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INTESTINAL DYSFUNCTION AND ALTERATION OF VARIOUS SYSTEMIC AND MORPHOMETRIC CHARACTERS IN ALBINO RATS (CHARLES FOSTER) UNDER STRESS OF INORGANIC ARSENIC (IAS) COMPOUNDS: A PILOT STUDY.

MOURI GHOSH [1] AND GOUTAM PAUL [2*]

[1]Research Scholar, Toxicology Unit, Environmental Physiology Division, Dept. of Physiology, University of Kalyani, Kalyani- 741235, India
[2] Professor & Former Head, Toxicology Unit, Environmental Physiology Division, Dept. of Physiology, University of Kalyani, Kalyani- 741235, India

ABSTRACT

The absorption of both necessary nutrients and harmful xenobiotics by mammalian small intestine is linked to its morphometry and wall movements. We exposed male albino rats to different dose and durations of sodium arsenite and arsenate to explore their effects on the intestinal performance. The treated rats exhibited decreased body mass, intestinal hypercontractility, diarrhea, lipid membrane damage and decreased tissue aminotransferase. Morphometric studies revealed increased intestinal length and intestine-somatic index in arsenic-exposed groups. The first set of results indicated rapid transit suggesting inhibited absorption of nutrients, which causes metabolic stress and malnutrition. Whereas the results of morphometric studies indicate an attempt of the body's innate protective mechanism to increase the effective area of absorption. Thus we conclude that the various assorted systemic and morphometric side effects of arsenic poisoning are strongly associated with intestinal dysfunction related to intestinal wall movement and disrupted absorption capabilities.

KEYWORDS: Arsenic, intestinal dysfunction, morphometry, aminotransferase, side effect, lipid peroxidation

GOUTAM PAUL
Professor & Former Head, Toxicology Unit, Environmental Physiology Division, Dept. of Physiology, University of Kalyani, Kalyani- 741235, India

INTRODUCTION

The primary function of mammalian small intestine is to absorb nutrients and water which is achieved by intestinal wall movement resulting in mixing of the foodstuff with digestive enzymes and increasing their contact with absorptive cells of the mucosa. It also serves as the principal site for absorption of potentially harmful xenobiotics[1]. One such compound, arsenic, currently the crowning poison enlisted by ATSDR[2] is responsible for millions of death in India and Bangladesh[3]. The morphometry of small intestine plays a major role in the intestines functional competency[4]. Inorganic arsenic (iAs) exerts its toxicity by producing reactive oxygen intermediates which causes cellular damage and increased lipid peroxidation (LPO) leading to loss of cell membrane integrity in various organs in a tissue specific way. Arsenite exposure has been associated with significantly elevated LPO in liver of mice at 3.2 mg/l[5] and male Wister rats at 15.86 mg/kg[6]. Liver of catfish has shown to yield increased LPO products after both arsenite and arsenate treatment[7], but liver of rats treated for 2 days with 10 mg/kg sodium arsenate has shown no significant increase in LPO[8]. Yet 2 days treatment with 5 mg/kg arsenite has resulted in significantly increased cardiac LPO and serum aspartate aminotransferase (AST) activity in male Wister rats[9]. The somatic indices and levels of enzymes such as AST and ALT have long been used as environment risk assessment tools[10,11]. Studies attest to dose dependent increase of the human serum AST and ALT in various arsenic affected areas[12]. Yet chronic exposure of sodium arsenite (4µg/ml) to adult female albino rats producing significant changes in uterine physiology has been found ineffective against the liver toxicity enzymes AST and ALT[13]. Yet again, chronic exposure to arsenite has revealed a significant decline in AST and ALT activities, along with increased LPO levels in mice liver[14]. On the other hand, liver of mice treated with 4.2 mg/Kg arsenate has shown significant increase in both AST and ALT activity[28]. So far no conclusive data on the effect of iAs on protein metabolism and morphometry of intestinal tissue has been published. The diverse nature of tissue specific cellular response in response to iAs exposure makes it tough to extrapolate the results in order to visualize the performance of small intestine exposed to arsenic. In order to conduct any basic research on the influence of iAs on small intestine we cannot disregard its various systemic side effects, as the resultant structural and functional modification of the system will be a consequence of those assorted altered characteristics. Considering this the current project has been designed to understand the altered behavioral patterns, systemic and morphometric characters, metabolic stress to the tissue and oxidative stress related damage to the cell membrane due to toxic insult of iAs to intestine; and inter-relate the findings with each other and intestinal function.

MATERIALS AND METHODS

(i) Test compounds and animal

Sodium arsenite ($NaAsO_2$) and sodium arsenate ($Na_2HAsO_4 \cdot 7H_2O$) were obtained from LOBA CHEMIE (P)Ltd. Male Charles foster rats (*Rattus norvegicus*, 35±5 day, 100±17 g) were purchased from a reliable local breeder.

(ii) Animal husbandry

All animals were allowed to acclimate to the facility (12L:12D, 22 ± 1°C and 50 ± 5% humidity) for 2 weeks before experiment and maintained as per Kalyani University Animal Ethics Committee (KUAEC). Rats were housed in groups of 3-4/cages (Tarson, 2008) on fresh straw and fed 15 g germinated gram and 15-20g pellets/cage/day, shaped out of a dough consisting of gram flour, milk powder, sugar, bread crumb and rusk with water *ad libitum*.

(iii)Animal Exposure

Animals were randomly assigned to 8 treated and 4 control groups of 10 rats each, as detailed in Table 1. Doses were administered

through oral gavages, in 0.5 ml. For biochemical, morphometry and motor activity studies, 7 rats out of 10 from each group were randomly selected. After treatment rats were killed following overnight fast by cervical dislocation according to KUAEC.

Table 1

Concentrations and exposure-time of test compounds used in the present investigation.

Group legend	Treatment regime
Control groups	
AN	Distilled water(DW), one day
5N	DW, 5 day
10N	DW, 10 day
20N	DW, 20 day
Acute test groups	
AIII	41 mg/Kg body weight (BW) $NaAsO_2$, one day
AV	41 mg/Kg BW $Na_2HAsO_4 \cdot 7H_2O$, one day
Chronic test groups	
5III	10 mg/Kg BW/day $NaAsO_2$, 5 day
10III	10 mg/Kg BW/day $NaAsO_2$, 10 day
20III	10 mg/Kg BW/day $NaAsO_2$, 20 day
5V	10 mg/Kg BW/day $Na_2HAsO_4 \cdot 7H_2O$, 5 day
10V	10 mg/Kg BW/day $Na_2HAsO_4 \cdot 7H_2O$, 10 day
20V	10 mg/Kg BW/day $Na_2HAsO_4 \cdot 7H_2O$, 20 day

(iv) Visible side effects, intestinal morphometry and behavioral studies.

All test and control group rats were observed for various behavioral and systemic side effects. The absolute body weight (BW) of the rats ware noted before administration of first dose and before sacrifice. The length, wet weight of intestine and gutted BW were recorded after sacrifice and intestinosomatic index (ISI), body mass index (BMI) and Lee index were calculated after the following formulas:

ISI=100 X (intestinal wet weight)/(gutted BW)

$BMI=(BW)/(\text{body length})^2$

Lee Index=$(BW)^{1/3}$ / (nose-to-anus-length)

(v) Tissue collection, fractionation and storage

After midline abdominal incision intestinal segments were collected for motor activity study or snap-frozen and stored at -20°C until further use in biochemical assays. 10%(w/v) crude intestinal homogenate was prepared in standard Dulbucco's buffer (pH 8.0) supplemented with 0.1 mM EDTA, 1 mM β-mercaptoethanol and 0.1 mM PMSF (homogenisation: 30 strokes; centrifugation: C-24BL rotor, low speed, 20 minutes - REMI elecktronic Ltd, 2011).

(vi) Recording of intestinal wall movement

2-cm intestinal segment was quickly removed, suspended in a 40-ml single chamber organ bath perfused with constantly aerated modified Ringer's solution (0.9 g% NaCl, 0.042 g% KCl, 0.024 g% $CaCl_2$, 0.001 g% $MgCl_2$, 0.25 g% $NaHCO_3$ and 0.5 g% dextrose at pH 7.4, 37°C). Intestinal wall movement was recorded with isotonic transducer (IT-2245) coupled to RMS-Polyrite D analyzing software (RMS (P)Ltd, Chandigrah, 2010).

(vii) Biochemical studies related to metabolic stress

Aspartate transminase (AST) and Alanine transaminase (ALT) were colorimetrically determined at 546 nm by measuring the amount of pyruvate or oxaloacetate produced by forming 2, 4-dinitrophenylhydrazine[15]. Protein amount was determined using standard method of Lowry[16]. Total malondialdehyde (MDA) content of crude homogenates were estimated as an index of lipid peroxidation[17].

(viii) Statistical analysis

Statistical analysis was performed using Duncan's multiple range test (DMRT)[29, 30] to determine differences among means at 5% level of significance. Results are mean±SD of 7

observations (however, only for behavioral and systemic changes, n=10). Common letters between any two means (in tables) or bars (in figure) indicate their similarity while two different letters indicate significant difference.

RESULTS

1. Behavioral and systemic changes.

Increased passage of watery and mucus rich stool with diarrhea, abdominal tenderness (manifested in squeaking if the side of abdomen is pressed and a tendency to stay in fetal position), fall of body temperature (as decreased tail temperature, increased piling, shivering, piloerection) decreased food and water intake, bald patches and skin lesions were noted in test groups. Control rats remained highly energetic with voracious appetite and furry. Mortality rate was highest in arsenite treated groups. The clinical features of arsenicosis are detailed in table 2.

Table 2
Visible side effects observed in the 8 treated and 4 control groups.

Symptom	5N	5V	5III	10N	10 V	10III	20N	20V	20III	AN	AV	AIII
Piloerection			+		+	+		+			++	+++
Shivering						+		+	+		+	++
Piling								+	+			
Tail temperature					-	-		-	-			-
Bald patches						+		+	+			
Black face						+		+	+			
Skin leision					+	+		++	++			
Stool softening		+	+		++	++		+			+	+
Stool color change			+		+	+		+	+		+	+
Diarrhea		+	+		+	+++		+	++		+	+
Mucus from mouth								++	++			
Mucus with stool					++	++		++	++		++	+++
Staying in fetal position					+	+		+	+		++	+++
Squeaking if abdomen is pressed		+	+		++	++		++	++		++	++
Eye fluttering								+	+		+	+
Pupil dilatation											+	++
Pupil fixation											+	+
Motor incoordination					+	++		+++	+++			
Restlessness					++							
Akinesia								+	+		+	+
Urination					+	+		+	+		+	+
Appetite					-	-		-	-			
Seizures									+		+	++++
Mortality rate	0/ 10	0/ 10	0/ 10	0/ 10	0/ 10	1/ 10	0/ 10	1/ 10	3/ 10	0/ 10	3/ 10	7/ 10

One '+' and '-' signs denote arbitrary unit of increase and decrease respectively.

2. Intestinal wall movement.

Arsenic induced significant prokinetic activities of the intestine with duodenum showing maximum hypercontractility

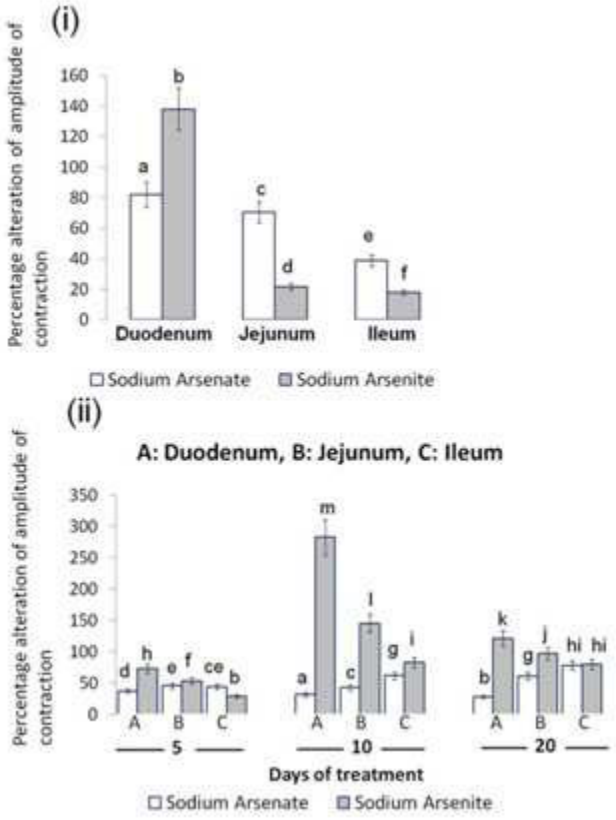

Figure 1

Effect of acute (i) and chronic (ii) exposure of iAs on the motility of different parts of intestine. Data are mean±SD (n=7). Results of DMRT are represented by small letters. Common letter between any two bars indicates their similarity; different letters indicate significant difference at 5% level.

3. Anthropometric and intestinal morphometric parameters

The absolute BW was significantly reduced by 4.6%, 3.6%, 16.4% and 8.5% in 10III, 10V, 20III and 20V rats, respectively compared to respective controls. The 20III group showed reduction by a significant 8.6% from the 20V group. The BW gains of rats were significantly reduced by 24.35% and 45.98% in 20III and 20V rats, respectively (figure 2). The intestinal wet weight of 20III and 20V rats were significantly increased by 23.67% and 14.18% respectively, compared to 20N; however, of the rest, only the 10III rats showed statistically significant increase (figure 3). The significant increase in intestinal length, moderate decrease in BMI and very mild decrease in Lee index of rat (table 3) in response to iAs resulted in significantly increased ISI (figure 4) in all the chronic test group rats.

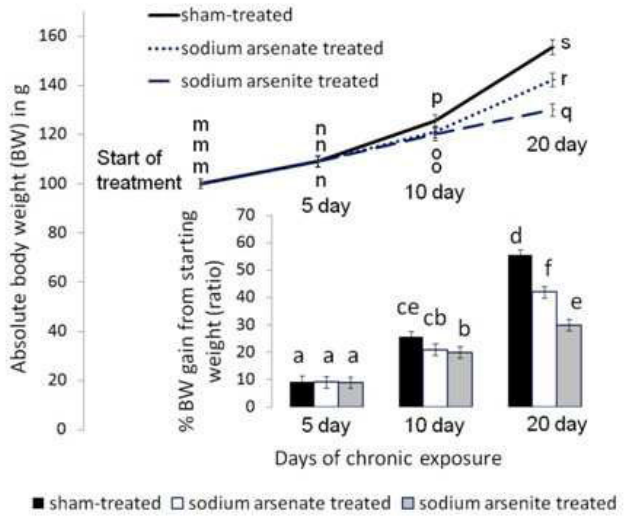

Figure 2

Effect of iAs on absolute body weight (line diagram) and percentage gain of body weight (bar diagram) of rat compared to sham treated control. Data are mean±SD (n=7). Results of DMRT are represented by small letters. Common letter between any two bars indicates their similarity; different letters indicate significant difference at 5% level.

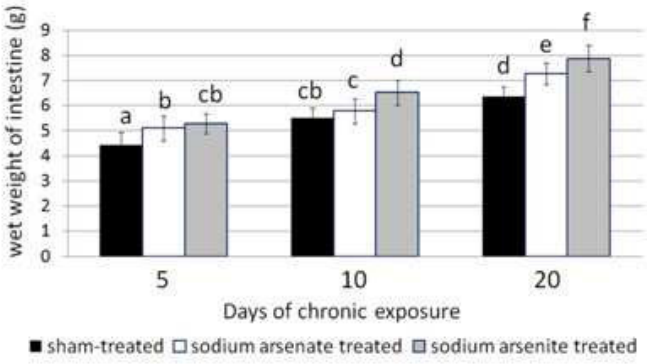

Figure 3

Effect of iAs on intestinal wet weight of rat compared to sham treated control. Data are mean±SD (n=7). Results of DMRT are represented by small letters. Common letter between any two bars indicates their similarity; different letters indicate significant difference at 5% level.

Table 3

Intestinal length (cm), BMI and Lee index of iAs and sham-treated rats.

Para-meters	Rats treated with	Chronic Group			Acute Group
		5 DAY	10 DAY	20 DAY	
Intestinal Length	Sham (DW)	$101.58^{a} \pm 3.1$	$100.41^{a} \pm 3.1$	$100.55^{a} \pm 3.4$	$100.58^{a} \pm 2.1$
	Sodium Arsenate	$100.78^{a} \pm 4.01$	$107.02^{bc} \pm 3.89$	$109.1^{c} \pm 4.2$	$102.52^{a} \pm 3.02$
	Sodium Arsenite	$101.12^{a} \pm 3.5$	$107^{bc} \pm 3.9$	$110.01^{c} \pm 3.2$	$103.79^{ab} \pm 3.2$
BMI	Sham (DW)	$0.5465^{b} \pm 0.01$	$0.6187^{d} \pm 0.01$	$0.7585^{g} \pm 0.02$	$0.5263^{a} \pm 0.01$
	Sodium Arsenate	$0.5455^{b} \pm 0.01$	$0.5961^{d} \pm 0.01$	$0.6939^{f} \pm 0.02$	$0.5263^{a} \pm 0.01$
	Sodium Arsenite	$0.5445^{b} \pm 0.01$	$0.5911^{d} \pm 0.01$	$0.6404^{e} \pm 0.01$	$0.5263^{a} \pm 0.01$
Lee index	Sham (DW)	$0.3595^{b} \pm 0.01$	$0.3656^{bc} \pm 0.01$	$0.3813^{c} \pm 0.02$	$0.3378^{a} \pm 0.01$
	Sodium Arsenate	$0.3593^{b} \pm 0.01$	$0.3610^{b} \pm 0.01$	$0.3810^{bc} \pm 0.02$	$0.3378^{a} \pm 0.01$
	Sodium Arsenite	$0.3591^{b} \pm 0.01$	$0.3600^{b} \pm 0.01$	$0.3644^{bc} \pm 0.01$	$0.3378^{a} \pm 0.01$

Data are mean±SD (n=7). Results of DMRT are represented by small letters. Common letter between any two means indicates their similarity; different letters indicate significant difference at 5% level.

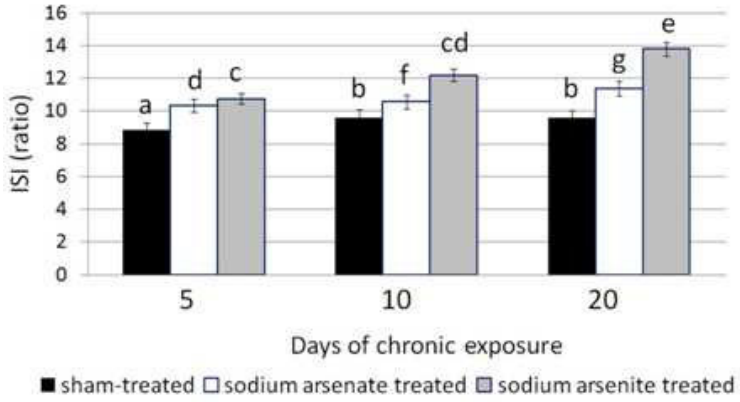

Figure 4

Effect of iAs on the intestine somatic index (ISI) of rat compared to sham treated control. Data are means ± SD (n = 7). Results of DMRT are represented by small letters. Common letter between any two bars indicates their similarity; different letters indicate significant difference at 5% level.

4. Biochemical analyses

The activities of total cellular AST and ALT were all decreased and LPO was increased significantly in all the test groups, more for arsenite than arsenate. Alteration in total protein content was insignificant at $p<0.05$ (table 4).

Table 4

Estimated AST, ALT (mM/min/mg tissue) and LPO (nM MDA/10µg tissue).

Assay	Rats treated with	Chronic Group			Acute Group
		5 DAY	10 DAY	20 DAY	
LPO	Sham (DW)	0.99^{a} ±1.2	1.0^{a} ±0.1	1.02^{a} ±0.1	0.99^{a} ±1.2
	Sodium Arsenate	1.82^{b} ±0.5	2.63^{d} ±0.06	2.99^{e} ±0.04	1.85^{b} ±0.08
	Sodium Arsenite	1.91^{b} ±0.09	2.96^{e} ±0.06	3.24^{f} ±0.07	2.13^{c} ±0.07
AST	Sham (DW)	14.9^{ab} ±2.1	14.9^{a} ±1.1	14.7^{ab} ±2.1	14.4^{b} ±2.1
	Sodium Arsenate	11.2^{c} ±1.2	10.7^{c} ±1.1	9^{d} ±1.04	11.19^{c} ±1.01
	Sodium Arsenite	9.3^{d} ±1.1	7.6^{e} ±1.08	6.9^{e} ±0.8	9.01^{d} ±1.3
ALT	Sham (DW)	12.32^{a} ±1.71	12.31^{a} ±1.71	12.311^{a} ±1.71	12.313^{a} ±1.71
	Sodium Arsenate	10.561^{b} ±0.5	9.321^{c} ±0.6	6.022^{e} ±0.59	10.491^{b} ±0.55
	Sodium Arsenite	9.421^{c} ±0.57	7.292^{d} ±0.56	6.021^{e} ±0.46	10.153^{b} ±0.53

Data are mean±SD (n=7). Results of DMRT are represented by small letters. Common letter between any two means indicates their similarity; different letters indicate significant difference at 5% level.

DISCUSSION

Inorganic arsenic at both low and high concentrations was shown to have profound effects on the behavioral pattern and systemic processes of rat. All iAs exposed rats came down with diarrhea. This can be associated with the increased intestinal motility and resultant rapid transit. So we may infer that as the intestinal content was hurried down, duodenum was maximally exposed to iAs; and as such, would be most susceptible to iAs. This is reflected in the maximum hypercontractility of duodenum. Chronic diarrhea resulting in decreased absorption of nutrients from the intestinal wall and loss of appetite in the iAs treated rats will also contribute towards metabolic stress. The body weight loss and akinesia could be attributed to appetite loss and energy deficit due to metabolic stress. Chronic diarrhea had been frequently associated with malnutrition[18], metabolic and oxidative stress in

rats accompanied with decreased body weight and increased length and weight of jejunum and ileum[19, 26]. Documents have been published in support of increased growth of the small gut of rats induced by increased muscle tension[20] and iAs have been implicated in raising smooth muscle tension in guinea pigs[21]. The increase in intestinal body observed in this study is probably a compensatory attempt of the body to increase absorption of nutrients through the intestinal wall. The binding of iAs to sulfhydryl group of keratin might be a possible explanation for the hair loss noted in the treated rats, a result which is similar to many previous published observations[22, 27]. In liver disease, ALT is usually more affected than AST[23] whereas in protein energy malnutrition (PEM) the opposite is found to be true[24]. In case of arsenicosis however the tissue levels of both enzymes were severely decreased. The serum AST and ALT in patients with PEM were found significantly higher in direct proportion with the severity of the disease[24, 25]. Arsenicosis possibly lead to release of huge amount of amino acids from the damaged tissue which will need to be metabolized. The increased LPO in arsenicosis can be directly responsible for the damage in the lipid bi-layer integrity and as such might be responsible for the increased "leakage" of the enzymes, probably as the body's innate effort to maintain homeostasis through protein synthesis from tissue breakdown and amino acid metabolism.

CONCLUSION

Thus we may conclude that the intestinal dysfunction in arsenicosis is linked to metabolic stress and altered morphometry of the intestine. The myriad of phenotypical abnormalities can be extrapolated to similar clinical findings in arsenic toxicity in humans. Also the rapid intestinal transit raised a need to devise a delivery system for protective drugs that are not affected by the intestinal hurry and malabsorption.

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