STUDY OF HISTOPATHOLOGICAL EFFECTS OF ARSENIC ON KIDNEYS OF ALBINO RATS

Running Title: Gupte B, et al; Histopathological effects of arsenic on kidneys of albino rats

[1] Bonita Gupte, [2] Sangeeta Gupta, [3] Vanita Gupta, [4] Bhawna Sharma, [5] Vikrant Singh[*], [6] Rishu Kour

[1] Senior Resident, [2] Professor, [3] Professor, [4]Assistant Professor, [5] Assistant Professor, [6] Assistant Professor.

[1]Department of Anatomy,

[1]Acharya Shri Chander College of Medical Sciences, Jammu, India.

[*]Corresponding Author: Vikrant Singh

Abstract

Context:

Arsenic is a common pollutant of water in developing counties and leads to major health problems and clinical conditions. It affects all organs of the body and can be lethal in higher doses.

Aims:

To study the histopathological effects of arsenic on kidney of albino rat and compare it with rats that are not exposed.

Methods and Material:

18 rats, each divided in the group of 3, having 6 rats each. Group A acted as control group and, Group B and Group C, acted as test group which received Sodium Arsenite, in drinking water. Kidney tissue was sectioned, stained and examined under Light microscope.

Results:

The kidneys of rats exposed to low dose of arsenic showed decrease in the size of the glomerulus, increased periglomerular space with mild congestion of vessels and haemorrhage. Those exposed to high dose of arsenic, revealed a disrupted architecture, loss of brush border in cells of PCT, vacuolation and cloudy swelling, cell sloughing and desquamation. Large areas of haemorrhagic foci and mononuclear infiltrate were spotted.

Conclusions:

We conclude from the present work that experimental administration of Arsenic to rats was associated with histological changes in the form of renal toxicity in the test groups.

Key-words:

Histo-pathology, Kidney, Rat, Sodium Arsenite.

Introduction

Heavy metals have been recognized as strong biological poisons because of their present nature, toxicity, tendency to accumulate in organisms and undergo food chain amplification.[1] Industrial pollution of the environment with metal compounds is becoming a serious problem.[2] Anthropogenic activities have modified the global cycle of non-toxic essential elements like Arsenic, Mercury, Cadmium, and lead, and among these metals, arsenic is possibly the most abundant pollutant as well as a potential human carcinogen.[3]

Arsenic is the 20th most abundant element in the earth's crust, and is primarily associated with igneous and sedimentary rocks where it occurs mostly as inorganic forms at an average concentration of 2-5mg/kg.[4] Arsenic occur in both organic and inorganic forms in nature but inorganic form of arsenic [As(III) and As(V)] represent a potential threat to the environment, animals and humans.[5] It is naturally occurring ubiquitous element with metalloid properties and widely present in soil, rocks, sediments and metal ores in the form of oxides and sulphides or as salts of iron, calcium, copper in most parts of the world.[6] Arsenic is found unnaturally in mine waste sites and agricultural runoff. [7]

Arsenic continues to be essential constituent of many non-western traditional medicines. Some Chinese traditional medicines contain *realgar* (arsenic sulphide) and are available as pills, tablets and other preparations. They are used for psoriasis, syphilis, asthma, rheumatism, cough, haemorrhoids and pruritus and also prescribed as health tonics, analgesic, anti-inflammatory agents, and as treatment for some malignant tumours.[8] In India herbal medicines containing arsenic are used in some homeopathic preparations[9] and haematological malignancies.[10] Arsenic trioxide is widely used to induce remission in patients with Acute Myelocytic Leukaemia, based on its mechanism as an inducer of apoptosis.[11]

The major cause of human arsenic toxicity is in the form of contamination of drinking water from natural geological sources rather than from mining, smelting or agricultural sources (pesticides or fertilizers).[12] Arsenic contamination in drinking water has become a significant concern in Bangladesh, West Bengal, India, China, Mongolia, Nepal, Cambodia, Myanmar, Afghanistan, DPR Korea, and Pakistan. [13]Most cases of acute arsenic poisoning result from accidental ingestion of insecticides and pesticides and less commonly from attempted suicide. [14] The lethal dose of arsenic in acute poisoning ranges from 100mg to 300mg. [15] The clinical features invariably relate to the gastrointestinal system and include nausea, vomiting, abdominal colic, and profuse watery diarrhoea.[16]

Chronic exposure to inorganic arsenic that occurs as a natural contaminant in drinking water is associated with development of skin cancer.[17] Deposition of high concentrations of arsenic in the liver, kidney, lungs, hair and nails have been well reported.[18] After ingestion, inorganic arsenic appears rapidly in the circulation, where it binds primarily to haemoglobin.[19] Oxidative stress and malfunctioning of various organs including liver, lungs, kidney and spleen are attributable to overproduction or an ineffective elimination of reactive oxygen species ROS. [20]

Toxic effects of sodium arsenate in drinking water of rats showed proximal tubular congestion, atrophy, along with glomerular swelling, interstitial fibrosis and nephropathy. [21]

There has been dearth of knowledge in the arsenic affected renal organ tissue histology. A better understanding of the effect of arsenic at target organs with an emphasis on observation of tissue architecture at critical sites will aid in defining mode of action for arsenic-induced toxicity in mammals and to reduce the uncertainty in the risk assessment for this metalloid.

In light of the above observations, the present study has been designed to examine the histological damages caused by arsenic exposure on kidney tissue architecture in adult Wistar rat.

Aims and objectives

1. To study normal histomorphological characteristics of kidney in male Albino rats.
2. To study the dose related effects of arsenic exposure in the form of sodium arsenite in drinking water on kidney of albino rat with special reference to histomorphological changes.

Materials and Methods:

In the present study, Albino rats served as experimental animals. The study was conducted after getting clearance from Institutional Animal Ethics Committee (IAEC/Research/2018/06 dated 27-10-2018). The study was done from April 2019 to June 2019. Eighteen (18), Healthy Wistar male rats, in the age group of 10-12 weeks, in the range of 125-160 grams were taken. The rats were procured from the Central Animal House of Government Medical College, Jammu.

Animals were weighed on the first day using AmtiQ electronic weighing machine (Aarav Enterprise, India). The animals were handled with humane care in accordance with the guidelines of the Institutional Animal Ethical Committee. All the animals were kept in standard cages. These cages were made of solid plastic (sides and base) and stainless steel grid top. Rice husk was used as bedding material. The animals were kept under standard laboratory conditions (temperature 25 ± 2ºC with 12 hours' light/ 12 hours' dark cycle) with free access to standard pellet diet and water maintained throughout the study. After two weeks of laboratory acclimatization under optimal conditions of light, temperature and access to distilled water, the rats were randomly divided into three groups of six rats –A, B and C. Identification number was given to rats of each group.

Group A rats were named A1 to A6. Group B rats were named as B-1 to B-6.Group C rats were named as C-1 to C- 6.

The animals were observed for any abnormal physical or behavioural changes throughout experimental period.

Sodium Arsenite (NaAsO2) (AVI Chemicals, India) used in the study was whitish/greyish powder with molar mass of 129.91gms/mole, density of 1.87gms/cm^3 and solubility of 156gms/100ml.

Technique: Sodium Arsenite was dissolved in 2ml of distilled water. Sodium Arsenite was not given to Group A (only fed on distilled water). Sodium Arsenite used in Group B was 50mg kg body weight and Group C received 100 mg/kg body weight of sodium arsenite

Rats were given oral Sodium Arsenite solution, using a 2 cc syringe fitted with a long hypodermal needle (Nirlife, Gujarat, India). All animals received 2cc of the Sodium Arsenite solution daily. Fresh dosing solution was made every day in the morning and immediately 2ml was administered orally for 28 days

Group A- served as control and was administered 2ml distilled water orally with the help of a syringe. (Nirlife, Gujarat, India).

Group B-rats were administered 50ppm (50mg/kg) of sodium arsenite daily in distilled water for 4 weeks. First dose was administered on 14/04/2019 and followed for 28 days, daily.

Group C-rats were administered 100ppm (100mg/kg) of sodium arsenite in distilled water for 4 weeks. First dose was administered on 14/04/2019 and followed for 28 days, daily.

After the last dose of drug, on 12/05/19, animals were anaesthetized using Chloroform. Rats were sacrificed and 10% buffered formalin-fixed kidney samples were processed, cut at 5 µm and stained with H and E stain, subjected to Histopathology.

Results:

Histopathological examination

Group A:

The microscopic section of kidney of rats (Group A) fed with normal distilled water showed a normal renal architecture (Fig: 1 and 2). The cortex was organized in to cortical labyrinth and medullary rays. The cortical labyrinth consisted of glomeruli, PCT, DCT along with the interlobular blood vessels. The medullary rays were seen as elongated regions or projections of medullary tissues and contained straight portions of proximal and distal tubules of superficial and mid cortical nephrons and collecting ducts. The interstitial space was scanty and contained peritubular capillary plexuses and interstitial cells. In outer part of the medulla, the vascular bundles were seen. Surrounding the vascular bundles were straight portions of proximal tubules and ascending thick limbs of the nephrons and away from the vascular bundle. In the inner medulla (papilla), the thin ascending and descending segments intermingled with the vascular bundles. The collecting ducts were lying peripheral to the vascular bundles and the interstitial tissue increased from outer to inner medulla. Near the papillary tip the vasa recta ended the epithelium of ducts of Bellini continued with urothelium of collecting system. The renal pelvis and renal vessels were seen to lie in the renal sinuses embedded in fatty tissue. The renal corpuscles (Fig: 2) were normal in structure, with the glomeruli invaginating the Bowman's capsule. The glomeruli had a tuft of capillaries lined by endothelium (Fig: 2) and filled with red blood cells and were united by minimal connective tissue containing mesangial cells. The proximal convoluted tubules were more numerous and were lined by a single layer of low columnar or pyramidal cells, which had round nuclei and granular cytoplasm staining deeply with eosin. The cell boundaries of lining epithelium were not clearly defined and the apical surfaces of the cells showed brush border which almost filled the lumen. The nuclei were euchromatic and central in position. The Distal convoluted tubules were less numerous and they were lined by cuboidal cells which lacked a brush border and contained lightly stained cytoplasm with central euchromatic nucleus. (Fig 2)

In the medulla, the thick ascending and descending segments of Loop of Henle formed by the straight portions of PCT and DCT were lined by similar cells as convoluted portions of the cortex. The collecting tubules and the ducts of Bellini were lined by cuboidal or columnar epithelium with clear lightly stained cytoplasm and distinct cell outlines. They had larger lumen and were regular in shape and lacked brush border. The thin segments of loop of Henle were lined by simple squamous epithelium with central bulging nucleus.

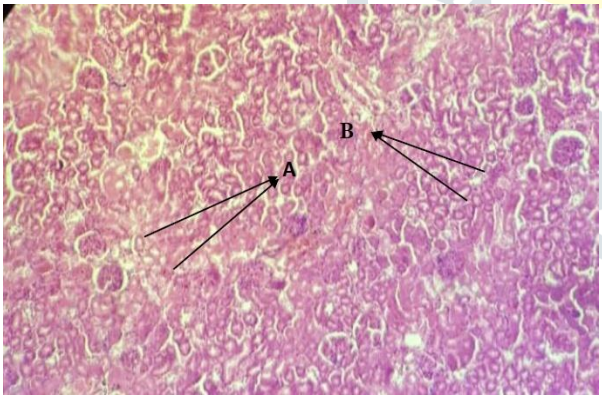

Fig 1: Photograph of the slide of Kidney of Group A, showing renal corpuscles (A) and (B) (H and E Stain 100x)

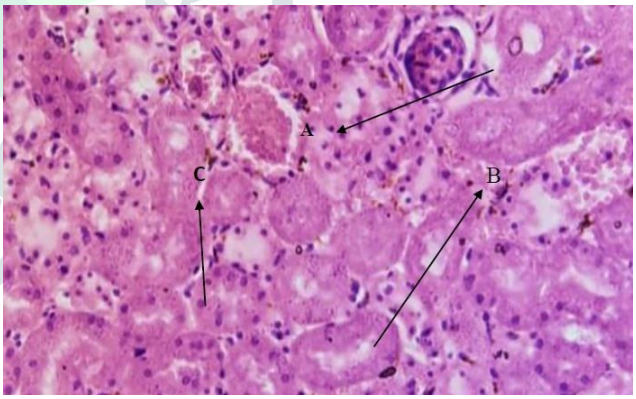

Fig 2: Photograph of the slide of Kidney of Group A, showing Glomerulus (A), Proximal Convoluted Tubule (B) renal tubules and Distal Convoluted Tubule (C) (H and E Stain 400x)

Group B:

The sections of kidney of group B rats revealed that the basic architecture of cortex and medulla of kidney was preserved. Decrease in the size of glomerulus and increased peri-glomerular space was seen. (Fig: 3). Small area of hemorrhagic foci could be seen in the connective tissue. (Fig: 4).

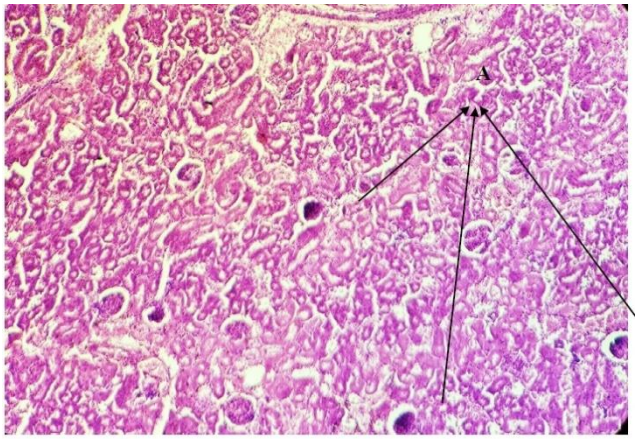

Fig 3: Slide of Kidney (Group B) showing decrease in the size of the glomeruli and increase in the glomerular space (A).
(H and E Stain 100x)

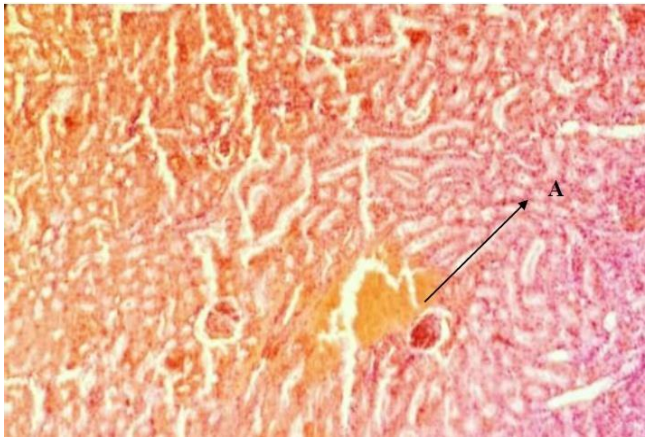

Fig 4: Photograph of the slide of Kidney of Group B showing area of focal hemorrhage (A)
(H and E Stain 100x)

Diffuse haemorrhages along with collection of inflammatory cells were seen in the interstitium. (Fig: 5) The PCT were more numerous. Most of these PCT appeared normal and were lined by single layer of low columnar cells, with round nuclei but the cell boundaries were not clearly defined. These nuclei were central in position and took an even stain. DCT were very few in number. They lacked the normal brush border. No significant changes were seen in the medulla.

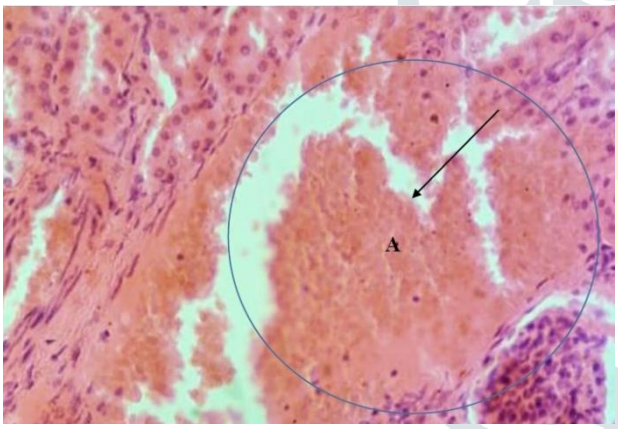

Fig 5: Photograph of the slide of Kidney of Group B showing wide haemorrhagic foci (A)
(H and E Stain 400x)

Group C:

The sections of the kidney of group C revealed that the basic architecture of the cortex was disrupted. Diffuse interstitial inflammation (Fig: 6) is seen. . There was wide hemorrhagic area, increase in the size of glomeruli and decreased periglomerular space, mononuclear cells of inflammation spread around hemorrhagic area (Fig: 7). There was extensive interstitial hemorrhage and vascular congestion. (Fig: 7, 8) There is decrease in the size of glomerular tuft and marked increase in the bowman's space. In some corpuscles, there was increase in the size of Bowman's capsule and hence, there was decrease in the bowman's space. The lumen of the PCT and DCT contained eosinophilic material and cellular cast (cellular fragments). (Fig: 9)In the PCT, the cells show loss of integrity of cell border. The tubular cells were vacuolated and showed cloudy swelling. (Fig: 10) An area of coagulative necrosis is seen in the tubules. The cells of the tubules showed separation from basement membrane and whole of the cellular lining collected in the centre of the lumen. Pyknotic nuclei were seen in the parenchyma. (Fig: 9).

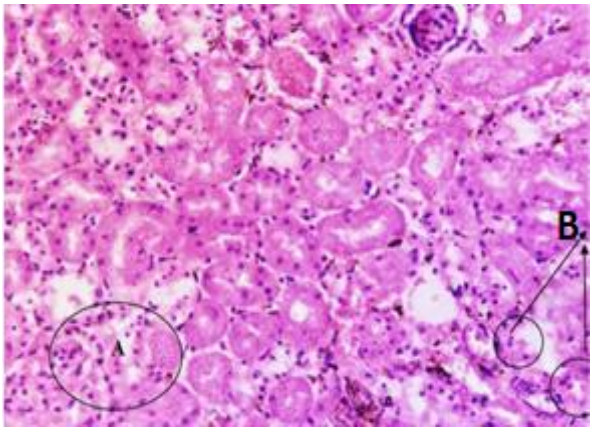

Fig 6: Photograph of the slide of Kidney of Group C, showing cells of Inflammation distributed randomly (A) and congested blood vessels (B) (H and E Stain 400x)

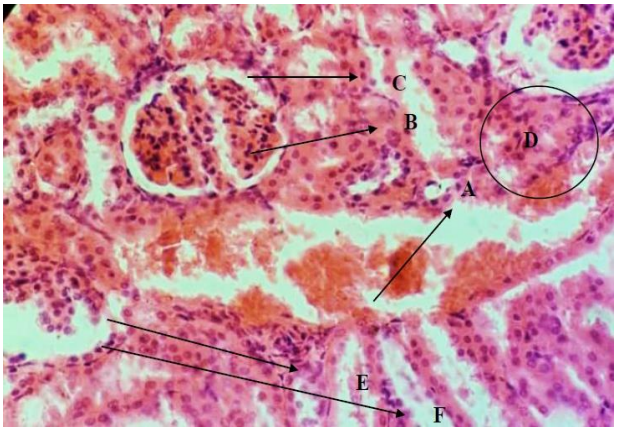

Fig 7: Photograph of the slide of kidney of Group C, showing wide hemorrhagic area (A), increase in the size of glomeruli (B) and decreased periglomerular space (C), mononuclear cells of inflammation spread around hemorrhagic area (D) (H and E Stain 400x)

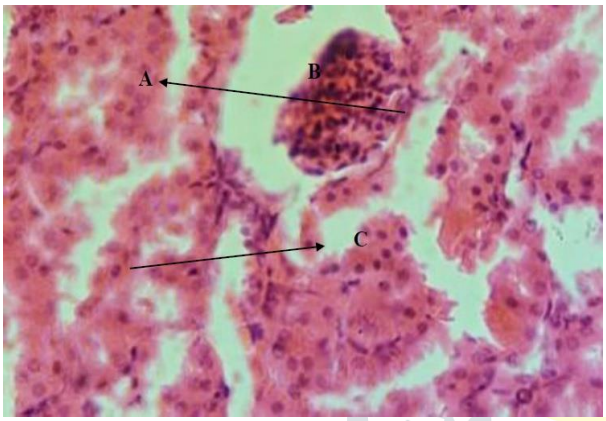

Fig 8: Photograph of the slide of kidney of Group C, showing wide hemorrhagic area (A), (H and E Stain 400x)

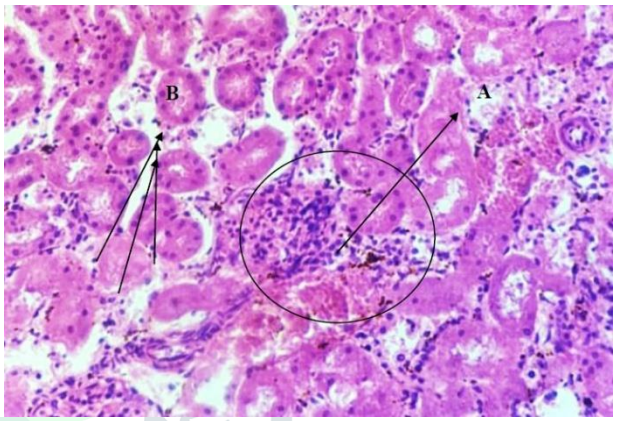

Fig 9: Photograph of the slide of Kidney of Group C, showing Glomerular congestion (A), increased glomerular space (B) and disruption of renal tubule with loss of brush border lining the tubule (C) (H and E Stain 400 x)

Discussion

Historically, the use of arsenic-based pesticides has led to considerable contamination of domestic and agricultural land, through their use as lawn herbicides, and insecticides for rice and cotton [22]
Recently, the environmental fate and behaviour of arsenic has received increasing attention due to a crisis in South-East Asia (West Bengal, Bangladesh and Vietnam). Tens of millions of people have been exposed to high levels of arsenic in ground-water, the region's primary source of drinking-water [23]
Arsenic is metabolized in the liver and its methylated species are excreted through urine by kidney. It has been described that kidney is one of the organs which is more vulnerable to arsenic chronic exposure.[24] It is considered as primary organ of target in arsenic toxicity.[25] In chronic arsenic ingestion, arsenic accumulates in the liver, heart, kidney, heart and lungs and smaller amounts in the muscles, nervous system, gastrointestinal tracts and spleen.[21]
Our investigation revealed normal histopathological findings of the renal tissue of Group A. The glomeruli and the tubules were normal.
Kidneys of rats belonging to Group B revealed a decrease in the size of Glomerulus and an increased periglomerular space.These findings are in accordance with the results of Hemalatha et al [26] who had exposed the rats to sodium arsenite in the dose of 300 mg/kg , daily for 2 weeks.
In the present study, kidneys of rats of Group B revealed, mild haemorrhage and a few inflammatory cells. These changes are in accordance with the changes seen in the study done by Chowdhury et al (2016), who exposed the rats to Arsenic in the dose of 50ppm, as Sodium Arsenite, orally, daily for 28 days and observed similar results in rats of his test group.[27]
In the present study, on kidneys of rats of Group B revealed PCT cells revealed tubular and intratubular degeneration, which is in concordance with study of Pagrut NK et al [28] who found very mild degenerative changes in tubules of kidneys of rats fed at a dose of 5 mg/kg orally for 4 weeks
In the present study, kidneys of rats of Group C revealed varied size of glomerulus i.e., both large and small glomerulus, with either decrease or increase in the periglomerular space. These changes are in accordance with the experiment done by Hemalatha et al [26] who exposed the rats to arsenic as Sodium Arsenite in the dose of 300mg/kg, orally, daily for a period of 2 weeks.
We observed infiltration of inflammatory cells in the arsenic-exposed groups though the severity of inflammation was more evidenced in higher dose of arsenic. Earlier studies of Pagrut NK et al[28] and Parrish AR et al[29] have confirmed moderate to severe fatty change, mononuclear cell infiltration in the tubules and reduced glomerular space. These findings are consistent with our results, thus implicating that high doses of sodium arsenite has degenerating effects on renal tubules .However fatty changes observed in the tubules could not be documented in our study.

In the present study, kidneys of rats of Group C revealed severe hemorrhage, which is in accordance with the study done by Chowdhury et al, in which rats were exposed to daily dose of 150ppm of sodium arsenite, daily for 28 days. [27]
In the present study, kidneys of rats of Group C revealed loss of brush border of PCT, desquamation and sloughing of epithelium. These changes are in accordance with the changes seen in study done by Forkan et al [31], who revealed these changes in rats treated with arsenic in the dose of 50ppm, for a period of 90days, daily. Also, these changes were seen in another study done by Noman et al [30]who saw these changes in rats, treated with Sodium arsenite (10mg/kg), daily for a period of 8 weeks.

In the present study, kidneys of rats of Group C revealed inflammatory cells and necrosis. These studies are in accordance with the study done by Chowdhury et al[27], who exposed the rats to Sodium arsenite in the dose of 150ppm, daily for 28 days. These changes can also be correlated to the changes seen in the study done by Noman et al [30]who gave the arsenic orally in the dose of 10mg/kg, daily for 8 weeks.

In the present study, vascular congestion was seen which is in accordance with the study done by Hemalatha et al,[26]who delivered similar changes in rats treated with arsenic in the dose of 10mg/kg, daily for 4 weeks. Another study done by Noman et al[30]also revealed a similar picture in rats exposed to daily dose of 10mg/kg of arsenic, daily for 28 days.

The changes observed in kidney of the rats of test group can be correlated with the studies done before, because of the same dosage and time duration followed, which resulted in the tissue injury from the arsenic.

While comparing the histopathological injury of organs with their accumulated arsenic, it was seen histological damage actually escalated with the increasing concentration of arsenic. This finding is coexistent with the fact that the damages to individual organ does not correlates with how much, arsenic is being gathered in that organ, here the architecture of tissue and interaction of arsenic with those tissue play a vital role.[31]

Conclusion:

We conclude from the present work that experimental administration of Arsenic in rats was associated with histological changes in the test groups. The kidneys were involved in both the test groups and the magnitude of histopathological changes were proportional to the dose administered low (50 mg / kg) versus higher dose of Arsenic (100mg/kg b.w) These findings can be corroborated with the possible role of arsenic in systemic disorders of human populations who are continuously being exposed to arsenic through drinking water and food stuff as well.

References

1. Dinodia GS, Gupta RK, Jain KL. Effect of cadmium toxicity on liver glycogen in some fresh water fishes. Proc. XI National Symp. Environ 2002; 236-38.
2. Foulkes EC. Biological effects of heavy metal in Metal carcinogenesis. Vol II. CRC Press, Florida, USA 1990.
3. Roy P, Saha A. Metabolism and toxicity of arsenic: a human carcinogen. Current science 2002; 82 (1):38-45.
4. Tamaki S, Frankenberger WT. Environmental biochemistry of arsenic. Reviews of Environmental Contamination and Toxicology 1992; 124: 79–110.
5. Singh TS and Pant KK. Equilibrium, kinetics and thermodynamic studies for adsorption of As (III) on activated alumina. Sep. Purif. Technol, 2004; 36: 139-147.
6. Aronson SN. Arsenic and old myths. R.I. Med 1994; 77: 233-34.
7. Shalat, S L, Walker DB, Finnell RH. Role of arsenic as a reproductive toxin with particular attention to neural tube defects. Journal of Toxicology and Environmental Health. 1996; 48: 253-272.
8. Ko RJ. Causes, epidemiology and clinical evaluation of herbal poisoning. Clin. Toxicol 1999; 37:697-08.
9. Kew J, Morris C, Aihie A, et al. Arsenic and mercury intoxication due to Indian ethnic medicine. BMJ 1993; 306:506-07.
10. Treleaven J, Meller S, Farmer P, Birchall D ,Goldman J, Pillar G,et al. Arsenic and Ayurveda. Leuk Lymphoma 1993; 10:343-45.
11. Shen ZX, Chen GQ, Ni JH, Li XS, Xiong SM, Qiu QY *et al*. Use of arsenic trioxide in the treatment of acute myelocytic leukaemia (AML): II. Clinical efficacy and pharmacokinetics in relapsed patients. Blood 1997; 89:3354-60.
12. Matschullat J. Arsenic is the geosphere –a review. The science of the Total Environment 2000; 249:297-12.
13. Mukherjee A, Sengupta MK, Hossain MA, Ahamed S , Das B , Nayak B et al. Arsenic contamination in groundwater: a global perspective with emphasis on the Asian scenario. *J. Health Pol. Nutri.*2006; 24:142–63.
14. Kingston RL, Hall S, Sioris L. Clinical observations and medical outcomes in 149 cases of arsenate ant killer ingestion. J Toxicol Clin Toxicol 1993; 31:581-91.
15. Schoolmeester WL, White DR. Arsenic poisoning. South Med J 1980; 73: 198-08.
16. Mueller PD, Benowitz NL.Toxicological causes of acute abdominal disorders. E*merg Med Clin No Am* 1989; 7:667-82.
17. Tseng WP, Chu HM, How SW, Fong JM, Lin CS, Yeh S. Prevalence of skin cancer in an epidemic area of chronic arsenicism in Taiwan J Natl Cancer Inst 1968; 40:453-63.
18. Pershagen G. The epidemiology of human arsenic exposure. In: BA Flower (ed.) Biological and environmental effects of arsenic (1st edn.) Elsevier, Amsterdam 1983.
19. Axelson O. Arsenic compounds and cancer. J. Toxicol. Environ. Health. 1980; 6:1229.

20. S. J. Flora, S. Chouhan, G. M. Kannan, M. Mittal, H.Swarnkar .Combined administration of taurine and monoisoamyl DMSA protects arsenic induced oxidative injury in rats. Oxid. Med. Cell. Longev. 2008; 1: 39-45.
21. Yuping LM, Sultan M, Habeebu P, et al. Chronic combined exposure to cadmium and arsenic exacerbates nephrotoxicity, particularly in mettalothionein-I/II null mice. Appl. Toxicol 2000; 147: 157-66.
22. Murphy EA, Aucott M. An assessment of the amounts of arsenical pesticides used historically in a geographical area. Science of the Total Environment 1998;218: 89–101
23. Christen K. The arsenic threat worsens. Environmental Science and Technology 2001; 35: 286A–291A
24. Mettam G, Nandi D, Patra RC, Swarup D .Oxidative stress indices and plasma biochemical parameters during oral exposure to arsenic in rats. Food Chem Toxicol 2006, 44: 1579.
25. Hsueh YM, Chung CJ, Shiue HS, Chen JB, Chiang SS, Yang MH, et al. Urinary arsenic species and CKD in a Taiwanese population: A case-control study. Am J Kid Dis 2009; 54:859–70.
26. Hemalatha P, Reddy GA, Rani UM, Anandkumar A, Shivkumar P. Arsenic induced Histological Alterations in Various Organs in Rats. Int. J. Lif Sc. Bt & Pharm. Res 2013: 119-27.
27. Chowdhury D.U.S., Islam S., Akter R., Khaleda L., Rahman Z., Al-Forkan M. A study on the effect of arsenic on tissue histology and its deposition pattern in various organs of wistar albino rats. Eur. J. Pharmacol. Med. Res. 2016; 3(5):580–587.
28. Nileshkumar Pagrut, Subha Ganguly, Shashi Tekam, Rinku Pendharkar and Vikas Kumar. Histological alterations in arsenic induced various organs in rats. Journal of Entomology and Zoology Studies 2018; 6(3): 1428-1429
29. Parrish AR, Zheng XH, Turney KD, Younis HS, Gandolfi AJ. Enhanced transcription factor DNA binding and gene expression induced by arsenite or arsenate in renal slices. Toxicol Sci. 1999; 50:98–105. [PubMed: 10445758]
30. Noman MSA, Dilruba S, Mohanto CN. Arsenic induced Histological Alterations in Various Organs of Mice. J Cytol Histol 2015; 6(3).
31. Al-Forkan M, Islam S, Akter R, Shameen Alam S , Khaleda L , Rahman Z and Salma Chowdhury DU. A Sub-Chronic Exposure Study of Arsenic on Haematological Parameters, Liver Enzyme Activities, Histological studies and Accumulation Pattern of Arsenic in organs of Male Albino Wistar rats. J Cytol Histol 2016; 5:1-7.