy, hyperkeratosis, cardiovascular diseases etc.[9]

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Arsenic (As) is an element with atomic number 33 and relative atomic mass 74.92. Arsenic can exist with many minerals, usually in conjunction with Sulphur and metals and also as a pure elemental crystal. It was first documented by Albertus Magnus in 1250, the Arsenic is a metalloid. Arsenic is present in the environment and humans, all over the world are exposed low to high amounts, mostly through food, water and air. The presence of high levels of arsenic in groundwater, the main source of drinking water in many countries around the world, has drawn the attention of the scientific community. Millions are exposed to high levels of inorganic arsenic through drinking water. It has become a major public health problem in many countries in South and East Asia which is a great burden on water supply authorities. The

Environment Protection Agency lowered the permissible level of Arsenic in drinking water in the USA in 2001 from 50 ppb to 10 ppb. The most affected areas in the world are Bangladesh and West Bengal, India. In 42 districts in southern Bangladesh and nine adjacent districts in West Bengal, 79.9 million and 42.7 million people respectively are exposed to groundwater Arsenic concentrations that are above the World Health Organization maximum permissible limit of 50 ug/l. Prolonged ingestion of water contaminated with Arsenic may result in the manifestations of toxicity in all systems of the body as subsequently discussed. Symptoms and diseases engendered by Arsenic include dermal lesions, anaemia, and an increased risk for cardiovascular disease, liver damage, diabetes and cancer. Arsenic induced cancer targets such organs as the lungs, skin, bladder, liver and kidney.[10] The most serious concern is the potential of Arsenic to act as a carcinogen. The complex chemistry of the metals makes them hard to completely understand their mechanisms, but in general, metals such as arsenic can affect multiple aspects of cellular function including proliferation, apoptosis, differentiation and cell transformation.

The toxicity of Arsenic and its derivatives depends chiefly on the oxidation state and chemical composition. Inorganic Arsenic is far more toxic than organic Arsenic as it readily reacts with sulfhydryl groups of various enzymes and proteins in a biological system. Thus, altering multiple cellular pathways including the promotion of apoptosis, inhibition of DNA repair and increasing oxidative stress ultimately leads to Arsenic mediated adverse health effects.[11,12]

It was demonstrated that ROS formed during oxidative stress can initiate lipid peroxidation (LPO) and oxidizes proteins to its inactive state and causes DNA strands to break, damaging normal cellular function.[13] It has been reported that Arsenic induced ROS enhances lipid peroxidation and cellular damage in liver and renal tissues. Ineffective elimination of ROS has been shown to induce oxidative stress and damage to various organs such as liver, lungs, kidney and spleen. Moreover, due to increased oxidative stress macromolecules such as lipid, protein and DNA may become vulnerable to its toxicity leading to disruption of cellular structural integrity and capacity.[14] Although there is proof that arsenic toxicity is associated with the induction of oxidative stress in vital organs like kidney through overproduction of reactive oxygen spices (ROS) and inhibition of antioxidant enzymes activity.[15] As per Varner *et al.* (1998)[16], the vital role of the kidney is xenobiotics metabolism. Since blood and kidney are susceptible to oxidative stress and xenobiotics metabolic disturbance. Thus, kidney damage due to oxidative stress further provokes the kidney dysfunction which may result in additional toxic metabolites load to the renal system and may induce more injury to the kidney due to high oxidative stress.

The kidney is one of the toxics target organ due to its capacity to extract and concentrate toxic substances by highly specialized cells and also due to its large blood flow.[17,18] It has been reported that the kidney is a primary organ for the excretion of metabolites and hence appears to be one of the main targets of Arsenic.[19] The toxicity of As in the kidney has been demonstrated in the human population and animals through renal pathology and functional changes.[20]

In addition, the mechanism of kidney damage by Arsenic has been investigated in numerous studies.[21] Oxidative stress is one of the major factors for kidney damage. LPO is an autocatalytic mechanics leading to oxidative destruction of cellular membranes and ultimately cell death.[22] Among the suggested mechanism, oxidative stress is one of the best-accepted theories.[23]

As above mentioned, the kidney is the organ for excretion of unwanted and toxic substances, it is more vulnerable to Arsenic toxicity. Moreover, oxidative stress is one of the major factors for nephrotoxicity. Hence, the present study has been designed to examine the impact of Arsenic on the kidney by evaluating histological damages in renal tissue and its correlation with lipid peroxidation parameter.

MATERIALS & METHODS

Experiments were carried out on Swiss Albino Mice. The animals were procured from the laboratory animal resources section of Mahavir Cancer Institute & Research Centre, Patna, India (CPCSEA Regd. No. 1129/bc/07/CPCSEA, dated 13/02/2008). The research work was approved by the IAEC (Institutional Animal Ethics Committee) with IAEC No. IAEC/2012/12/04.The experimental animal (Swiss albino mice) (n=20) selected for the study were 8 to 10 weeks old and their weight was measured as 28 ± 2 grams. Mice were kept in the polypropylene cages with paddy rusk at room temperature

25 ± 2ºC and humidity 50±5% in a controlled light (12 hrs light and 12 hrs dark). Animals were maintained in ideal condition as per animal ethical guidelines. After a week of acclimatization to the environment, the mice were separated into four groups.

The drug was procured from the pharmacy of Mahavir Cancer Institute & Research Centre, Patna. Sodium Arsenite @ 2mg/kg body weight was administrated orally to the experimental mice at the interval of 4 weeks, 6 weeks and 8 weeks during the designed protocol for the study.

The mice were segregated into four groups with 5 mice (n=5) in each group. Group A was kept as control and served with equal volume of distilled water by Gavage method. Group B,C & D were treated with Sodium Arsenite orally @2mg/kg body weight for 8 weeks.

Body weight:

Body weight of each group of mice were measured before and after the administration of Sodium Arsenite. Each group of mice were sacrificed after 4 weeks, 6 weeks, and 8 weeks of administration with Sodium Arsenite and kidney were dissected.

Lipid Peroxidation:

Blood serum was obtained by centrifuging the blood collected from each group at 3000 rpm for 10 minutes and store at -20ºC. The lipid peroxidation level was estimated through formation Thio Barbituric Acid Reactive Substance (TBARS) by standard method.[24]

Histological analysis:

The kidney tissues were fixed in formalin (10%) and 4-5 µm thick sections were sliced. The sections were further stained with Hematoxyline and Eosin and observed under light microscope.

RESULT

The mice were sacrificed in order to collect the tissue samples for the analyses of histological examinations and blood sample for lipid peroxidation evaluation. The body weights of the mice (control and treated) were recorded before the dissection. The mean ±SEM of the body weights of mice in control group (Group A) was found to be 27.80±1.056g. On the other hand, the mean ±SEM of body weights of mice treated for 4 weeks (Group B), 6 weeks (Group C) and 8 weeks (Group D) were calculated as 29±1.012g, 30.30±0.9028g and 31.80±0.9566g respectively (Table 1). The result clearly indicates that body increased with the increase in the duration of treatment with sodium arsenite (Figure 1).

Table 1: The body weight, organ weight of kidney and LPO parameters of control and Sodium Arsenite treated of swiss mice. (Values are expressed as Mean± SEM of p<0.05)

TEST	CONTROL (N=5) (Group A)	SODIUM ARSENITE @2mg/kgb. w		
		4th week(N=5) (Group B)	6th week(N=5) (Group C)	8th week(N=5) (Group D)
Body weight (g)	27.80±1.056	29.00± 1.012	30.30± 0.9028	31.80± 0.9566
LPO(nmol/ml)	27.26±1.138	35.38±1.824	37. 12 ±1.840	44.01±1.177

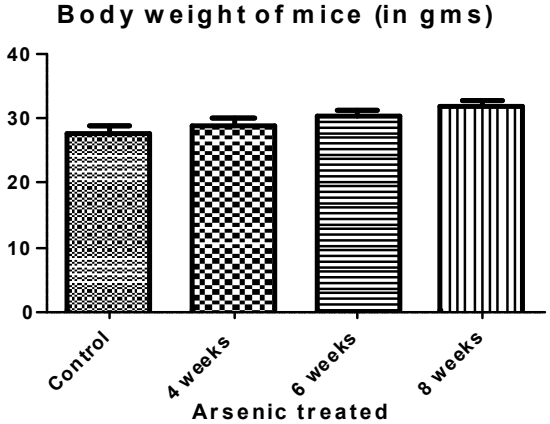

Figure 1: Comparison of body weight (mean±SEM) between control and arsenic treated mice of 4 weeks, 6 weeks and 8 weeks. *Significantly different from control group at p<0.05. p -value was performed by using one way ANOVA

The LPO levels in the blood serum collected from control and treated groups were analyzed. The mean±SEM of LPO level in the control group (Group A) was observed to be 27.26±1.138 nmol/ml. In contrast, the mean±SEMs of LPO levels in group B, group C and group D were recorded to be 35.38±1.824 nmol/ml, 37.12±1.840nmol/ml, 44.01±1.177nmol/ml respectively (table 1). The LPO level in control was observed to be lowest as compared to group B, C and D, which reflects that longer duration of arsenic treatment induces higher oxidative stress level (figure 2).

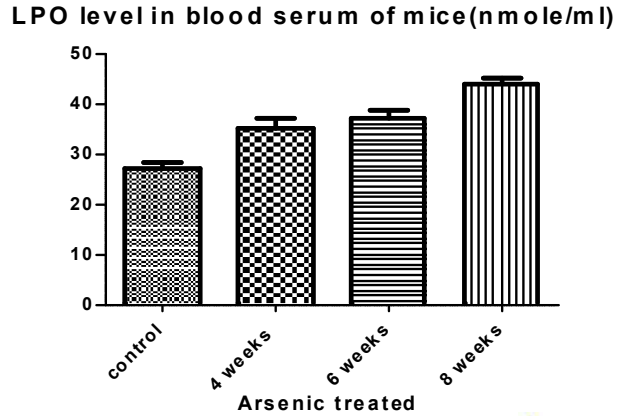

Figure 2: Comparison of LPO levels(mean ± SEM) between control and arsenic treated mice of 4 weeks, 6 weeks and 8 weeks. *Significantly different from control group at p<0.05. p-Value was performed by using one way ANOVA.

Histology:

To understand the arsenic induced tissues alterations, we have performed histopathology of organs (kidney). We have observed deformed glomeruli, clustered nuclei, diminished glands, weakening periplasm in arsenic treated mice kidney tissue (fig 4-6). In contrast, tissue architecture, glomeruli were well structured in control of kidney tissue (fig 3). It shows prominent to necrosis in tissue (fig 6). The degree of biochemical parameters can predict the level of histological damage in the kidney. The LPO level was found to be significantly increased only in as exposed group as compared to the control group (fig 2).

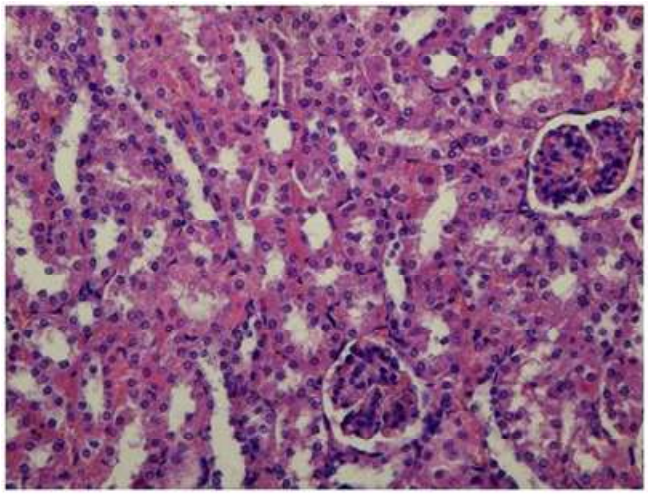

Figure 3: Photomicrograph of a normal kidney showing well - structured glomeruli, proper nuclei arrangement, well-shaped glands H&E (x100).

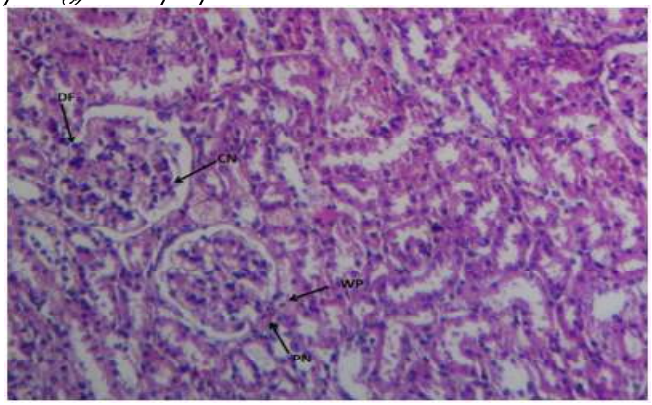

Figure 4: Photomicrograph of kidney 4th weeks showing deformed glomeruli, pervasive nuclei (PN), deformed glands (DF), clustered nuclei (CN), weakening of periplasm (WP), H&E (X100)

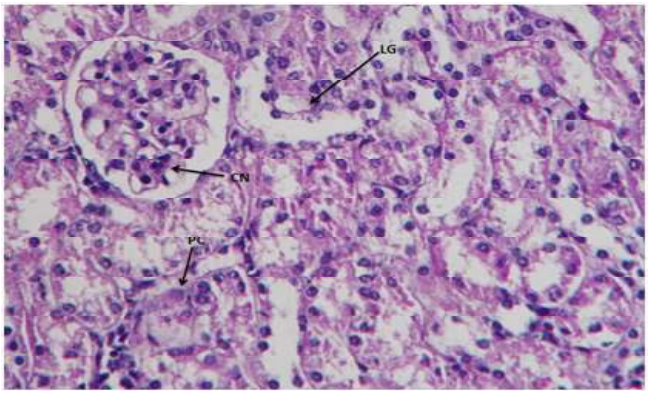

Figure 5: Photomicrograph of kidney 6th weeks showing unstructured glomeruli, irregular arrangement of nuclei also clustered nuclei (CN), loss of glands (LG), pervasiveness of cytoplasm (PC) (X100).

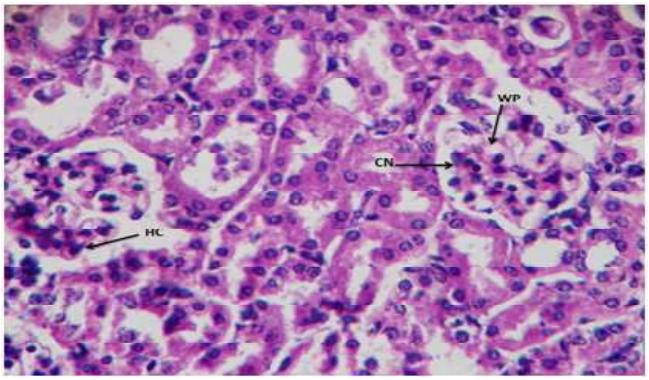

Figure 6: Photomicrograph of kidney 8th weeks showing necrosis, nuclei in the glands are spread out and dispersed (Shows low mitotic count - apoptosis may be implicated), clustered nuclei (CN) may also be seen somewhere, glomeruli completely diminished, hyperchromatins (HC) can also be seen in glomeruli region, cytoplasm is seen but not clearly defined as nuclei are absent, weak periplasm (WP), H & E (X100).

DISCUSSION

The purpose of this study was to correlate the high lipid per oxidation with histopathological alterations induced by sodium arsenite in the kidney of mice. Sodium arsenite contributes to the increased lipid per oxidation and altered histological appearances in the experimental mice. An earlier report suggests, Arsenic is an exogenously oxidative inducer, which can trigger the production of reactive oxygen species (ROS), causing damage to DNA, lipids and proteins.[14] Because the kidney is rich in phospholipids, and play a vital role in xenobiotic metabolism, is highly vulnerable to oxidative damage.[16]

In the present study, increased LPO levels in mice serum were found highly significant in sodium arsenite group (mean value 35.38, 37.12 and 44.01 nmol/ml for 4 weeks, 6 weeks and 8 weeks respectively) as compared to the control group (27.26 nmol/ml). Increased oxidative stress in tissue due to arsenic exposure is seemed to be the major cause of arsenic induced toxicity in mice. Santra *et al.* (2000)[25] has also reported oxidative stress in an experimental animal model due to arsenic exposure. Arsenic mediated oxidative stress can be indicated to induce the organ degeneration during the exposure. During the excretion process by the kidney, a significant amount of Arsenic inflows along with blood and other metabolites which further might interact with cellular protein or intercellular lipid or DNA molecules and thereby produce free radicals. This process may be responsible for the architectural changes of the kidney.[26] We have observed the degenerative changes, a cluster of nuclei, glomerulus diminished, the pervasiveness of cytoplasm; in kidney tissue in arsenic treated mice (figure 4-6). These damaging changes were observed as reflected action of toxic metabolites of Sodium arsenite. Microscopic changes were more pronounced in treated mice suffering from mild to high necrosis, indicating the toxic effect of chemical agents in the present study. Singh *et al.* (2011)[27] has also been reported renal failure, tubular necrosis due to arsenic toxicity. Apart from renal disorders, the toxic effect of arsenic has also been associated with hepatic disorders in an animal model study.[28]

It has been reported that exposure to arsenic is associated with metabolic disorders, hypertrophy of adrenal glands[29] and anaemia[30]. Exposure of sodium arsenite (2 mg/kg b.wt) to mice for the definite time intervals results in a significant increase of LPO levels in their serum.

CONCLUSION

Increased LPO leads to degeneration of lipids in the bilayer lipid layer of the plasma membrane which results in weak periplasm and hence nuclei inconsistently and irregularly present. Effect of LPO is applied in all the slides where irregularly spaced nuclei are observed i.e. 4th & 8th week treated slides.

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