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ORIGINAL ARTICLE

VITAMINS C AND E AMELIORATE THE SODIUM ARSENATE INDUCED INTERSTITIAL FIBROSIS AND TUBULAR ATROPHY IN FETAL KIDNEYS OF ALBINO MICE

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ABSTRACT

Objectives: Epidemiological evidence indicates that exposure to arsenic contaminated drinking water during pregnancy may lead to various congenital abnormalities. The fetal renal tissue is impaired in relation to the developmental stage of kidney and to the amount and duration of arsenic exposure. This study evaluated the role of vitamin C and E in preventing the sodium arsenate induced mutilation of developing renal tissue of albino mice.

Materials & Methods: Albino female mice of BALB/c strain (24), were randomly divided into 4 groups after confirmation of pregnancy. The control group A_1 received 0.1 ml/kg body weight distilled water; group A_2 was given a single dose of sodium arsenate (35 mg/kg) body weight on 8th gestational day (GD) by intra-peritoneal (I/P) injection. The groups A_3 and A_4 were treated with sodium arsenate (35 mg/kg) body weight on 8th GD and vitamin C, (9mg/kg/day) and vitamin E (15 mg/kg/day) by I/P injections, from 8th GD for the rest of the pregnancy period. The fetal kidneys were extracted on 18th GD. Three coronal sections stained with PAS and Masson's trichrome from each kidney, were randomly chosen and examined for tubular injury and interstitial fibrosis.

Results: The mean scoring for histological changes in fetal kidney tubules/10 fields among various study groups was statistically significant ($p < 0.000$). Group A_2 showed severe tubular necrosis and interstitial fibrosis confirmed by Masson's trichrome staining. Group A_3 (vit C) and A_4 (vit E) depicted mild to moderate improvement in tubular necrosis and interstial fibrosis.

Conclusions: This study supports the role of vitamin C and E in ameliorating the developing renal tissue from the oxidative stress induced by sodium arsenate.

Key Words: Antioxidants, interstitial fibrosis, sodium arsenate, α-tocopherol.

INTRODUCTION

Drinking water contamination with heavy metals has been associated with a risk of various congenital anomalies like limb defects, cardiac defects, chromosomal and urinary system anomalies.[1] High levels of arsenic in drinking water have been correlated with the progressive chronic kidney disease.[2] The mechanism of arsenic induced tissue injury is due to the production of reactive oxygen species(ROS) and free radicals which induce DNA damage in animals and humans.[3] Antioxidants and antioxidant enzymes have a synergistic action in scavenging the free radicals and thus play a significant role in preventing many diseases like cancers, atherosclerosis, diabetes and neurodegenerative diseases.[4]

The interstitium of kidney consists of extravascular spaces of renal parenchyma, bounded on all sides by vascular and tubular basement membranes.[5] The renal interstitium is involved in all functions of kidney and affects the GFR, growth and differentiation of parenchymal cells; therefore any changes in the interstitium points towards the renal disease.[6] The destruction of renal tubules and interstitial capillaries with accumulation of extracellular matrix proteins results in interstitial fibrosis.[7]

Arsenic induced effects on animals and humans had been investigated by several researchers. Liu et al. (2000) demonstrated that cadmium administration accentuates the arsenic toxicity and caused a severe glomerulonephritis and interstitial fibrosis in mice kidneys.[8] Chu et al. (2012) revealed that arsenic trioxide lead to cardiac fibrosisin guinea pigs.[9] Ferzand et al (2008) investigated the histopathological changes in arsenic fed mice and establishedthe severe necrotic and degenerative changes in liver and kidney.[10]

The antioxidants like ascorbic acid and α-tocopherol prevents the lipid peroxidation and reduces the oxidative stress.[11] The search for finding the cheap and

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effective measures for combating the arsenic toxicity was commenced by various researchers.[12] Banerjee et al. (2009) reduced the arsenic induced fibrosis in liver with vitamin C.[13] Pan et al. (2011) diminished the liver fibrosis in rats with grape seed extract which is rich in antioxidants.[14] Umar (2007) used spinach extract to lessen the ROS and thus removed the arsenic burden in rats.[15] This study aims to determine the antioxidant potential of vitamin C and E in preventing the interstitial fibrosis in developing kidneys of fetuses, exposed to arsenic through contaminated drinking water. These vitamins are freely available, inexpensive and can be used during pregnancy.

MATERIALS AND METHODS

Albino mice of BALB/c strain (twenty four females and eight males), 10 weeks old and weighing 30-35 gm were kept under controlled environments (temperature 22 ± 1˚C) and humidity (40%-60%); in experimental research laboratory of University of Health Sciences Lahore. The animals were allowed to acclimatize for 7 days and were provided with standard rodent diet and distilled water ad libitum. The photoperiod was kept as 12 hour light/dark cycle. Three female mice were kept overnight with a single male for purpose of mating. The day, when vaginal plug appeared, was regarded as gestational day (GD) one. The pregnant females were randomly divided into four groups of six females each and placed in marked cages (n = 6). A_1 served as a control and the other three (A_2, A_3 and A_4) as experimental groups. Group A_1 received 0.1 ml/kg body weight distilled water by I/P injection for 18 days. Group A_2 animals were injected with a single I/P injection of sodium arsenate (35 mg/kg body weight) dissolved in distilled water, on 8th day of gestation. Groups A_3 and A_4 females received sodium arsenate (35 mg/kg) on the 8th GD and vitamin C (9 mg/kg/day) and E (15 mg/kg/day) by I/P injections respectively, from 8th day for the rest of the pregnancy period.[16] The gestation period of mice is about 19 to 21 days and sodium arsenate was given on 8th day of gestation which is the start of embryonic period in which organogenesis takes place. Intra peritoneal injections given to avoid confounding factors of arsenate absorption by oral route and maximal transfer by placental circulation to fetuses. The doses were given according to previous literature.

The fetuses were extracted from the uterus by anaesthetizing the animals on 18th day of gestation to elude the risk of losing the fetal tissue by spontaneous abortions. The fetuses were dissected and the fetal kidneys were washed with distilled water and fixed in 10% formalin solution for histological study by standard procedures. Periodic acid – Schiff stained sections of 5 µm thickness, were studied under a light microscope (Leica DM 1000) for thickening of basement membranes, mesangial cell proliferation and degenerative changes of tubules. The interstitial fibrosis was confirmed by Masson's trichrome staining. Three coronal sections from each kidney were randomly chosen and examined both qualitatively and quantitatively for the tubules and the interstitium. The observation bias was eliminated by coding the origin of group and it was un-coded after the results were interpreted.

Tubular Indices

The tubules were randomly selected from ten fields in corticomedullary region. They were assessed for cellular distension, cellular vacuolation and condition of their lumen as histological variables and scored as:

1 – 3 = Mild change
4 – 6 = Moderate change
7 – 9 = Severe change

Mean scores of histological changes were calculated. The frequency of histological changes in renal tubules was expressed in percentage.

Interstitial Indices

Interstitial fibrosis was estimated similarly as tubular injury score, in cortex and medulla separately and expressed as mild, moderate and severe. The interstitial fibrosis was confirmed by Masson's trichrome staining**.**

Statistical Analysis

The statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 18.0. Mean and standard deviations were calculated for the numerical variables. ANOVA was used to assess the mean difference among the groups. The post-hoc Tukey test was applied to assess the difference of means between the groups. The p value of ≤ 0.05 was considered statistically significant.

RESULTS

The control group A_1 showed the normal organization of renal corpuscles and tubules (Fig. 1a). The group A_2 treated with sodium arsenate, illustrated the diffuse enlargement of glomerular tuft, capillary lumens were difficult to observe. There was proliferation of mesangial and endothelial cells and confluence of podocytes with parietal and tubular epithelium leading to obliteration of Bowman's spaces. Thickening of glomerular capillaries basement membranes were observed (Fig. 1b). Degenerative changes were observed in all segments of proximal convoluted tubules of group A_2. The tubules also showed vacuolization and apoptotic changes and deposition of PAS +ve material on luminal surfaces (Fig. 1b).

In group A_3, the renal corpuscles showed clear Bowman's spaces, glomerular capillaries had thin basement membranes (Fig 1c yellow arrow). Mesangial cell proliferation was not observed, the tubules showed the deposition of PAS +ve material on the luminal

surface (Fig 1c). The group A_4, demonstrated the extenuation of glomerular tuft but the mesangial cellularity was not observed, Bowman's spaces were clear, glomerular capillaries had thin basement membranes and endothelial thickening were not observed (Fig. 1d). The tubules showed the presence of PAS +ve material on the luminal surface (Fig. 1d).

Micrometric analysis of the tubular changes revealed moderate to severe tubular atrophy in group A_2. Whereas, the groups A_3 & A_4 showed the reduction in tubular degenerative changes from mild to moderate. The data are shown in Table 1. The mean scoring for histological changes in fetal kidney tubules/10 fields among various study groups was statistically significant Table 2. The difference between the groups by test Tukey was significant among all groups (Table 2a).

The presence of interstitial fibrosis was confirmed by Masson's trichrome stain. Blue staining reaction was observed in group A_2 where interstitial fibrosis was exhibited as patchy, striped pattern with tubular atrophy. There was preferential involvement of medulla (Fig. 2b). The groups A_1 and A_3 showed normal staining reaction, while group A_4 depicted mild to moderate interstitial fibrosis (Fig. 2c and 2d**),** Table 1**.**

Table 1: *Comparison of Tubular Histological changes/10 Fields from Mice Fetal Kidneys among Various Groups.*

Tubular Injury Score	*A_1 Control*		*A_2 Sodium Arsenate*		*A_3 Sodium Arsenate + Vit. C*		*A_4 Sodium Arsenate + Vit. E*		*Total*
	Freq	*%*	*Freq*	*%*	*Freq*	*%*	*Freq*	*%*	
Mild change	40	100.0	-	-	40	100.0	7	17.5	54.4%
Moderate change	-	-	21	52.5	-	-	33	82.5	33.8%
Severe change	-	-	19	47.5	-	-	-	-	11.9%
Total	40	100.0	40	100.0	40	100.0	40	100	100.0%

Table 2: *Comparison of Mean Scoring for Histological changes in Fetal Kidney Tubules per 10 Fields among Various Groups.*

Parameter	*A_1 Control (n = 40)*		*A_2 Sodium Arsenate (n = 40)*		*A_3 Sodium Arsenate + Vit C (n = 40)*		*A_4 Sodium Arsenate + Vit E (n = 40)*		*p-value*
	Mean	*S.D*	*Mean*	*SD*	*Mean*	*SD*	*Mean*	*SD*	
Scoring of histological changes in tubules.	0.9	0.2	6.9	0.5	2.0	0.3	4.7	0.6	0.000*

*The mean difference is statistically highly significant.

Table 2a: *Multiple Comparison of Mean Scoring for Histological changes in Fetal Kidney Tubules per 10 Fields among Various Groups according to Tukey Test.*

Comparison among Groups		*Mean Difference*	*Level of Significance*
Groups (α)	*Group Compared (β)*	*(α-β)*	*p-value*
(A_1)	(A_2)	-6.0	0.000*
	(A_3)	-1.1	0.000*
	(A_4)	-3.8	0.000*
(A_2)	(A_3)	5.0	0.000*
	(A_4)	2.3	0.000*
(A_3)	(A_4)	-2.7	0.000*

*The mean difference is statistically highly significant among all groups.

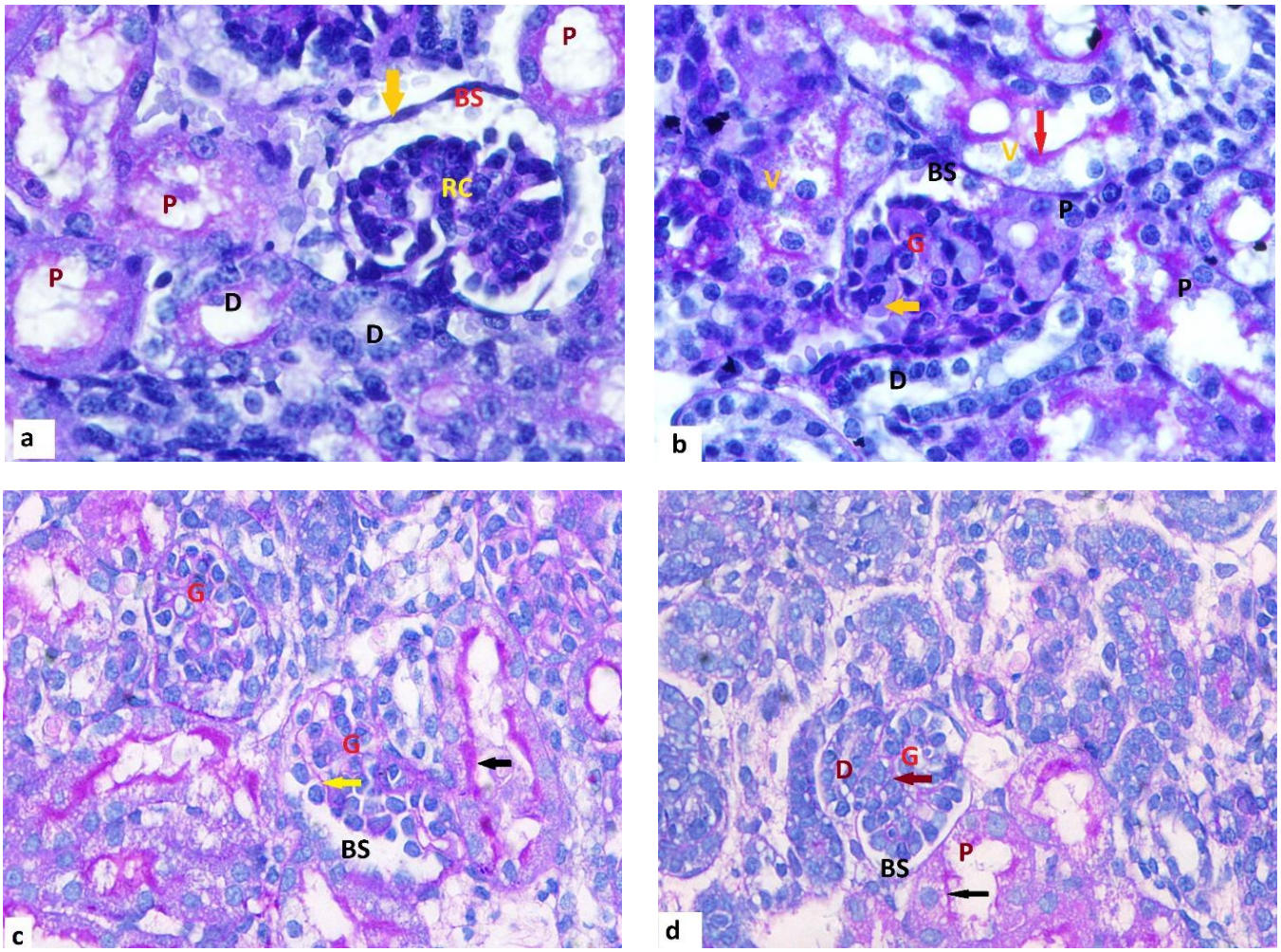

Fig. 1: *Photomicrographs of mice fetal kidney; PAS; X400. a. Shows (Gp A_1), the renal corpuscle (RC) as a spherical structure composed of thin capsule lined by squamous epithelium (yellow arrow), clear appearing Bowman's space (BS), surrounded by proximal (P) and distal (D) convoluted tubules. b. (GpA_2) shows glomerulus (G) with global mesangial hypercellularity obliterating the Bowman's space (BS), endothelial thickening (yellow arrow), tubular vacuolization (V) and deposition of PAS +ve material on luminal surface (red arrow). c. (Gp A_3) shows renal corpuscles with glomerulus (G), surrounded by clear Bowman's spaces (BS), the endothelial basement membranes are thin (yellow arrow). The tubules show PAS positive material on luminal surface (blackarrow). d. (Gp A_4) shows renal corpuscle with extenuation of glomerular tuft (G), the endothelial basement membranes are thin (brick red arrow). The Bowman's space (BS) is enclosed between the two layers. The tubules show PAS positive material on luminal side (black arrow).*

BS	=	Bowman's space	P	=	Proximal convoluted tubule
D	=	Distal convoluted tubule	RC	=	Renal corpuscle
G	=	Glomerulus	PAS	=	Periodic Acid Schiff
Gp	=	Group	V	=	Vacuolization

DISCUSSION

Sodium arsenate induces oxidative stress and increases the production of reactive oxygen species which induces the renal tissue damage. Nephrotoxicity of arsenic is due to its accumulation in the renal tissue during its excretion and thus affects the tubules.[17] The current study encompassed the role of vitamins C and E as antioxidants in preventing the arsenic induced renal tissue impairment. In sodium arsenate treated group (A_2) both glomerular and tubular damage was observed in histological preparations (Fig. 1b). The tubular damage was probably due to direct injury triggered by ROS and free radicals generated by sodium arsenate.[18] Glomerulonephritis along with tubular atrophy with arsenic had been reported earlier by Liu et al. (2000).[8]

Vitamin C, due to its antioxidant potential, scavenges the free radicals from kidney and reduces the arsenic burden.[19] Therefore, group A_3 treated with sodium arsenate and vitamin C simultaneously showed marked improvement in mesangial cellularity, basement

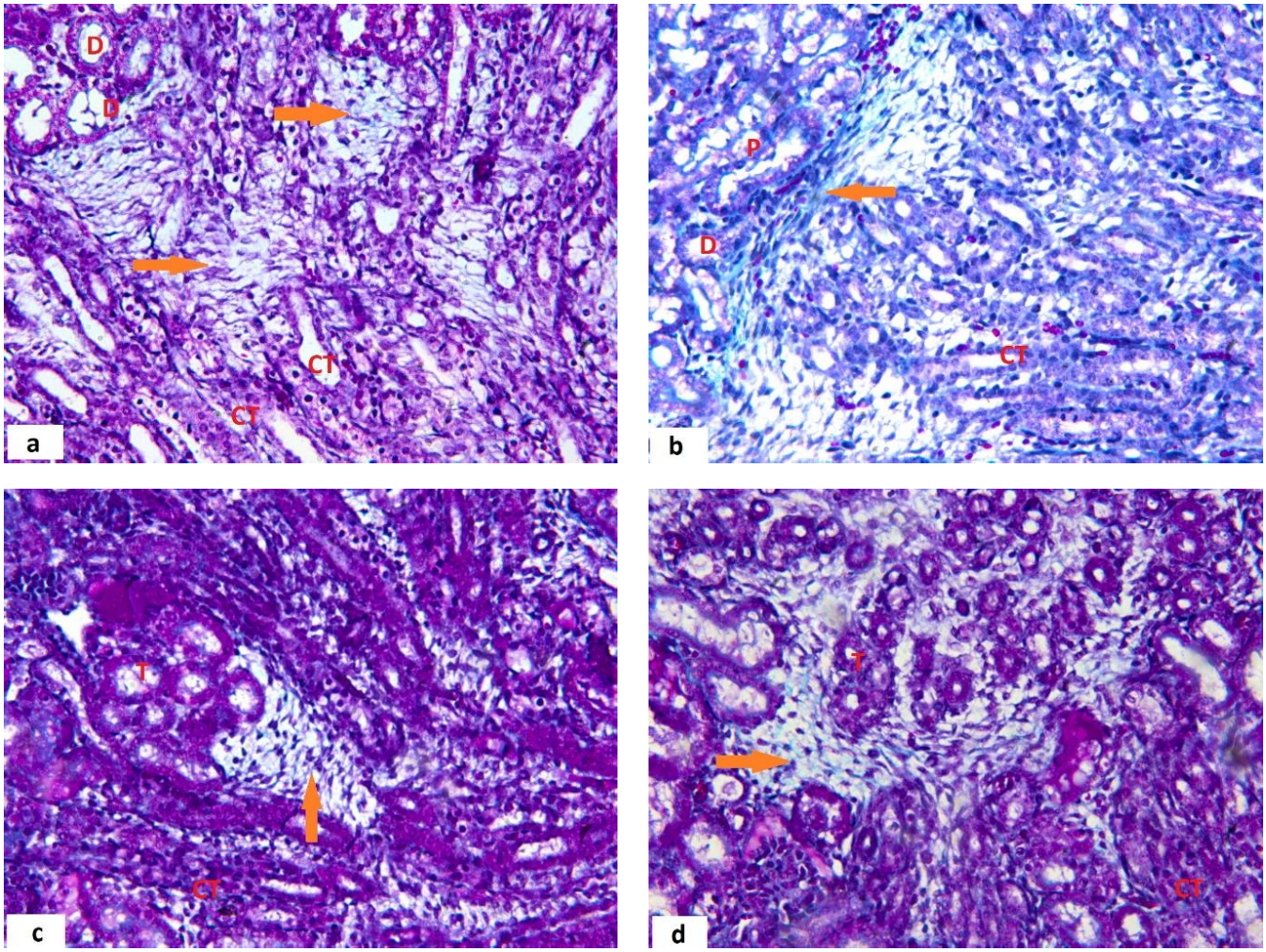

Fig. 2: *Photomicrographs of mice fetal kidney; Masson's trichrome; X200. a. Shows (Gp A_1), well defined distal tubules (D), collecting tubules (CT) surrounded by interstitial connective tissue (arrows). b. Shows striped pattern of interstitial fibrosis (arrow) in (Gp A_2) with tubular atrophy confirmed by blue staining reaction. c. (Gp A_3) shows medullary tubules (T) and collecting tubules (CT) surrounded by interstitial tissue (arrow).d. (Gp A_4), shows medullary tubules (T) and collecting tubules (CT) surrounded by interstitial connective tissue (arrow).*
Gp = Group D = Distal tubules CT = Collecting tubules T = Tubules

membranes became thin and tubular atrophic changes were also improved (Fig. 1c). Al-Attar (2011) described that supplementation of vitamin E along with heavy metals in mice minimizes the histological alterations in kidney and protect the organ from toxic agents and free radicals.[20] Vitamin E neutralizes lipid peroxidation and unsaturated membrane lipids due to its oxygen scavenging effect.[21,22] In this study, the group A_4 injected with vitamin E along with sodium arsenate showed marked improvement in glomerular and tubular atrophic changes (Fig. 1d).

Micrometric analysis of tubular injury scores showed moderate to severe necrotic and degenerative changes in tubules of group A_2 treated with arsenic only (Table 1). These effects of arsenic were attributed due to its physical accumulation in renal tissue and injury caused by ROS. Birri et al. (2010) gave male Wister rats different doses of arsenic in drinking water and demonstrated deleterious effects on tubules with periglomerular accumulation of arsenic along with copper.[23] The tubular injury score among groups A_3 and A_4 showed marked improvements in tubular degenerative changes with simultaneous administration of vitamins C & E along with sodium arsenate respectively (Tables 1, 2, 2a). These effects of vitamin C&E were ascribed because these vitamins boost the antioxidant system by decreasing the arsenic burden in tissues and preventing the lipid peroxidation.[11] Chinoy and Shah (2004) in their histocytometry study of kidney tubules and glomeruli revealed that administration of antidotes along with As_2 O_3 brought about a significant recovery in degenerative changes.[24]

The group A_2 (arsenate only group) showed moderate to severe interstitial fibrosis (Fig. 2b) which was a

result of direct toxic effects of sodium arsenate and due to the production of ROS which lead to increase in collagen production and thus affects the renal functions. Chu et al, (2012) demonstrated similar effects with $As_2 O_3$ causing cardiac fibrosis in guinea pigs.[9] Liu et al (2000) had earlier established that combination of arsenic and cadmium produced massive renal interstitial fibrosis in mice.[8] Children exposed to arsenic soiled drinking water in Chile were reported to have chronic lung disease and pulmonary interstitial fibrosis.[25]

The co-administration of vitamin C or E with arsenate in groups A_3 & A_4 respectively showed marked improvements in interstitial fibrosis (Fig. 2c & d). Sing et al. (2010) demonstrated in their study that arsenic exposed mice lungs showed necrosis and degenerative changes in bronchial epithelium and thickening of alveolar septa. These effects were antagonized by jaggery feeding which is rich in antioxidants.[26] Yu et al. (2013) experimented with Chinese Dragon-Li cats and administered $As_2 O_3$ along with resveratrol, the protective effects of resveratrol increased the glutathione peroxidase activity and methylation of arsenic which is excreted in urine thus reducing the arsenic burden in renal tissue.[27] The involvement of interstitial tissue and destruction of tubules with sodium arsenate was suggestive of the progressive kidney damage; however, vitamins C and E decreased the interstitial fibrous tissue, indicating recovery from arsenic induced injury.

This study ***concludes*** that vitamins C and E had ameliorated the sodium arsenate induced injurious effects on fetal renal tissue in albino mice.However, further studies on animals and humans are needed to understand the effects of arsenic exposure and arsenic contaminated drinking water on pre-conceptional and postconceptional periods.

Disclosure of Interest

The authors report no conflict of interest.

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REFERENCES

1. Dolk H, Vrijheid M. The impact of environmental pollution on congenital anomalies. British Medical Bulletin, 2003 Dec. 1; 68 (1): 25-45.
2. Soderland P, Lovekar S, Weiner DE, Brooks DR, Kaufman JS. Chronic kidney disease associated with environmental toxins and exposures. Advances in chronic kidney disease, 2010 May 31; 17 (3): 254-64.
3. Jomova K, Jenisova Z, Feszterova M, Baros S, Liska J, Hudecova D, Rhodes CJ, Valko M. Arsenic: toxicity, oxidative stress and human disease. Journal of Applied Toxicology, 2011 Mar. 1; 31 (2): 95-107.
4. Fang YZ, Yang S, Wu G. Free radicals, antioxidants, and nutrition. Nutrition, 2002 Oct. 31; 18 (10): 872-9.
5. Lemley KV, KRIz WI. Anatomy of the renal interstitium. Kidney international, 1991 Mar. 1; 39 (3): 370-81.
6. Kaissling B, Le Hir M. Characterization and distribution of interstitial cell types in the renal cortex of rats. Kidney international, 1994 Mar. 1; 45 (3): 709-20.
7. Fukagawa M, Noda M, Shimizu T, Kurokawa K. Chronic progressive interstitial fibrosis in renal disease—are there novel pharmacological approaches? Nephrology Dialysis Transplantation, 1999 Dec. 1; 14 (12): 2793-5.
8. Liu J, Liu Y, Habeebu SM, Waalkes MP, Klaassen CD. Chronic combined exposure to cadmium and arsenic exacerbates nephrotoxicity, particularly in metallothionein-I/II null mice. Toxicology, 2000 Jul. 5; 147 (3): 157-66.
9. Chu W, Li C, Qu X, Zhao D, Wang X, Yu X, Cai F, Liang H, Zhang Y, Zhao X, Li B. Arsenic-induced interstitial myocardial fibrosis reveals a new insight into drug-induced long QT syndrome. Cardiovascular research, 2012 Oct. 1; 96 (1): 90-8.
10. Ferzand R, Gadahi JA, Saleha S, Ali Q. Histological and haematological disturbance caused by arsenic toxicity in mice model. Pakistan journal of biological sciences: PJBS. 2008 Jun; 11 (11): 1405-13.
11. Ramanathan K, Balakumar BS, Panneerselvam C. Effects of ascorbic acid and a-tocopherol on arsenic-induced oxidative stress. Human & experimental toxicology, 2002 Dec; 21 (12): 675-80.
12. Kalia K, Flora SJ. Strategies for safe and effective therapeutic measures for chronic arsenic and lead poisoning. Journal of occupational health, 2005; 47 (1): 1-21.
13. Banerjee P, Bhattacharyya SS, Bhattacharjee N, Pathak S, Boujedaini N, Belon P, Khuda-Bukhsh AR. Ascorbic acid combats arsenic-induced oxidative stress in mice liver. Ecotoxicology and environmental safety, 2009 Feb. 28; 72 (2): 639-49.
14. Pan X, Dai Y, Li X, Niu N, Li W, Liu F, Zhao Y, Yu Z. Inhibition of arsenic induced-rat liver injury by grape seed exact through suppression of NADPH oxidase and TGF-β/S mad activation. Toxicology and applied pharmacology, 2011 Aug. 1; 254 (3): 323-31.
15. Umar BU. Effect of hexane extract of spinach in the removal of arsenic from rat. Bangladesh Journal of Pharmacology, 2007; 2 (1): 27-34.
16. Stump DG, Holson JF, Fleeman TL, Nemec MD, Farr CH. Comparative effects of single intraperitoneal or oral doses of sodium arsenate or arsenic trioxide during in utero development. Teratology, 1999 Nov. 1; 60 (5): 283-91.
17. Singh AP, Goel RK, Kaur T. Mechanisms pertaining to arsenic toxicity. Toxicology international, 2011 Jul; 18 (2): 87.
18. Hughes MF. Arsenic toxicity and potential mechanisms of action. Toxicology letters, 2002 Jul. 7; 133 (1): 1-6.
19. Rana SV. Protective effect of ascorbic acid against oxidative stress induced by inorganic arsenic in liver and kidney of rat.
20. Al-Attar AM. Antioxidanteffect of vitamin E treatment on some heavy metals-induced renal and testicular injuries in male mice. Saudi journal of biological sciences, 2011 Jan. 31; 18 (1): 63-72.
21. Aldana L, Tsutsumi V, Craigmill A, Silveira MI, de Mejia

EG. α-Tocopherol modulates liver toxicity of the pyrethroidcypermethrin. Toxicology letters, 2001 Nov. 30; 125 (1): 107-16.
22. John S, Kale M, Rathore N, Bhatnagar D. Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. The Journal of nutritional biochemistry, 2001 Sep. 30; 12 (9): 500-4.
23. Birri PN, Pérez RD, Cremonezzi D, Pérez CA, Rubio M, Bongiovanni GA. Association between As and Cu renal cortex accumulation and physiological an histological alterations after chronic arsenic intake. Environmental research, 2010 Jul. 31; 110 (5): 417-23.
24. Chinoy NJ, Shah SD. Beneficial effects of some antidotes in fluoride and arsenic induced toxicity in kidney of mice. Fluoride, 2004 Aug. 1; 37 (3): 151-61.
25. Mazumder DG. Effect of drinking arsenic contaminated water in children. Indian pediatrics, 2007 Dec. 1; 44 (12): 925.
26. Singh N, Kumar D, Lal K, Raisuddin S, Sahu AP. Adverse health effects due to arsenic exposure: modification by dietary supplementation of jaggery in mice. Toxicology and applied pharmacology, 2010 Feb. 1; 242 (3): 247-55.
27. Yu M, Xue J, Li Y, Zhang W, Ma D, Liu L, Zhang Z. Resveratrol protects against arsenic trioxide-induced nephrotoxicity by facilitating arsenic metabolism and decreesing oxidative stress. Archives of toxicology, 2013 Jun. 1; 87 (6): 1025-35.