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Effect of arsenic trioxide on renal functions and its modulation by *Curcuma aromatica* leaf extract in albino rat

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Abstract: *The protective effects of Curcuma aromatica leaf extract were studied on nehrotoxicity induced by arsenic trioxide in albino rats. LD_{50} estimated for arsenic trioxide was 14.98 mg kg^{-1} body weight. Nephrotoxicity was assessed by estimating the serum levels of urea, uric acid and creatinine, the markers of renal dysfunctioning. The applied doses of arsenic trioxide administered orally were 0.007 , 0.01 , 0.02 and 0.15 mg 100 g^{-1} body weight for sub acute (21,14 and 7 days) and acute (1 day) treatments respectively. Arsenic trioxide intoxication significantly increased the serum level of urea, uric acid and creatinine in comparison to control due to renal dysfunctioning. Pretreatment with dose of 50 mg kg^{-1} body weight of leaf extract of Curcuma aromatica restored the increased serum levels of urea, uric acid and creatinine to normal. The results reveal that Curcuma aromatica leaf extract has a potential to modulate the renal dysfunctioning caused by arsenic trioxide.*

Key words: *Albino rat, Arsenic trioxide, Curcuma aromatica, Leaf extract, Renal functions*
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Introduction

Arsenic is one of the most dangerous occupational and environmental toxins. Both natural and anthropogenic sources are responsible for the distribution of many toxicants, mainly heavy metals throughout the environment. Arsenic is abundant in the crust of the earth and is found in all environment. It is found in soil, minerals, surface and groundwater(Antman, 2001).Arsenic trioxide is a trivalent inorganic compound of arsenic. Groundwater arsenic contamination has assumed an alarming proportion in large part of West Bengal, India and adjoining areas of Bangladesh, so much so that it has been earmarked as "the biggest arsenic calamity in the world" (Das *et al.*, 1994).Groundwater contamination of arsenic in West Bengal was first reported in December 1983 and in west of Bangladesh in late 1993. The arsenic contamination was also observed in three districts Ballia, Varansi and Gazipur of UP in the upper and middle Ganga plain, India (Ahamed *et al.*,2006). Approximately 20 incidents of groundwater arsenic contamination have been reported from all over the world (Mukherjee *et al*., 2006). Due to groundwater contamination, a large number of populations in Bangladesh are suffering from melanosis, leuco-melanosis, keratosis, hyperkeratosis, dorsum, non petting oedema, gangrene, skin cancer and skin lesions in sole and palm (Karim, 2000).

Arsenic compounds show toxicity in many organs of body as kidney, liver, lung, gastro intestinal tract and respiratory tract (Vahter, 2007). Kidney shows many renal functions as excretion of nitrogenous waste products, acid-base balance and balance of electrolytes and water. Arsenic trioxide toxicity disturb these renal functions. A disruption in kidney function has immediate effects on the composition of circulating blood (Martini, 1989). However to evaluate the effect of arsenic on kidney functions, it becomes a must to determine the urea, uric acid and creatinine levels in serum (Saxena *et al.*, 2006). In recent years in scientific investigations attention has been paid towards the "health – promoting" activity of herbal plants, *Curcuma aromatica* that showed highly promising results in combating arsenic intoxication in albino rat. *Curcuma aromatica* (Fig. 1) is a member of family Zingiberaceae. It is commonly known as wild turmeric, jangli haldi, aranyaharidra and vanarishta. For the last few decades, extensive work has been done to establish the biological activities and pharmacological actions of *Curcuma aromatica* root and its extract, such as antiinflammatory, antioxidant, anticarcinogenic, anticoagulant, antifertility, antidiabetic, antibacterial and antifungal activities *etc*. Its antioxidant role has been clinically exploited to control oxidative stress–related pathogenesis (Chattopadhyay *et al*., 2004). However, turmeric leaf extract has not yet been studied extensively, though few reports on the role of leaf extract of *Curcuma* in perfumary, soap and cosmetic industries are available (Behura *et al*., 2002). The pharmacological activities of *C. aromatica* leaf extract are to be explored out and for which the present experiment has been planned.

With such a background our initial aim was to find out suitable herb to modulate arsenic poisoning, which should be (i) easy to administer (ii) effective in low dose (iii) inexpensive and (iv) without any toxic effects of their own.

Materials and Methods

Animals: 48 wistar strain albino rats of 100 ± 15 weight were procured from inbred colony of Department of Zoology, Dr. B.R.A. University Agra. Rats were selected randomly and acclimatized

for and almost uniform for better one month results in the laboratory conditions. The rats were kept in polypropylene cages at room temperature, a 12 hr light 12 hr dark cycle and fed on Goldmohar brand rat feed and were provided water *ad libitum*.

The chemical arsenic trioxide was purchased from Merck, India. The LD_{50} for arsenic trioxide was determined by log dose/ probit regression line method (Finney, 1971) as 14.98 mg kg^{-1} body weight. 50 mg kg^{-1} body weight of *Curcuma aromatica* leaf extract was dissolved in 1 ml distilled water.

Experimental design: The animals were divided into four sets, one acute (1 day) and three subacute (7, 14 and 21 days). Each set contained 12 rats. The individuals of each set were further divided into four groups, 3 rats in each of them. The rats were provided water, arsenic, *Curcuma* leaf extract and *Curcuma* + arsenic in different sets respectively and their respective amounts have been shown in Table 1.

The rats were weighted before and after 1, 7, 14 and 21 days of treatment to asses the body weight. After 1, 7, 14 and 21 days the albino rats were anaesthesized under light chloroform anaesthesia and dissected carefully. To determine the renal function, the blood samples were collected from the ventricle of heart and serum was separated for the determination of Serum uric acid by uricase trinder method, Serum urea by urea berthelot method (Newman and Price, 1999) and Serum creatinine by alkaline picrate method (Tietz *et al*., 1994).

Statistics: Data were analysed by analysis of variance (ANOVA) followed by multiple comparison using SNK (Student-Newman-Keul's) procedure to compare pair wise significant difference of the experimental groups (Glantz,1992) at $p<0.05$.

Results and Discussion

Analysis of variance for acute and subacute treatments revealed inter group variation in serum urea, uric acid and creatinine at the significance level of $p < 0.05$.

Arsenic treated group showed a significant increase in serum uric acid, urea and creatinine content as compared to control after acute and subacute treatments ($p<0.05$) (Table 2,3 and 4). However, there was no significant difference in serum uric acid, urea and creatinine content of curcuma and curcuma + arsenic treated groups when compared with controls.

When arsenic treated group was compared to the curcuma + arsenic treated group, a significant ($p<0.05$) difference was obtained, after acute and subacute treatments and reveals modulation in arsenic toxicity by *Curcuma aromatica* leaf extract.

Among possible target organs of heavy metals like arsenic, the kidney and central nervous system appear to be the most

Table - 1 : Showing the given doses of arsenic and *Curcuma aranatica* leaf extract for acute and subacute treatment

Groups	12 rats / set			
	Set : 1 (3) Acute (1 day)	Set : 2 (3) Subacute (7 days)	Set : 3 (3) Subacute (14 days)	Set : 4 (3) Subacute (21 days)
Control water (12)	1 ml	1 ml	1 ml	1 ml
Arsenic treated (12)	1.5 mg kg^{-1} b.wt.	0.2 mg kg^{-1} b.wt.	0.1 mg kg^{-1} b.wt.	0.07 mg kg^{-1} b.wt.
Curcuma treated (12)	50 mg kg^{-1} b.wt.	50 mg kg^{-1} b.wt.	50 mg kg^{-1} b.wt.	50 mg kg^{-1} b.wt.
Curcuma + Arsenic treated (12)	50 mg kg^{-1} b.wt. 1.5 mg kg^{-1} b.wt	50 mg kg^{-1} b.wt. 0.2 mg kg^{-1} b.wt.	50 mg kg^{-1} b.wt. 0.1 mg kg^{-1} b.wt.	50 mg kg^{-1} b.wt. 0.07 mg kg^{-1} b.wt.

Values in parathesis indicate number of rats

Table - 2: Serum uric acid content (mg 100 ml^{-1}) after treatment with *Curcuma aranatica* leaf extract followed by arsenic trioxide

Treatment (days)	Individuals / group	Control	Arsenic treated	*Curcuma* treated	*Curcuma* + Arsenic treated
		Mean ± S.Em. (Range)	Mean ± S.Em. (Range)	Mean ± S.Em. (Range)	Mean ± S.Em. (Range)
1 (acute)	3	1.62 ± 0.01 (1.60-1.64)	1.72 ± 1.21* (1.69-1.75)	1.63 ± 0.03 (1.62-1.65)	1.68 ± 0.09 (1.67-1.69)
7 (sub-acute)	3	1.64 ± 0.01 (1.62-1.67)	(1.69-1.73) 1.71 ± 1.05*	1.65 ± 0.01 (1.63-1.67)	1.65 ± 0.01 (1.64-1.69)
14 (sub-acute)	3	1.63 ± 0.01 (1.60-1.66)	1.73 ± 2.01* (1.70-1.74)	1.64 ± 0.005 (1.63-1.65)	1.66 ±0.01 (1.64-1.69)
21 (sub-acute)	3	1.65 ± 0.01 (1.63-1.68)	1.76 ± 2.52* (1.73-1.78)	1.66 ± 0.008 (1.65-1.68)	1.67 ± 0.02 (1.66-1.70)

The values are mean of three replicates. * Significant at $p < 0.05$ level

Table - 3: Serum urea content (mg 100 ml^{-1}) after treatment with *Curcuma aranatica* leaf extract followed by arsenic trioxide

Treatment (days)	Individuals / group	Control	Arsenic treated	*Curcuma* treated	*Curcuma* + Arsenic treated
		Mean ± S.Em. (Range)	Mean ± S.Em. (Range)	Mean ± S.Em. (Range)	Mean ± S.Em. (Range)
1 (acute)	3	16.13 ± 0.17 (15.8-16.4)	18.43 ± 0.23* (18.13-19.1)	16.06 ± 0.12 (15.9-16.3)	17.0 ± 0.11* (16.7-17.2)
7 (sub-acute)	3	15.96 ± 0.14 (15.7-16.2)	19.48 ± 0.28* (18.9-19.8)	16.2 ± 0.37 (15.5-16.8)	17.03 ± 1.17 (16.8-17.4)
14 (sub-acute)	3	16.26 ± 0.14 (16-16.5)	19.53 ± 0.35* (19-20.2)	16.36 ± 0.88 (16.2-16.5)	16.4 ± 1.20 (16.2-17.6)
21 (sub-acute)	3	16.36 ± 0.08 (16.2-16.6)	18.8 ± 1.22* (18.3-20.4)	16.33 ± 0.14 (16.1-16.6)	16.63 ± 1.18 (16.3-17.8)

The values are mean of three replicates. * Significant at $p < 0.05$ level

Table - 4: Serum creatinine content (mg 100 ml^{-1}) after treatment with *Curcuma aranatica* leaf extract followed by arsenic trioxide

Treatment days	Individuals / group	Control	Arsenic treated	*Curcuma* treated	*Curcuma* + Arsenic treated
		Mean ± S.Em. (Range)	Mean ± S.Em. (Range)	Mean ± S.Em. (Range)	Mean ± S.Em. (Range)
1 day (acute)	3	0.40 ± 0.01 (0.38-0.42)	0.51 ± 0.02* (0.46-0.54)	0.38 ± 0.02 (0.34-0.40)	0.43 ± 0.01 (0.41-0.47)
7 days (sub-acute)	3	0.41 ± 0.02 (0.37-0.45)	0.53 ± 0.02* (0.50-0.58)	0.40 ± 0.005 (0.39-0.41)	0.45 ± 0.01 (0.43-0.49)
14 days (sub-acute)	3	0.40 ± 0.01 (0.38-0.43)	0.56 ± 0.02* (0.51-0.60)	0.40 ± 0.01 (0.36-0.42)	0.46 ± 0.01 (0.44-0.49)
21 days (sub-acute)	3	0.42 ± 0.01 (0.40-0.45)	0.58 ± 0.02* (0.55-0.63)	0.41 ± 0.01 (0.38-0.44)	0.47 ± 0.01 (0.45-0. 50)

The values are mean of three replicates. * Significant at $p < 0.05$ level

sensitive ones. Having been absorbed from the alimentary tract, most of the metals form durable combination with the protein thionein, forming metallothionein, which plays an important role in further metabolism of these metals (Maitani *et al.*,1987; Peraza *et al.*,1998).The kidney and liver are considered to be the most susceptible organs for metals, because these organs contain most of the metallothionein binding toxic metals (Choudhary *et al.*, 2001; Hollis *et al.*, 2001).

Natural and anthropogenic activities have been contributing arsenic spread into the environment. Contaminated water is an important route of human exposure (Chakraborti *et al.*, 2002). Absorption of arsenic primarly occurs through the gastrointestinal tract after its ingestion causing GI lesions which increase permeability of the small blood vessels, through which arsenic enters into the blood and binds to haemoglobin. After then arsenic reaches the liver where its biotransformation takes place and is finally sent to kidney through blood to be excreted out. In kidney, arsenic exerts its toxic effects through several mechanisms, the most significant of which is the reversible combination with suflydryl group of proteins present in glomerular filtration membrane (Yoon *et al.*, 2008). It causes oxidative stress by producing reactive oxygen species (Flora *et al.*, 2007; Vahter, 2007) which damage proteins. Due to lipophilic nature, arsenic also attaches to lipid, increases the lipid peroxidation (Farombi *et al.*, 2007) resulting in deposition of lipid droplets in the slit pores of glomerular filtration membrane. Both the above reasons are responsible for decreased glomerular filtration rate (GFR), causing retention of nitrogenous waste products into the blood.

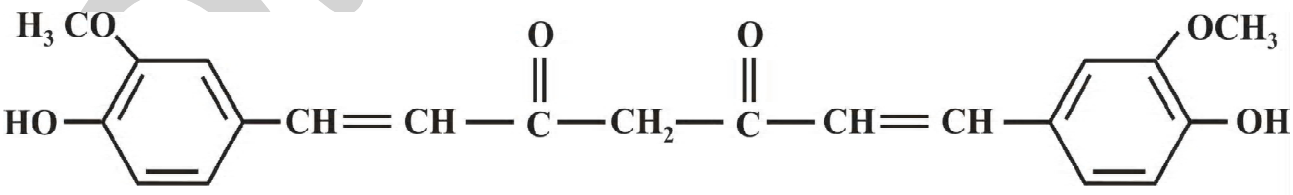

Fig. 1: Structure of curcumin

Curcuma aromatica has been used in traditional medicine for various diseases as anorexia, cough, renal disorders, hepatic disorders and diabetic wounds *etc*. The major composition in *C. aromatica* leaf extract is of 1,8-Cineole (28.01%), Linalool (7.67%), ag-Phallandrene and Curcumin(3-4%) (Behura *et al*., 2002). Curcumin has been found to show antioxidant activity (Phan *et al.,* 2001).

The probable antioxidant mechanism of curcumin is attributed to its unique conjugated structure (Fig. 1), which includes two methoxylated phenols and an enol form of β-diketone; the structure shows typical radical – trapping ability as a chain breaking antioxidant (Masuda *et al*., 2001). Generally, the non enzymatic antioxidant process of the phenolic material present in *Curcuma aromatica* is thought to be mediated through the following two stages: (1) Phenolic antioxidant reduces a substance free radical and becomes oxidized and form an antioxidant radical. (2) This antioxidant radical combines with another free radical and forms a non radical material. Thus the formation of this non radical material is responsible to normalize the glomerular filtration rate and maintain the serum level of nitrogenous waste products.

The serum uric acid concentration is determined largely by the efficiency of renal clearance and rate of purine metabolism (Anetor,2002; Chandra Sekhar *et al*., 2003; Dioka *et al*.,2004 ; Kalia and Flora, 2005). Uric acid has important antioxidant property *in vivo* (Hink *et al*., 2002) and *in vitro* (Ames *et al*., 1981). Uric acid is the final product of purine metabolism. Moreover, formed from guanine and hypoxanthine via xanthine in reactions catalysed by guanase and xanthine oxidase of liver, small intestine and kidney. Arsenic intoxication changes the activity of guanase and xanthine oxidase which results into the increased serum level of uric acid. Besides arsenic intoxication also increases production of oxygen free radicals and increases oxidative stress in lipid peroxidation. Then lipid droplets deposit in the pores of endothelial layer of glomerulus under peroxidative changes in presence of reactive species of oxygen which eventually produce endothelial injury, due to it glomerular filtration rate (GFR) decreases. However, renal clearance of uric acid decreases with simultaneous increase in serum uric acid. But curcumin from *Curcuma aromatica* leaf extract reduces the lipid peroxidation (Hussain, 2002) due to which the serum level of uric acid becomes normalized and due to its antioxidant property it inhibited generation of reactive oxygen species (Jainu and Devi, 2005; Amin *et al*., 2006).

Urea is end product of protein metabolism, gets increased and serum level of urea increases (Aphosian, 1989). Anetor (2002) revealed that production of oxygen free radicals by arsenic induces tubular necrosis which inturn increases tubular permeability, resulting in diffusion and backleak of the filtrate across the tubular basement membrane back into the interstitium and circulation, leading to an apparent decrease in GFR. Under these circumstances, backleak of filtrate results in decreased excretion and increased retention of nitrogenous waste *i.e.* urea in serum (Klassen, 1996; Verbeke *et al*., 1996). But after administration of *Curcuma aromatica* leaf extract, arsenic probably attaches to the thiol group of *Curcuma aromatica* protein instead of body protein, as a result of which protein metabolism becomes normal leading to increased serum level of urea to be normal. Due to antioxidant property, *Curcuma aromatica* prevent tubular necrosis and back-leak of filtrate and normalize urea level of serum.

The increased level of serum creatinine after arsenic trioxide intoxication is due to enhanced formation of metabolic waste product of muscle metabolism. Further, creatinine is anhydride of creatine. Muscle contains phosphocreatine which undergoes spontaneous cyclization with loss of inorganic phosphorous to form creatine. Conversion of creatine to creatinine is a non enzymatic irreversible process. Due to affinity for thiol group of various proteins found in the cell membrane of muscles, arsenic damages the cells due to which the enzyme CPK (Creatine phosphokinase) gets released from the cells which is responsible for the conversion of phosphocreatine in to creatine. Thus increases the level of creatinine. In presence of *Curcuma aromatica*, arsenic does not attach to the proteins of muscle cells but to the *Curcuma* protein, thus the serum level of creatinine gets normal (Nayan and Janardhanan, 2000).

Thus *Curcuma aromatica* leaf extract may find application as a novel drug in the near future to control various diseases due to its multidirectional activities.

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