ALTERATIONS IN HEPATOSOMATIC INDEX AND BEHAVIOUR UNDER STRESS OF ARSENIC TRIOXIDE AND THEIR MODULATION BY *CURCUMA AROMATICA* PLANT EXTRACT IN ALBINO RATS (*BERKENHOUT*)

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Summary

Effect of oral administration of arsenic trioxide at different doses i.e. 0.15mg/100gm for acute (1 day) and 0.02mg/100gm, 0.01 mg/100 gm and 0.007 mg/100 gm for sub-acute (7, 14 and 21 days) sets after LD_{50} (14.98 mg/kg body weight) determination was studied on hepatosomatic index and behaviour with respect to control rats. The modulating role of *Curcuma aromatica* (50 mg/kg body weight) was also studied on arsenic trioxide toxicity. The results revealed significant decrease in body weight, liver weight and hepatosomatic index after arsenic trioxide intoxication and significant increase after *Curcuma aromatica* treatment while, it has been almost similar to control group in *Curcuma aromatica* and arsenic trioxide treatment. However, behaviour changes (scratching, excitation, thirst, salivation, tremors, food avoidance and pilling) have been observed at short term and long term exposure of arsenic trioxide, *Curcuma aromatica* and their combination. The results indicate protective action of *Curcuma aromatica* on hepatosomatic index and behaviour under stress of arsenic trioxide.

Key words: Body weight, liver weight, scracthing, lethargy

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Introduction

Heavy metals are the most noxious pollutants owing to their diverse effects. Some metals are soluble in water and readily absorbed into the living organisms. Metal ions of high toxicity are known to cause deleterious impact on organs and blood level. They form metal complexes with the structural proteins, enzymes and nucleic acids and interrupt their functions (1). They have received particular attention among the non-degradable toxic chemicals due to their adverse effects aquatic as well as terrestrial forms (2).

Arsenic is a naturally occurring metal that is present in food, soil and water. It is released in the environment from natural and man-made sources (3). Arsenic, ranked first in a list of hazardous substances by the agency for Toxic substances and Disease Registry and United states Environmental pollution agency (4). It is highly toxic element and its presence in food composites is a matter of concern to the wellbeing of both humans and animals. Recent reports have showing some of our food stuff's are also contaminated with arsenic (5).

Arsenic is well known toxicant and carcinogen found naturally in surface and ground water around the world. Exposure can cause skin lesions, adverse reproductive outcomes and cancer (6). It also perturbs mitotic spindle and induces G2/M prolongation, leading to genomic instability (7).

On the other hand, *Curcuma aromatica* is an herb belongs to *Zingiberaceae* family commonly known as Jangli Haldi. It have been claimed to be useful in the treatment of endoparasite, bacteria fungus and in wound healing (8).

Considering all these facts present study is designed to find out toxic effects of arsenic trioxide on hepatosomatic index and behaviour in albino rats and their subsequent modulation by *Curcuma aromatica* plant extract.

Material and methods

Collection of plant materials: The leaves of *Curcuma aromatica* herb were collected from Makhdoom village (Farah), Mathura district in the favourable season. The leaves were authenticated with taxonomy in literature.

Preparation of plant extract: All the collected herb leaves were shade dried and grind to coarse powder. The coarse powder (100 gm) of shade-dried leaves of the *Curcuma aromatica* was extracted using methanol in soxhlet extractor for a period of 22 h, as per standard methods. Prepared extract was concentrated by rotatory evaporator (Heidolph, Germany) under reduced pressure and optimum temperature.

Experimental animals: 24 albino rats of 100±10 gm weight, almost equal size and age were selected randomly. The rats were maintained in polypropylene cages (45 cm X 27 cm X 15 cm) at room temperature and provided 10 hours photoperiod. The cages were cleaned regularly. The rats were fed on Goldmohar brand rat feed procured from Hindustan India Ltd., Mumbai and provided water *ad libitum*.

Experimental chemical: Experimental chemical (AS_2O_3) used for treatment was of analytical grade and procured from Merck, India Ltd., Mumbai, while other chemicals from SRL, Mumbai and Span, Gujarat.

Experimental protocol: The rats were randomized in four sets, one for acute (1 day) and three for sub-acute (7, 14 and 21 days) studies of 6 rats each. Each set were further divided into two groups, one group served as control group given distilled water only and other served as treated group given arsenic trioxide orally per Os. Rats were weighted before and after autopsy.

The body weight was weighted before and after treatment in each group, while excised liver weight was weighted after autopsy at predetermined time interval with standard protocol. Liver weight-body weight ratio was also calculated with the help of body weight and liver weight. Livers were washed with saline, blotted off blood between filter papers and weighted.

Behaviour changes have been noticed throughout the study to distinguish normal and stressful experimental animals (9). The data were analyzed statistically by using Fisher's student't' test (10).

Results

Results of the present study demonstrate significant deleterious effect of acute and sub-acute doses of arsenic trioxide on body weight, liver weight and hepatosomatic index along with behaviour in albino rats.

Significant ($p<0.01$) reduction in body weight has been observed after acute and sub-acute intoxication of arsenic trioxide. However, non-significant increased in *Curcuma aromatica* and combination of *Curcuma aromatica* and arsenic trioxide treated sets (Table 1).

Arsenic trioxide significantly ($p<0.05$) increased liver weight in treated rats through out the experiment, while non-significantly in *Curcuma aromatica* treated rats. However, non-significant increase in *Curcuma aromatica* and arsenic trioxide combination sets (Table 2).

Further, liver weight-body weight ratio (hepatosomatic index) has also been found increased significantly ($p<0.01$) after arsenic trioxide intoxication, while non-significantly increased in *Curcuma aromatica* and combination of *Curcuma aromatica* and arsenic trioxide (Table 3).

Moreover, arsenic interferes with a number of body functions such as central nervous system and peripheral nervous system of albino rats. This interference with nervous system brings about changes in behavioural responses.

Spontaneous (5 min to 15 min), short term (15 min to 1 hr) and long term (1 hr to 2 hrs) behavioural responses (scratching, excitation, thirst, salivation, tremors, food avoidance and pilling) were observed after treatment with arsenic trioxide, *Curcuma aromatica* and combination of *Curcuma aromatica* and arsenic trioxide (Table 4).

Table 1 Body weight (g) of *Rattus norvegicus* in different treatment sets

Treatment duration	Treatment sets	Control	Arsenic trioxide treated	*Curcuma aromatica* treated	Arsenic trioxide and *Curcuma aromatica* treated
I day	Acute	106±3.05	93±0.66***	109±2.08*	105±3.50*
7 days	Sub acute	107±1.45	92±4.37***	108±0.88*	106±3.0*
14 days		104±2.33	91±1.52****	106±1.76*	102±1.52*
21 days		110±0.66	90±1.66*****	110±1.15*	108±1.52*

Table 2 Liver weight (g) of *Rattus norvegicus* in different treatment sets

Treatment duration	Treatment sets	Control	Arsenic trioxide treated	*Curcuma aromatica* treated	Arsenic trioxide and *Curcuma aromatica* treated
1 day	Acute	3.38±0.24	4.21±0.11**	3.22±0.12*	3.49±0.21*
7 days	Sub acute	3.34±0.17	4.31±0.17***	3.33±0.09*	3.41±0.11*
14 days		3.33±0.12	4.32±0.10****	3.32±0.13*	3.38±0.16*
21 days		3.34±0.12	4.34±0.12****	3.33±0.16*	3.37±0.13*

Table 3 Hepatosomatic index of *Rattus norvegicus* in different treatment sets

Treatment duration	Treatment sets	Control	Arsenic trioxide treated	*Curcuma aromatica* treated	Arsenic trioxide and *Curcuma aromatica* treated
1 day	Acute	0.031±0.001	0.044±0.0008****	0.029±0.005*	0.032±0.001*
7 days	Sub acute	0.030±0.001	0.048±0.001****	0.030±0.0008*	0.032±0.0005*
14 days		0.031±0.0003	0.046±0.0008****	0.030±0.0008*	0.032±0.001*
21 days		0.029±0.0008	0.047±0.0008*****	0.029±0.0006*	0.031±0.001*

Abbreviation used
*=>0.05
**=<0.05
***=<0.02
****=<0.01
*****=<0.001

Pilling

Scratching

Food avoidance

Plate-I

Letharginess

Table 4 Behavioural responses of albino rat after acute and sub-acute treatment with arsenic trioxide, *Curcuma aromatica* and their combination

Responses	Acute treatment									Sub-acute treatment																							
	1 day									7 days									14 days									21 days							
	£			ß			¥			£			ß			¥			£			ß			¥			£			ß			¥		
	S	St	Lt	S	St	Lt	S	St	Lt	S	St	Lt	S	St	Lt	S	St	Lt	S	St	Lt	S	St	Lt	S	St	Lt	S	St	Lt	S	St	Lt	S	St	Lt	S	St	Lt
Scratching	*	$	$	$	◦	◦		#	◦	*	$	$	#	◦	◦	$	◦	◦	*	*	$	#	#	◦	#	#	◦	*	*	$	#	#	◦	$	#	◦
Excitement	*	$	$	◦	◦	◦	#	◦	◦	*	$	$	◦	◦	◦	#	◦	◦	*	$	$	◦	◦	◦	◦	◦	◦	*	*	$	◦	◦	◦	#	◦	◦
Thirst	*	$	$	$	$	$	#	#	◦	$	$	◦	#	◦	◦	#	◦	◦	*	$	◦	#	◦	◦	#	#	◦	*	$	$	#	#	◦	#	#	◦
Salivation	$	#	◦	$	◦	◦	◦	◦	◦	$	$	◦	#	#	◦	#	#	◦	$	#	#	#	#	◦	$	#	◦	$	$	◦	#	◦	◦	#	#	◦
Food avoidance	#	#	◦	◦	◦	◦	◦	◦	◦	$	$	◦	◦	◦	◦	◦	◦	◦	$	$	◦	◦	◦	◦	◦	◦	◦	$	$	$	◦	◦	◦	#	◦	◦
Pilling	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	◦	*	◦	◦	◦	◦	◦	◦	◦	◦	*	◦	◦	◦	◦	◦	◦	#	$	*	◦	◦	◦	◦	◦	◦
Tremor	*	$	◦	◦	◦	◦	#	◦	◦	#	#	◦	◦	◦	◦	◦	◦	◦	$	#	◦	◦	◦	◦	#	◦	◦	*	*	$	◦	◦	◦	◦	◦	◦

Legends

£= Arsenic treated ß= *Curcuma aromatica* ¥= *Curcuma aromatica* + Arsenic trioxide

S= Spontaneous St= Short term Lt= Long term

*= Severe $= Moderate #= Mild ◦= absent

Discussion

Decrease in body weight may probably be due to reduction of food and water intake under the influence of arsenic trioxide. Present finding is in affirmation to NTP report (11), Ramalingam *et al*. (12) and Mahour and Saxena (13) following heavy metals, mercuric chloride and mercuric chloride intoxication respectively, while increase in body weight in *Curcuma aromatica* and combination of *Curcuma aromatica* and arsenic trioxide sets may probably be due to free radical scavenging activity of *Curcuma aromatica* (Curcumene, an alkaloid). Similar observations have been made by Mahour and Saxena (14) and Rathore and Vargase (15) after mercuric chloride and *Panax ginseng*, mercuric chloride and Liv-52 treatment respectively.

Mercuric chloride significantly increased liver weight after arsenic trioxide. This increase in liver weight may probably be due to formation of reactive oxygen species (free radicals) which leads to hepatic damage. Similar changes after combined effect of water contamination with cobalt and nickel on wistar rats have also been rendered (16). However, liver weight in *Curcuma aromatica* treated animals was found to be almost similar to control rats and thus could probably be due to antioxidant activity of *Curcuma atomatica* (curcumene, an active constituent). Antioxidant activity under the influence of *Feronia elephantum* and *Nigella sativa* earlier supplements the observations in the present investigation (Kapoor *et al*. (17) and Kamat *et al*. (18).
Determinations of liver weight-body weight ratio (hepatosomatic index) in all the groups as compared to control also substantiate the observations (13) *vide supra*.

According to Kalia and Flora (19) and Vahter (20), Arsenic mainly affects kidney and central nervous system, including brain that contains almost all the regulatory centres of behavioural patterns. As, feeding and thirst are regulated by hypothalamus (fore brain) and salivation by medulla oblongata (hind brain). Food avoidance, thirst and salivation have also been recorded (21) and (22) in present course of study after arsenic intoxication. However, were minimized by *Curcuma aromatica* as revealed by normalcy in the behaviour pattern *vide supra*.

Food avoidance as observed during investigation results in loss of weight and physiological weakness leads to letharginess (23).
The primary target for arsenic intoxication is skin and disturbs central nervous system resulting in peripheral neuropathy which leads to scratching and excitement (valentine *et al*. (24) and (25).

It is evident that *Curcuma aromatica* has a potency to protect losses revealed inform of hepatosomatic index and behavioural changes under arsenic trioxide stress.

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