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Arsenic-mineral interaction and ameliorative effect of vitamin E supplementation in arsenic exposed goats

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ABSTRACT

This study was aimed at investigating the arsenic-mineral interrelationship and ameliorative efficacy of dietary vitamin E supplementation in arsenic (As) exposed kids. Three treatments, groups (T1, control; T2, 60 mg As kg^{-1} diet as sodium arsenite; T3, 60 mg As kg^{-1} diet as sodium arsenite + 250 IU vitamin E kg^{-1} diet) were formed with seven kids in each group in a randomized block design. Blood samples were collected on day 0, 45, 90, 135 and 180 of the experiment and analyzed for hemato-biochemistry, serum minerals and thyroid hormones. At the end of the experiment, goats were sacrificed to analyze mineral status of different vital organs. Results showed that inclusion of As in the diet adversely affected different kidney and hepatic affliction markers (hemoglobin, total protein, globulin, urea, creatinine, thyroid hormones) as well as serum and tissue mineral (Cu, Zn, Fe, Mn, Se) concentrations. Supplementation of vitamin E (T3) at 250 IU kg^{-1} diet could partially ameliorate the adverse effects caused by 60 mg As kg^{-1} diet.

Key words: Arsenic, kids, mineral retention, serum minerals, thyroxine

INTRODUCTION

Arsenic (As) is one of the most prevalent toxic element around the world (Naujokas et al., 2013). It is now emerging as a serious candidate for causing damage to human and animal health (Naujokas et al., 2013; Mandal, 2017; Zubair et al., 2018a, b). Animals are the silent and worst sufferers of this problem (Keshavarzi et al., 2015; Mandal, 2017; Zubair and Martyniuk, 2018). The health effect is mediated either directly by retention of As in tissues or by interfering with other essential element metabolism (Goyer and Clarkson, 2008; Mohanta et al., 2016; Zubair and Martyniuk, 2018). Arsenic impairs organ functions particularly that of liver and kidney (Mohanta et al., 2014a; Mohanta and Garg, 2018; Zubair and Martyniuk, 2018) through oxidative stress (Mohanta et al., 2015; Ganger et al., 2016).

Moreover, toxic elements are known to interfere with metabolism of essential trace elements leading to disturbances in the homeostasis of essential elements (Goyer and Clarkson, 2008; Mohanta et al., 2014b), which are essential for many physiological functions in the animal's body. Therefore, we were interested in whether As exposure disrupts levels of essential trace elements and other toxic elements in the serum and tissues along with the blood biochemistry.

Vitamin E is well known to act as an anti-oxidant, as it protects body tissues against oxidative damage by reducing free radicals (Zubair et al., 2016; Zubair and Martyniuk, 2018). In view of these facts, present study was conducted to test the efficacy of vitamin E as an ameliorative agent against As toxicity for its effect on blood biochemical parameters, serum thyroid hormones and blood and tissues mineral retention in kids.

MATERIALS AND METHODS

The experiment was conducted following international guidelines after approval of Institutional Animal Ethics Committee and the Committee for the Purpose of Control and Supervision of Experiments on Animals. Twenty one male goat kids were procured from Sheep and Goat Section of ICAR-Indian Veterinary Research Institute, Izatnagar, India; dewormed for both ecto- and endo-parasites and acclimatized to the shed environment and feeds for one month's duration. The kids of 4-6 months age were then divided randomly into three equal groups on the basis of their body weight (6.71±0.49 kg). They were maintained under similar managemental conditions in a well-ventilated shed and provided with individual feeding and watering troughs.

Kids were offered a common basal diet comprising of concentrate mixture (27% ground maize grain, 25% soybean meal, 45% wheat bran, 2% mineral mixture and 1% common salt) calculated according to body weight, 100 g wheat straw and about 250-300 g green maize (*Zea mays*) fodder daily to meet their nutrient requirements (NRC, 2007). The analyzed chemical composition of concentrate mixture is presented in Table 1. Experimental feeding was similar in all the groups except that of As, which was added at 60 mg (as aqueous solution of sodium arsenite) kg^{-1} in the diet of T_2 and T_3 animals, while kids in group T_3 were additionally supplemented with 250 IU vitamin E per kg diet. Feed was offered daily at 9.30 AM and orts, if any, were recorded for the whole experimental period of 180 days. Clean and fresh drinking water was provided ad libitum.

Table 1. Chemical composition (% DM basis) of feeds and fodders offered in the experiment

Attribute	Concentrate mixture	Wheat straw	Green maize
Dry matter	94.49	95.84	26.09
Organic matter	94.38	92.80	93.35
Crude protein	22.44	3.43	5.67
Ether extract	2.05	0.90	2.18
Neutral detergent fibre	33.87	81.82	69.37
Acid detergent fibre	9.34	56.68	38.06
Calcium	1.43	1.43	0.97
Phosphorus	0.67	0.14	0.31
Zinc (ppm)	75.1	28.4	38.7
Copper (ppm)	19.3	19.7	25.8
Iron (ppm)	570	92	504
Manganese (ppm)	82.0	3.9	27.3
Cobalt (ppm)	4.38	4.37	3.87
Selenium (ppb)	279	115	68.1
Arsenic (ppb)	460	771	670

About 10 mL of blood samples were collected on day 0, 45, 90, 135 and 180 of the experiment through jugular vein from each kid. Out of this, 2 mL was collected into heparinized tube and the rest into clean and dry test tube and the other blood was separated by centrifuging at 3000 rpm for 10-15 minutes to get serum and stored in clean vials at -20°C for further analysis.

Haemoglobin (Hb) was estimated in the whole blood using Drabkin's solution by cyano-methemoglobin method. The serum samples were analyzed for different biochemical constituents (glucose, total protein, albumin, urea, creatinine and total cholesterol) using diagnostic kits (Span Diagnostics Limited, Surat, India). Serum globulin concentration was calculated as the difference between total protein (TP) and albumin.

Tri-iodothyronine (T_3) and thyroxin (T_4) in serum samples was estimated by radioimmunoassay kits (Immunotech, Radiova, Czech Republic) by competitive immunoanalytical assay. Unknown serum samples and standards were incubated together with ^{125}I triodotyrosine in monoclonal anti-T_3 T_4 antibody-coated tubes. After incubation, the contents of the tubes were aspirated and the bound activity was measured in a gamma counter (Packard, USA).

Serum and tissue samples were analyzed for Ca (Talapatra et al., 1940) and P (AOAC, 2012). Trace elements (copper, zinc, iron, cobalt and manganese) were estimated in their mineral extracts [prepared by wet digestion in triple-acid mixture of nitric acid, sulphuric acid and perchloric acid (4:2:1)] using atomic absorption spectrophotometer (AAS, Model 4141, Electronic Corporation of India Limited, Hyderabad, India).

The data generated in the present experiment were analyzed statistically using IBM SPSS 20.0 (2011). Analysis of variance and univariate model of Generalized Linear Model were used for comparing means among groups, and group and period effects, respectively (Snedecor and Cochran, 1994). Duncan test with least significant difference was used for post-hoc comparison.

RESULTS AND DISCUSSION

Effect of biochemical profile

The data regarding mean blood Hb concentration, serum TP, albumin, globulin, blood glucose, urea, creatinine and cholesterol are presented in Table 2. Blood Hb level showed a significant ($P<0.05$) depression in the 60 ppm As (T_2) group on 180th day of study indicating chronic As toxicity. Similar results were reported in goats fed 50 mg As kg^{-1} diet (Das et al., 2012). This reduction in Hb levels in As exposed animals might be due to suppression of the granulopoietic activity of bone marrow by residual As (Kaneko et al., 2008). It was further observed that vitamin E supplementation of 250 IU vitamin E kg^{-1} diet improved the Hb level, suggesting its partial ameliorative effect on blood Hb. In previous reports, supplementation of vitamin E (50, 100 or 150 IU) kg^{-1} diet could partially restored Hb level in goats (Das et al., 2012).

Serum albumin concentrations were similar ($P>0.05$) among three groups, but TP and globulin levels were significantly ($P<0.05$) reduced in the As exposed kids after 90 th day of exposure indicating an adverse effect of As on the animals. Reduced TP and globulin values were also observed in goats (Zubair and Martyniuk, 2018). However, no difference was observed in vitamin E supplemented animals and control group, which confirms an ameliorative effect against As toxicity. Similar ameliorative effect of vitamin E against As toxicity was observed in poultry (Kalavathi et al., 2011)

The mean values of serum glucose levels were comparable ($P>0.05$) among different groups. Contrary to our observations, increased blood glucose level was observed in goats upon As exposure (Pandey et al., 2005) and the authors attributed it to the stress elicited by As exposure that increased cortisol release by adrenal cortex and resulting into gluconeogenesis (Szinicz and Forth, 1988). However, in our previous study, we did not observe any variation in serum cortisol concentration on As exposure (60 mg of As kg^{-1} diet) in kids (Mohanta et al., 2015). This discrepancy in result might be due to higher concentration of As used i.e. 375 mg vs 30 mg per 15 kg BW (Biswas et al., 2000).

Serum urea and creatinine are sensitive indicators of glomerular filtration and renal function (Kaneko et al., 2008). Kidney dysfunction results in their decreased clearance and therefore increases their blood levels. Serum urea and creatinine concentrations were increased significantly ($P<0.05$) in the As exposed kids after 135th day of experiment, which approached normal values in the kids supplemented with vitamin E. Similar protective effects were observed by vitamin E supplementation in poultry (Kalavathi et al., 2011). Cholesterol levels in the kids were similar in all the three groups indicating that inclusion of 60 mg As kg^{-1} feed did not affect cholesterol values of kids.

Table 2. Effect of vitamin E supplementation on blood haemato-biochemical parameters of kids in different groups

Group	Period (days)					Mean*	SEM	P value
	0	45	90	135	180			
Haemoglobin (g/ dL)								
Control (T1)	10.0	10.4	10.8	10.6	10.0b	10.5	0.20	G=0.049
As 60 ppm (T2)	10.1	10.5	10.3	9.7	8.6a	9.8	0.20	P=0.005
As 60 ppm+Vit-E (T3)	10.0	10.3	10.6	10.2	9.7b	10.2	0.15	G*P=0.516
Total Protein (g/ dL)								
Control (T1)	6.9	7.02	7.35b	7.22b	7.18b	7.19b	0.16	G=0.022
As 60 ppm (T2)	6.92	7.04	6.88a	6.79a	6.58a	6.82a	0.13	P=0.000
As 60 ppm+Vit-E (T3)	6.89	6.98	7.01ab	6.95ab	7.02ab	6.99ab	0.15	G*P=0.157
Albumin (g/ dL)								
Control (T1)	3.23	3.27	3.25	3.24	3.15	3.23	0.09	G=0.039
As 60 ppm (T2)	3.26	3.25	3.28	3.35	3.26	3.29	0.09	P=0.000
As 60 ppm+Vit-E (T3)	3.27	3.21	3.26	3.31	3.27	3.26	0.08	G*P=0.098
Globulin (g/ dL)								
Control (T1)	3.67	3.75	4.10b	3.98b	4.03b	3.97b	0.12	G=0.020
As 60 ppm (T2)	3.66	3.79	3.60a	3.44a	3.32a	3.54a	0.16	P=0.000
As 60 ppm+Vit-E (T3)	3.62	3.77	3.75a	3.64ab	3.75b	3.73ab	0.17	G*P=0.066
Total cholesterol (mg/ dL)								
Control (T1)	79	90	112	115	110	107	3.03	G=0.697
As 60 ppm (T2)	75	110	115	113	107	111	2.59	P=0.000
As 60 ppm+Vit-E (T3)	81	95	111	114	108	107	3.69	G*P=0.481
Glucose (mg/ dL)								
Control (T1)	53.1	54.8	48.2	49.2	49.8	50.5	1.12	G=0.366
As 60 ppm (T2)	52.3	55.7	55.2	58.2	59.5	57.2	1.43	P=0.001
As 60 ppm+Vit-E (T3)	52.6	54.7	56.2	56.7	59.6	56.8	1.26	G*P=0.896
Urea (mg/ dL)								
Control (T1)	34.4	32.6	35.2	32.7a	32.5a	33.3a	0.60	G=0.038
As 60 ppm (T2)	33.9	35.8	39.0	40.6b	40.0b	38.9b	0.63	P=0.000
As 60 ppm+Vit-E (T3)	35.9	33.6	36.5	40.5b	36.3ab	36.7ab	1.16	G*P=0.910
Creatinine (mg/ dL)								
Control (T1)	1.10	1.29	1.32	1.41a	1.39a	1.25a	0.07	G=0.042
As 60 ppm (T2)	1.05	1.38	1.36	1.67b	1.67b	1.52b	0.05	P=0.000
As 60 ppm+Vit-E (T3)	1.28	1.30	1.44	1.54ab	1.48a	1.44ab	0.04	G*P=0.189

*Mean of 45, 90, 135 and 180 d values; T1, Goats in the control group; T2, goats additionally added 60 mg arsenic per kg diet; T2, Goats additionally given 60 mg arsenic and 250 IU vitamin E per kg diet; [ab] Means with different superscripts in column differ significantly ($P<0.05$)

Thyroid hormones, i.e. tri-iodothyronine (T_3) and thyroxine (T_4), and T_4: T_3 ratios were adversely affected by exposure to As in kids and vitamin E supplementation could partially reverse the effect of As (Table 3). In our earlier study, we observed similar adverse effect on thyroid hormones in guinea pigs (Mohanta et al., 2014a). Guo et al. (2018) observed higher arsenic level association with lowered thyroid hormone concentration in pregnant women. Similar effects were seen on the lowering of thyroid hormones on exposure to arsenic (Sun et al., 2016; Ahangarpour et al., 2018).

Table 3. Serum hormonal profile indifferent groups of goat kids

Group	Period (days)					Mean*	SEM	P value
	0	45	90	135	180			
T3 (nmol/L)								
Control (T1)	1.81	1.87	1.84^{a}	1.98	1.91^{a}	1.90	0.10	G=0.046
As 60 ppm (T2)	2.09	1.72	1.81^{ab}	1.96	1.40^{b}	1.72	0.07	P=0.002
As 60 ppm+Vit-E (T3)	2.14	1.97	2.23^{b}	1.75	1.53^{b}	1.87	0.10	G*P=0.014
T4 (nmol/L)								
Control (T1)	39.20	33.90	36.80	32.60^{a}	29.90^{a}	33.30^{a}	1.64	G=0.045
As 60 ppm (T2)	43.10	33.90	32.00	25.20^{b}	17.80^{b}	27.30^{b}	1.94	P=0.000
As 60 ppm+Vit-E (T3)	44.40	37.80	29.50	30.50^{ab}	19.30^{b}	26.80^{b}	1.66	G*P=0.115
T4: T3								
Control (T1)	21.66	18.13	20.00^{a}	16.46^{a}	15.65^{a}	17.56^{a}	0.93	G=0.011
As 60 ppm (T2)	20.62	19.71	17.68^{ab}	12.86^{b}	12.71^{b}	15.87^{b}	1.03	P=0.001
As 60 ppm+Vit-E (T3)	20.75	19.19	13.23^{b}	17.43^{a}	12.61^{b}	14.33^{b}	1.05	G*P=0.047

*Mean of 45, 90, 135 and 180 d values; T1, Goats in the control group; T2, goats additionally added 60 mg arsenic per kg diet; T2, Goats additionally given 60 mg arsenic and 250 IU vitamin E per kg diet; ^{ab}Means with different superscripts in a column differ significantly (P<0.05)

Effect on serum mineral content

Toxic elements may compete or interfere with essential elements (Molin et al., 2008) and/or create disturbances in the homeostasis of essential elements due to their common chemical properties, common sites or mechanisms of absorption and common functional interest etc (Goyer and Clarkson, 2008). The data pertaining to serum macro and micro-minerals in different groups as well as at different time interval is presented in graphical forms treating 0 day values as 100 (Fig. 1-6).

Serum Ca and P levels were marginally depressed, whereas levels of Cu and Mn were reduced significantly (P<0.05) after 90 days of As exposure (Fig. 1-4). A marginal decrease in blood Cu level was reported in goats when exposed to 2 mg As kg^{-1} BW (~38 ppm As) for a period of 6 months (Roy et al., 2009). However, no alterations in serum Cu and plasma ceruloplasmin were observed in pigs fed 30 mg As kg^{-1} diet for 78 days (Wang et al., 2006).

Zinc levels were increased after 45 days of As exposure, which remained elevated upto 90 th day and returned to normal at 135th day of observation but at 180th day again reduced significantly (P<0.05) (Fig. 5). This may be because of increased uptake of Zn to meet its high demand due to As toxicity (Kumar et al., 2011) as supported by non-significant increase of plasma Zn level in As exposed goats (Roy et al., 2009). In our earlier study also we obtained depressed Zn absorption and retention in goats (Mohanta et al., 2014b).

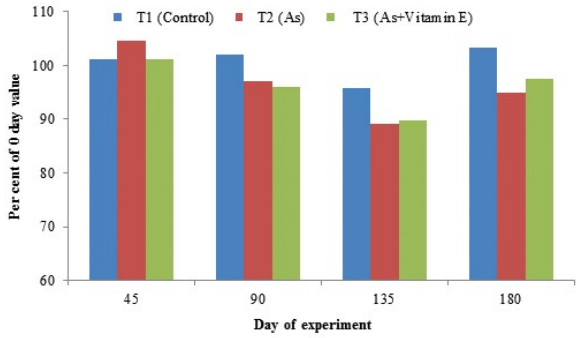

Fig. 1. Effect of arsenic exposure (60 mg kg^{-1} diet) and vitamin E supplementation (250 IU kg^{-1} diet) on serum Ca profile of kids

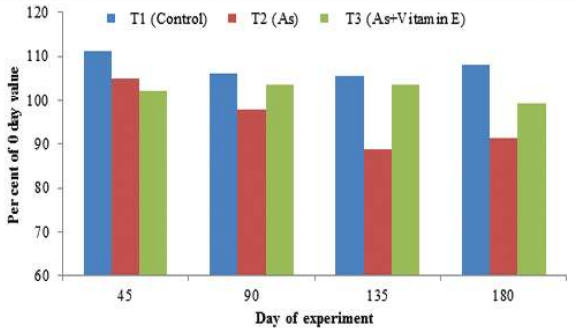

Fig. 2. Effect of arsenic exposure (60 mg kg^{-1} diet) and vitamin E supplementation (250 IU kg^{-1} diet) on serum P profile of kids

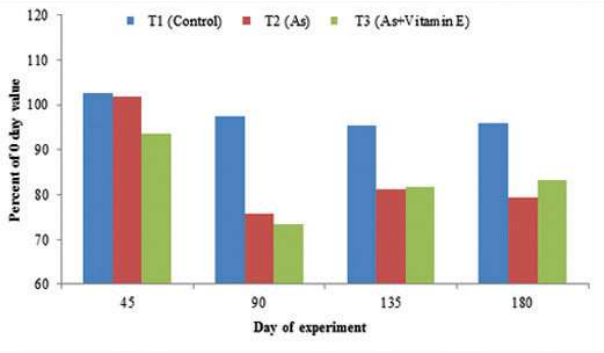

Fig. 3. Effect of arsenic exposure (60 mg kg^{-1} diet) and vitamin E supplementation (250 IU kg^{-1} diet) on serum Cu profile of kids

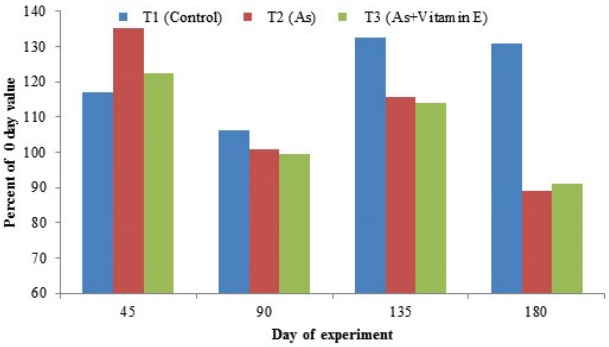

Fig. 4. Effect of arsenic exposure (60 mg kg^{-1} diet) and vitamin E supplementation (250 IU kg^{-1} diet) on serum Mn profile of kids

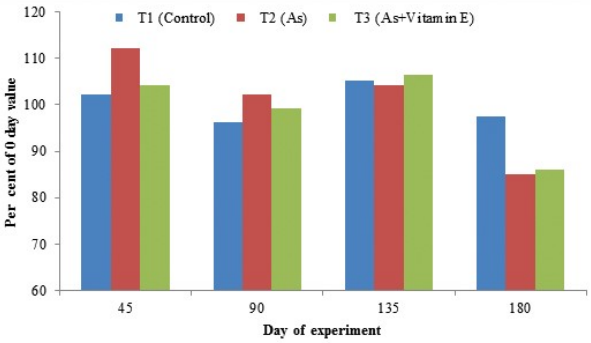

Fig. 5. Effect of arsenic exposure (60 mg kg^{-1} diet) and vitamin E supplementation (250 IU kg^{-1} diet) on serum Zn profile of kids

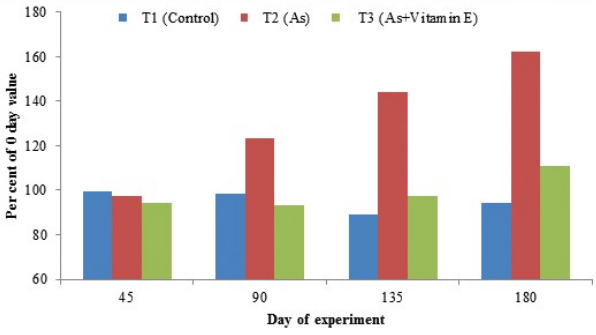

Fig. 6. Effect of arsenic exposure (60 mg kg^{-1} diet) and vitamin E supplementation (250 IU kg^{-1} diet) on serum Fe profile of kids

Serum Fe level was increased in As exposed kids from 135 day onwards, but returned to normal in vitamin E supplemented kids showing potential beneficial effect of vitamin E supplementation (Fig. 6). In other studies, a significant alteration in blood Fe levels were observed in pigs fed 20 and 30 mg As kg^{-1} diet (Wang et al., 2006).

In our earlier report, we found an increased serum arsenic concentration along with decreased selenium level in serum (Mohanta et al., 2015). Supplementation of vitamin E at present level could not bring back selenium content to normal level and the effect was partially ameliorative.

Clinical signs

In the present study, no signs of toxicity were observed during 180 days of study. However, a sign of mild dullness and depression was seen along with a relative rough and dull hair coat in As exposed animals. Contrary to our observations, upon exposure to high dose of As (25 mg As kg^{-1} BW), goats exhibited prominent clinical signs of As toxicity (Biswas et al., 2000; Pandey et al., 2005) like dullness and depression, which progressed into partial loss of appetite, reddish coloured urine, rough body coat with erected hairs, and profound muscular weakness. They further reported progressive signs of polyuria, incoordination, inability to get up and salivation along with increased respiration and heart rate (Biswas et al., 2000).

Effect on organ weight

Weight of liver, kidney, lungs, heart, testes, spleen and rumen was comparable among three groups (Table 4). However, a modest ($P>0.05$) increase was noticed in lung weight, whereas heart and testes weight was reduced ($P>0.05$) in As exposed groups, suggesting role of As in causing affection of these organs. Nevertheless, vitamin E supplementation could alleviate these alterations (Table 4).

Table 4. Effect of arsenic on weight of vital organs and their ash content in goat kids

Particular	T1 (Control)	T2 (Arsenic)	T3 (Arsenic+ Vitamin E)	SEM	P value
Mean BW (kg)	15.29	16.09	17.05	0.87	0.751
Organ weight (% BW)					
Liver	20.4	19.5	20.4	0.82	0.905
Kidney	3.6	3.4	3.2	0.14	0.510
Lungs	10.7	12.2	11.3	0.45	0.412
Testes	8.6	7.7	8.0	0.35	0.600
Stomach	32.3	32.8	31.5	2.04	0.973
Heart	4.6	4.0	3.9	0.16	0.214
Spleen	1.6	1.5	1.3	0.09	0.441
Ash (% fresh basis)					
Liver	1.34	1.30	1.42	0.03	0.338
Kidney	1.08	1.16	1.37	0.08	0.388
Lungs	1.13	1.07	1.09	0.02	0.510
Testes	1.08	1.03	0.91	0.06	0.479
Stomach	0.78	0.72	0.67	0.04	0.524
Heart	0.93	0.90	0.85	0.05	0.759
Spleen	1.31	1.44	1.40	0.04	0.496

T1, Goats in the control group; T2, goats additionally added 60 mg arsenic per kg diet; T2, Goats additionally given 60 mg arsenic and 250 IU vitamin E per kg diet

Effect on organ mineral content

The data pertaining to the status of ash, Ca, P, Fe, Cu, Zn and Mn, in different tissues from animals of different groups is presented in Table 5-10. Total ash and calcium content remained similar (P>0.05) in all the analyzed organs of kids among different groups (Table 4, 5) demonstrating that As supplementation did not affect concentration of Ca and total ash in different tissues.

Table 5. Tissue calcium content (%) of kids in different groups (on fresh basis)

Particular	T1 (Control)	T2 (Arsenic)	T3 (Arsenic+ Vitamin E)	SEM	P value
Liver	0.044	0.039	0.038	0.001	0.107
Kidney	0.045	0.043	0.042	0.004	0.952
Lungs	0.053	0.089	0.079	0.013	0.543
Testes	0.041	0.057	0.042	0.004	0.248
Stomach	0.157	0.117	0.102	0.014	0.261
Heart	0.058	0.109	0.091	0.018	0.571
Spleen	0.109	0.085	0.123	0.019	0.749
Muscle	0.043	0.041	0.040	0.02	0.774
Lymph node	0.054	0.047	0.058	0.04	0.845
Pancreas	0.084	0.078	0.105	0.01	0.721

T1, Goats in the control group; T2, goats additionally added 60 mg arsenic per kg diet; T2, Goats additionally given 60 mg arsenic and 250 IU vitamin E per kg diet

Phosphorus (P) content was depressed significantly (P<0.05) in lungs and kidney tissues of As exposed kids (Table 6). As and P both belong to the same group, resulting in a competition for their uptake and utilization (Gonzalez et al., 1995). But, the control and vitamin E supplemented kids showed similar P concentrations in these tissues showing potential ameliorative effect of vitamin E (Table 6).

Table 6. Tissue phosphorus content (%) of kids in different groups (on fresh basis)

Particular	T1 (Control)	T2 (Arsenic)	T3 (Arsenic+Vitamin E)	SEM	P value
Liver	0.339	0.336	0.316	0.01	0.511
Kidney	0.232b	0.189a	0.230b	0.008	0.028
Lungs	0.252b	0.226a	0.242b	0.004	0.016
Testes	0.214	0.224	0.221	0.005	0.711
Stomach	0.120	0.129	0.130	0.005	0.668
Heart	0.226	0.195	0.192	0.015	0.623
Spleen	0.279	0.316	0.431	0.059	0.593
Muscle	0.201	0.181	0.186	0.011	0.754
Abomasum	0.161	0.140	0.198	0.019	0.845
Lymph node	0.342	0.281	0.317	0.021	0.683
Skin	0.223	0.143	0.198	0.024	0.652

T1, Goats in the control group; T2, goats additionally added 60 mg arsenic per kg diet; T2, Goats additionally given 60 mg arsenic and 250 IU vitamin E per kg diet, [ab]Means with different superscripts in a column differ significantly ($P<0.05$)

Significant (P<0.05) increase of Fe content was observed in testis and muscle tissues of 60 ppm As exposed kids as compared to the control and vitamin E supplemented groups, indicating that supplementation of 250 IU vitamin E kg^{-1} diet could not overcome the adverse effect on tissue Fe level (Table 7). Similar to our findings, high dietary As (30 mg kg^{-1} diet) caused marked accumulation of Fe in the liver, bile, pancreas, spleen and thymus along with a significant reduction in kidney and heart Fe level in pigs (Wang et al., 2006). It showed that As affects Fe status of the vital organs and vitamin E supplementation could not produce any beneficial effect on tissue Fe level in kids. This is further supported by the elevated serum Fe concentration in As exposed kids (Fig. 6), in this study.

Table 7. Tissue iron content (mg kg^{-1}) of kids in different groups (on fresh basis).

Particular	T1 (Control)	T2 (Arsenic)	T3 (Arsenic+Vitamin E)	SEM	P value
Liver	60.05	47.92	57.52	5.95	0.874
Kidney	46.89	45.99	39.70	3.97	0.761
Lungs	90.26	99.82	80.87	9.48	0.755
Testes	13.9a	35.81b	31.56b	3.32	0.002
Stomach	75.20	91.42	72.11	6.37	0.455
Heart	37.61	40.55	37.99	1.83	0.807
Spleen	197.7	208.1	212.7	24.01	0.973
Muscle	70.09a	99.31b	111.3b	8.54	0.045
Skin	126.9	113.3	165.8	14.52	0.847
Hair	786.2	682.2	575.7	30.43	0.564
Lymph node	30.25	42.39	29.92	1.02	0.874

T1, Goats in the control group; T2, goats additionally added 60 mg arsenic per kg diet; T2, Goats additionally given 60 mg arsenic and 250 IU vitamin E per kg diet, ^{ab}Means with different superscripts in a column differ significantly (P<0.05)

Significant (P<0.05) reduction in Cu concentration was observed in liver, testes, muscle and lymph node in kids exposed to 60 mg As kg^{-1} diet for 180 days, which indicates adverse effects of As on Cu metabolism (Table 8). Similar to our findings, a significant depression in Cu level of liver was observed in As exposed goats (Roy et al., 2009). However, in monogastric animals, dietary As caused marked accumulation of Cu in liver and/or kidney of rats (Cui and Okayasu, 2008) and pigs (Wang et al., 2006); and reduction of Cu concentrations in liver (Uthus, 2001). It is interesting to observe recovery in the Cu level in most of the organs except liver following supplementation of vitamin E (Table 8).

In the present study, Zn concentrations in organs remained similar among three groups, except testis and heart, which was significantly (P<0.05) altered upon As exposure. Increased testicular Zn level may be attributed to its linkage with As for decreasing its toxicity. In contrast to our findings, significant increase in Zn level was observed in liver and kidney of goats fed 2 mg As kg^{-1} BW for 6 months (Roy et al., 2009). In contrast, hepatic Zn concentrations were unaffected and renal Zn level was increased in rats exposed to 10 or 100 mg inorganic As L^{-1} water for 16 weeks (Cui and Okayasu, 2008). However, in our earlier study, we obtained significantly lowered Zn absorption and apparent retention in goats (Mohanta et al., 2014b). Arsenic exposure increased uptake of 65 mg Zn in the liver, intestine, and kidney indicating increased requirement of Zn for synthesis of various enzymes or proteins like metallothioneins during As toxicity (Kumar et al., 2011). In vitamin E supplemented kids, tissue Zn level was comparable in all the organs except testis, where Zn concentration was still significantly higher than that of the control values (Table 9) indicating only partial amelioration.

Table 8. Copper content (mg kg^{-1}) of organs of kids in different groups (on fresh basis).

Particular	T1 (Control)	T2 (Arsenic)	T3 (Arsenic+Vitamin E)	SEM	P value
Liver	6.10b	1.21a	1.30a	0.86	0.010
Kidney	0.525	0.373	0.538	0.041	0.194
Lungs	2.09	1.66	1.84	0.10	0.256
Testes	1.08b	0.656a	1.17b	0.098	0.044
Stomach	0.618	0.950	1.45	0.199	0.250
Heart	1.94	1.62	1.77	0.10	0.498
Spleen	1.01	0.83	0.99	0.058	0.426
Muscle	0.498b	0.200a	0.545b	0.056	0.046
Skin	0.89	0.56	0.99	0.065	0.412
Hair	3.56	2.09	3.51	0.09	0.548
Lymph node	0.786b	0.456a	0.745b	0.049	0.045

T1, Goats in the control group; T2, goats additionally added 60 mg arsenic per kg diet; T2, Goats additionally given 60 mg arsenic and 250 IU vitamin E per kg diet, [ab]Means with different superscripts in a column differ significantly ($P<0.05$)

Table 9. Tissue zinc content (mg kg^{-1}) of kids in different groups (on fresh basis).

Particular	T1 (Control)	T2 (Arsenic)	T3 (Arsenic+Vitamin E)	SEM	P value
Liver	13.50	16.65	16.04	1.16	0.548
Kidney	12.27	11.89	11.01	0.59	0.712
Lungs	13.76	14.03	14.96	0.76	0.824
Testes	8.97a	12.70b	12.57b	1.98	0.031
Stomach	14.87	15.43	14.77	0.80	0.948
Heart	9.15a	1.76b	9.05a	1.17	0.044
Spleen	14.39	17.91	17.90	0.88	0.176
Muscle	16.14	13.52	21.20	0.75	0.584
Skin	9.83	6.89	11.13	0.63	0.756
Hair	62.19	68.44	66.03	9.62	0.845
Lymph node	11.17	15.15	13.39	1.09	0.880

T1, Goats in the control group; T2, goats additionally added 60 mg arsenic per kg diet; T2, Goats additionally given 60 mg arsenic and 250 IU vitamin E per kg diet, [ab]Means with different superscripts in a column differ significantly ($P<0.05$)

Manganese (Mn) content was significantly (P<0.05) increased in lungs and testes, whereas significantly (P<0.05) decreased in kidneys in the 60 ppm As exposed kids (Table 10). Arsenic exposure did not affect the concentration of Mn in kidney and liver in rats received a dose of 1, 10 and 100 mg L^{-1} of sodium arsenate in drinking water daily for 4 and 16-weeks (Cui and Okayasu, 2008). In vitamin E supplemented kids, Mn values were comparable to control, except that of kidney where the value was still significant (P<0.05) compared to control (Table 10) indicating partial protective effect of vitamin E at present dose rate.

Table 10. Tissue manganese content (mg kg^{-1}) of kids in different groups (on fresh basis)

Particular	T1 (Control)	T2 (Arsenic)	T3 (Arsenic+Vitamin E)	SEM	P value
Liver	1.22	1.03	1.10	0.09	0.708
Kidney	0.447b	0.283a	0.305a	0.030	0.033
Lungs	0.248a	0.519b	0.349ab	0.062	0.048
Testes	0.191a	0.512b	0.364ab	0.056	0.042
Stomach	5.23	7.67	5.49	0.78	0.415
Heart	0.238	0.322	0.223	0.034	0.492
Spleen	0.431	0.438	0.446	0.077	0.998
Muscle	4.73	6.70	7.51	0.58	0.085
Skin	8.56	7.65	11.19	0.980	0.657
Hair	53.05	46.03	38.85	6.10	0.654
Lymph node	0.431	0.387	0.383	0.036	0.583

T1, Goats in the control group; T2, goats additionally added 60 mg arsenic per kg diet; T2, Goats additionally given 60 mg arsenic and 250 IU vitamin E per kg diet, ^{ab}Means with different superscripts in a column differ significantly (P<0.05)

In our previous published article, we found an increased arsenic concentration in tissues and serum along with decreasing trend in selenium content (Mohanta et al., 2015). This is further proof of toxicity of arsenic and its role in tissue oxidative stress. Supplementation of vitamin E at present level could not bring back tissue selenium concentration to control level and the effect was only partially ameliorative.

Altered retention of essential and toxic elements in some organs indicates their altered metabolism in these tissues, as in kids as ruminants there may be different metabolic process involved for their absorption and retention in the body (Suttle, 2012; Mohanta et al., 2014b) and need further investigation to confirm the results. These alterations could partially be alleviated by addition of vitamin E at present dosage.

Gross pathology

Gross pathological lesions were not observed in the kids, except mild congestion in cortico-medullary junction of kidneys indicating mild effect of As in ruminants. On higher doses (25 mg kg^{-1} BW), kids exposed to As exhibited enlargement of the kidney, abomasum and liver along with haemorrhagic changes in abomasum, intestinal mucosa, kidney and spleen (Biswas et al., 2000).

CONCLUSION

The findings from the present study reveals that blood haemato-biochemistry, serum mineral concentrations, thyroid hormonal status and retention of mineral elements by different body organs of kids were adversely affected when exposed to 60 ppm of arsenic through their diet. Supplementation of vitamin E at 250 IU kg^{-1} diet

could partially reduce these adverse effects in the kids, demonstrating alleviation of oxidative stress through vitamin E alone could not alleviate the adverse effects, which may be due to insufficiency of antioxidant or presence of other dominant mechanisms that address arsenic toxicity.

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