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Research Article

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A study on arsenic-induced toxicity in rats

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ABSTRACT

Arsenic, a naturally occurring metalloid element that is present in food, soil and water [1 and 2], induces adverse health effects on all forms of life through polluting ground water and food chains. Because arsenic targets ubiquitous enzyme reactions, it affects nearly all organ systems in humans and other animals [3]. It is a known carcinogen that has been associated with cancers of the skin, lung, urinary bladder, and possibly liver, kidney and prostate in humans. In view of the significance of adverse effects induced by arsenic, the current research was planned with the objective to evaluate its toxic effects on performance, heart, kidney, lung, stomach, intestine and male reproduction in rats.

Key words: Arsenic, NAC and Organ toxicity,

MATERIALS AND METHODS

Male albino rats of *Wistar kyoto* strain weighing about 200-220 g were procured from National Institute of Nutrition, Hyderabad. The animals were housed in solid bottom polypropylene cages. Animals were placed on commercial standard mash feed for rat or mice and provided water *ad libitum.* Experiment was conducted according to the guidelines of Institutional Animal Ethics Committee. Following acclimatization for 10 days, the rats were randomly distributed into 4 groups with 6 animals each and were treated as follows for 4 wks: Group 1: sham control, 2: arsenic control (sod ium arsenite @ 10 mg/kg b. wt. orally for 4 wks), 3: N-Acetyl cysteine (NAC) pre-treatment (300 mg/kg b. wt orally) for two weeks followed by arsenic + NAC (as per above doses for 4 wks) and 4: arsenic + NAC (as per above doses for 4 wks).

Blood samples were collected from the rats on 29th day of experiment by retro-orbital puncture and sera samples were separated to estimate serum LDH, and creatinine. Body weights were recorded at weekly intervals in all the test groups. All rats were euthanized on 29th day and lung, stomach and intestines, testes were collected, weighed and stored at -20^{0}C for further
estimation of testicular LDH and oxidative stress markers in lung, stomach, and intestine. Statistical analysis of experimental data was carried out with SPSS version 15.

RESULTS AND DISCUSSION

The concentration of serum creatinine was increased significantly ($p<0.05$) in group 2 in this study, which may be due to the nephrotoxic potential of sodium arsenite as kidney is one organ that is rich in phospholipids that are prone to arsenic-induced lipid peroxidation of kidney leading to functional deterioration [4]. Thus, increase in serum creatinine in the present study can be related to renal dysfunction. Groups 3 and 4 showed a significant ($p<0.05$) decrease in serum creatinine as compared to the toxic control group 2 (Table 1), which may be attributed to antioxidant action of NAC, which is the precursor for GSH and has been reported to increase creatinine clearance [5]. A significant ($p<0.05$) increase in serum LDH activity was observed in group 2. Groups 3 and 4 showed decrease in the serum LDH activity as compared to group 2 (Table 1). NAC has been reported to possess cardio-protective actions and to decrease serum LDH [6], which may be the reason for findings of this study in groups 3 and 4.

Table 1: Serum parameters in different groups of rats

Group	Serum creatinine concentration (mg/dl) Mean ± SE	Serum lactate dehydrogenase activity (IU/L) Mean ± SE
1. Control	1.69 ± 0.41^{a}	156.69 ± 9.56^{a}
2. Arsenic control	6.60 ± 0.80^{d}	319.48 ± 16.76^{c}
3. NAC Pre-treatment (2 wks) followed by NAC + Arsenic (28 days)	2.63 ± 0.61^{b}	229.11 ± 11.66^{b}
4. NAC+ Arsenic (28 days)	4.27 ± 0.74^{c}	232.27 ± 12.34^{b}

Values are Mean ± SE (n = 6); One way ANOVA (SPSS)
Means with different alphabets as superscripts differ significantly (P<0.05)

In the present study, body weight gain of arsenic-treated groups was significantly ($p<0.05$) reduced (Table 2) [7]. Groups 3 and 4 showed increase in body weight gain as compared to group 2, which may be attributed to the beneficial anti-oxidant actions of NAC on different organ systems as NAC can enter into the cells and therefore improves the overall health of the animals that reflected in weight gains.

Table 2: Weekly body weight gain (g) in different groups of rats

Group	1st wk	2nd wk	3rd wk	4th wk
1. Control	11.88 ± 0.61^{aA}	13.13 ± 0.79^{aA}	12.3 ± 0.56^{aA}	9.25 ± 0.41^{aB}
2. Arsenic control	8.35 ± 0.59^{bA}	7.06 ± 0.64^{bA}	3.75 ± 0.45^{bB}	2.95 ± 0.25^{bB}
3. NAC Pre-treatment (2 wks) followed by NAC + Arsenic (4 wks)	10.89 ± 0.50^{aA}	9.90 ± 0.94^{acA}	8.63 ± 0.50^{dB}	7.81 ± 0.48^{dB}
4. NAC+ Arsenic (4 wks)	9.84 ± 0.50^{abA}	8.53 ± 0.59^{bcA}	6.13 ± 0.58^{cB}	5.83 ± 0.38^{cB}

Values are Mean ± SE (n = 6); One way ANOVA (SPSS)
Means with different alphabets as superscripts differ significantly (P<0.05)
Capital alphabets (within the group); Small alphabets (among groups)

Relative weight of testes (% of body weight) was significantly ($p<0.05$) decreased in group 2 in this study as compared to other groups, which is in accordance with the report of Das *et al.* (2009) [8]. The reduction in testicular weight by arsenic may be due to the oxidative stress the loss of germinal epithelium that eventually leads to arsenic-induced loss of testicular weight [9]. Significant improvement in the testicular weights in groups 3 and 4 (Table 3) is attributed to the GSH replenishing and antioxidant actions of NAC [10]. Intra-testicular LDH was increased in group 2 as compared to control group in this study. This may be attributed to the fact that the excess of oxy-free radicals that are generated due to the drugs/toxicants interact with cellular constituents and possibly induce damage to the lysosomal membranes leading to the release of marker enzymes like LDH and other hydrolytic enzymes that aid in further progression of cellular damage [9]. Groups 3 and 4 showed significant decrease in the activity of intra-testicular LDH.

Table: 3 Testicular parameters in different groups of rats

Group	Testicular LDH activity (IU/L)	Relative weight of testes (% of body weight)
1. Control	236.19 ± 12.55^{a}	1.29 ± 0.04^{c}
2. Arsenic control	516.15 ± 27.42^{c}	0.64 ± 0.03^{a}
3. NAC Pre-treatment (2 wks) followed by NAC + Arsenic (28 ays)	325.60 ± 14.40^{b}	0.89 ± 0.09^{b}
4. NAC+ Arsenic (28 days)	382.45 ± 19.24^{b}	0.82 ± 0.07^{b}

Values are Mean ± SE (n = 6); One way ANOVA (SPSS);
Means with different alphabets as superscripts differ significantly (P<0.05)

In the present study, concentration of TBARS and protein carbonyls ($p<0.05$) were increased in the lung, intestine and stomach of arsenic toxic group suggesting an ongoing oxidative stress (Table 2) [11-15]. Free radicals cause

peroxidation of lipids resulting in formation of aldehydes such as TBARS [16], while oxidation of proteins results in formation of carbonyls [17]. Therefore, the presence of excess of TBARS and protein carbonyls signifies excess free radical production. Concentration of GSH decreased ($p<0.05$) in the lung, intestine and stomach of arsenic toxic group suggesting an ongoing oxidative stress. Arsenic produces oxidative damage by disturbing the prooxidant–antioxidant balance, because it has very high affinity for sulfhydryl groups in reduced glutathione (GSH), which might have implications in the maintenance of thiol-disulfide balance [18]. Arsenic also induces oxidative tissue damage through interference with glutathione (GSH) utilization [19].

Table: 4 Oxidative stress markers in different organs in different groups of rats

Group	Lung			Stomach		Intestine	
	TBARS	GSH	Protein Carbonyls	TBARS	GSH	TBARS	GSH
1. Control	0.82±0.13^{a}	62.20±5.85^{d}	1.49±0.11^{a}	1.03±0.19^{a}	16.21±1.38^{d}	1.65±0.32^{a}	14.21±1.38^{d}
2. Arsenic control	3.25±0.33^{c}	32.57±2.45^{a}	5.52±0.40^{d}	2.86±0.42^{c}	7.53±0.69^{a}	7.06±0.98^{c}	5.53±0.49^{a}
3. NAC Pre-treatment (2 wks) followed by NAC + Arsenic (28 days)	1.48±0.21^{ab}	51.25±4.66^{c}	2.47±0.20^{b}	1.59±0.21^{ab}	13.10±1.08^{c}	3.02±0.44^{ab}	11.10±1.07^{c}
4. NAC+ Arsenic (28 days)	2.70±0.29^{bc}	40.99±3.52^{b}	3.24±0.27^{c}	2.04±0.33^{b}	10.29±0.95^{b}	4.32±0.52^{b}	8.29±0.75^{b}

Values are Mean ± SE (n = 6); One way ANOVA (SPSS);
Means with different alphabets as superscripts differ significantly (P<0.05)

CONCLUSION

It is concluded that arsenic-induced toxicity in rats was manifested by reduced weight gains, reduced GSH concentration in various organs and reduced relative testicular weights, besides increased serum creatinine, LDH, testicular LDH and TBARS and protein carbonylsin various organs under study. Supplementation of NAC was found beneficial in reducing the toxicity. Pre-treatment with NAC was found more protective as compared to NAC co-treatment against arsenic-induced toxicity.

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