Int J Pharm Bio Sci 2016 April; 7(2): (B) 441 - 445

Original Research Article **Life Sciences**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

BIOSAFTY ASSESSMENT OF *Curcuma aromatica* LEAF EXTRACT ON HISTOPATHOLOGICAL CHANGES IN KIDNEY OF ALBINO RATS UNDER THE STRESS OF ARSENIC TRIOXIDE

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ABSTRACT

The purpose of this study was to investigate the impact of arsenic on kidney and its treatment with *Curcuma aromatica* to reduce arsenic toxicity. For experimental purpose wistar albino rats (100- 110g body weight) were divided into four groups (controlled, arsenic treated, *Curcuma* treated and arsenic + *Curcuma* treated group). Rats were given 0.15, 0.02, 0.01, 0.007 mg /100 gm body weight of arsenic and 50 mg/100 gm body weight of *Curcuma* orally after 1, 7, 14, 21 days respectively. The rats were etherised and the kidneys were carefully dissected out and processed for histological study. Many histopathological changes were observed in cortical region of kidney due to arsenic exposure whereas *Curcuma aromatica* leaf extract reversed such changes to near the control levels. Therefore it reveals the protective effect of *Curcuma aromatica* leaf extract under stressed condition of arsenic trioxide in albino rat.

KEY WORDS: **Arsenic trioxide, Kidney, Albino rat, *Curcuma*, Histopathology**

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INTRODUCTION

It has become evident that increasing human activities have modified the global cycle of heavy metals and metalloids, including the toxic non-essential elements like As, Hg, Cd and Pb. Arsenic exhibits a complex metabolism and is possibly the most abundant pollutant as well as a potential human carcinogen. Arsenic contamination have been reported from many parts of the world[1] such as Australia, New Zealand, Chile, Taiwan, Mongolia, India, China, Thailand, U.S.A., U.K. as well as Bangladesh. But in terms of severity of the problem, Bangladesh tops the list followed by India and china[2,3]. In India harmful concentration of arsenic in ground water, exceeding the Bureau of Indian Standards permissible limit (of 0.01 mg/litre), is spread across 31 districts of the U.P. state as Ballia, Lakhimpur-Kheri, Bahraich, Ghazipur, Gorakhpur, Bareilly, Siddharthanagar, Basti, Chandauli, Unnao, Moradabad, Sant Kabir Nagar, Sant Ravidas Nagar, Gonda, Bijnor, Mirzapur, Shahjahanpur, Balrampur, Meerut, Rae Bareli, Faizabad, Kanpur Nagar, Sitapur, Lucknow and Pilibhit. Arsenic poisoning is a serious instantaneous concern for the people and other life forms regarding the poisoning through crops and vegetables.[4] The most common forms of arsenic are water-soluble arsenite (As III) and arsenate (As V), and trivalent arsenic is known to be more toxic than the pentavalent arsenic[5]. Arsenic compounds show toxicity in many organs of body as kidney, liver, lung, gastrointestinal tract, respiratory tract and other tissues, skin diseases are the common effects of arsenic poisoning and it also accelerates the risk of cancer in liver, bladder, kidney and skin[6,7]. Arsenic toxicity can be evaluated by measuring the structural and functional changes in the kidney of rats. Albino rats (*Rattus norrvegicus*) are considered to be the best on account of easy handling, rearing and availability. Our initial aim was to minimize the arsenic toxicity through herbal remedy. The history of the use of herbs dates back to the time of the early man[8]. Among herbal plants used in the treatment of ailments, *Curcuma aromatica,* an idiosyncratic plant surrounded by age old traditions of ancient cult and magic rites. *Curcuma aromatica,* member of family Zingiberaceae, commonly called as Jangli haldi, Banhaldi, Kasturi manjal, Ambehaldi, Van haridra, wild turmeric etc[9]. *Curcuma aromatica* is widely used as a flavoring agent, condiment, a source of yellow dye, tonic, astringent and antidote to snake bite. Medicinally it possesses antimicrobial, antifungal, antioxidant and antiinflammatory activities. An assessment has been done to use curcuma leaf extract have active ingredient of great importance against arsenic induced toxicity.

MATERIALS AND METHODS

Albino rats (*Rattus norvegicus*) weighting 100 ± 10 gm of both sexes were procured from inbred colony and acclimatized at room temperature with 12 hr dark/light cycle. The animals were fed on Goldmohar brand rat feed and water was provided *ad libitum*. The experimental compound arsenic trioxide was obtained from Merck, India. The LD_{50} for arsenic trioxide was determined by log dose/probit regression line method and the estimated LD_{50} was 14.98 mg/kg body wt. *Curcuma* leaves were obtained from Dept. of Forestry, Dr. B.R. Ambedkar University, Agra and crude extract of *Curcuma* leaves was used. A safety trial was performed to determine the dose (50 mg/100 gm body wt.) of leaf extract. Animals were divided into 4 groups of 5 rats each. Group I (control) received only 1 ml of distilled water, Group II received leaf extract (aqueous) (50mg/100gm body wt.), Group III received arsenic trioxide (aqueous) (0.15, 0.02, 0.01 and 0.007 mg/100gm body wt.) for 1, 7, 14 and 21 days respectively, as derived from estimated LD_{50} and Group IV received leaf extract followed by arsenic trioxide. To observe the histological changes, rats were etherized and the kidneys were carefully dissected out. Small pieces of fresh tissue of kidney were fixed in bouins fluid and processed for histological study. For nephromorphometry a total of 10 renal capsules were randomly measured in the each experimental animal section and control animal section. Corpuscular changes in the treated and control groups were detected morphometrically by measuring the area of the bowman's capsule and glomerulus using the getner microscope and occulometer. Since it was intended to detect changes in terms of the ratio between the area of bowman's capsule and the area of glomerulus (B/G ratio). The B/G ratio were subjected to statistical analysis. One-way analysis of variance (ANOVA) followed by student Newman – Keul's test (SNK) was done for statistical analysis.

RESULTS

The effect of arsenic and therapy by curcuma aromatic on renal histology was observed as follow

Control group

The kidney of the control set did not reveal pathogenecity and the mean value of B/G ratio has been observed as 1.20 -1.25. The sections of kidney reveal following histological structures: Glomeruli, bowman's capsule, capsular space and nuclei have been observed normal. Renal tubules i.e. PCT and DCT have been normally located and lined by well formed epithelium and brush border (Fig.1).

Arsenic trioxide treated group

A significant ($p < 0.01$) reduction in B/G ratio (1.01-1.06) with that of control group has been observed. (Table 1 and Fig. 9). Kidney of arsenic treated rats showed the following histological changes-

Shrunken lobulated glomeruli and damaged Bowman's capsule have been revealed in cortical region which reduced the capsular space between bowman's capsule and glomerulus. Focal necrosis and pyknosis have also been observed. Tubules exhibit cellular swelling, fatty degeneration and tubular dilation. Structural damage is

evident in form of loss of cell-cell contact,loss of microvilli and cytoplasmic blabbing in the epithelium of PCT, pyknosis, necrosis and karyolysis of nucleus(Fig.2).

Curcuma leaf extract treated group

The kidney of curcuma treated rats reveals normal structures of the cortex after acute and subacute studies. The cortex exhibit normal glomerulus, proximal and distal tubules (Fig. 3).

Curcuma + arsenic treated group

Histological changes induced by arsenic toxicity in renal cortex i.e. glomerulus and renal tubules and in B/G ratio have been reduced after acute and subacute treatment with curcuma before arsenic trioxide intoxication (Fig. 4).

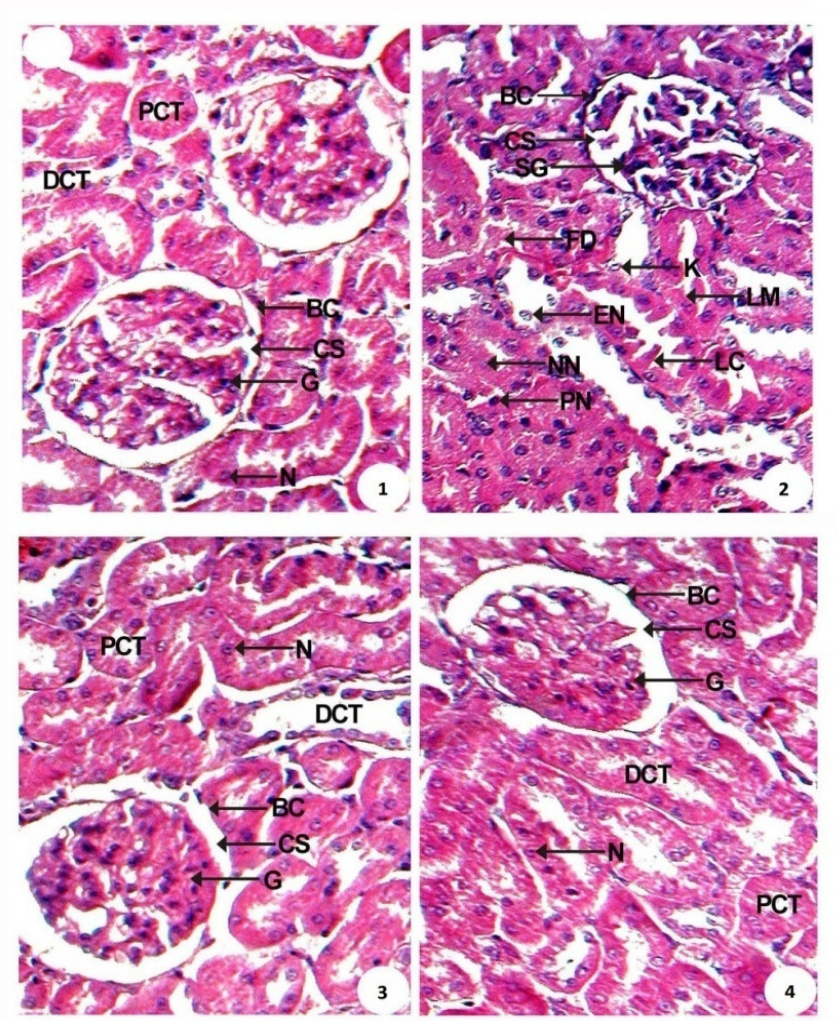

Figure 1

Photomicrograph of cortical region showing normal structure of renal glomeruli (G), bowman's capsule (BC) capsular space (CS), proximal convoluted tubule (PCT) and distal convoluted tubule (DCT) well lined by epithelium with distinct lumen and nuclei (N) in control group. Fig.2 Cortical region showing damage in glomeruli (G), Bowman's capsule (BC), highly reduced capsular space (CS), fatty degeneration in tubules (FD), loss of cell-cell contact (LC), loss of microvilli (LM), excentric nuclei (EN), pyknosis (PN), necrosis (NN) and karyloysis (K) in nucleus following arsenic intoxication. Fig.3 Cortical region of extract treated rats showing normal structure of glomerulus and renal tubules similar to control rats. Fig.4 Cortical region of curcuma + arsenic rats showing no damage in glomerulus (G), Bowman's capsule (BC) and renal tubules (PCT and DCT) [400X].

Figure 9
Alterations in serum B/G ratio in Rattus norvegicus after acute and subacute treatments with Curcuma followed by arsenic trioxide

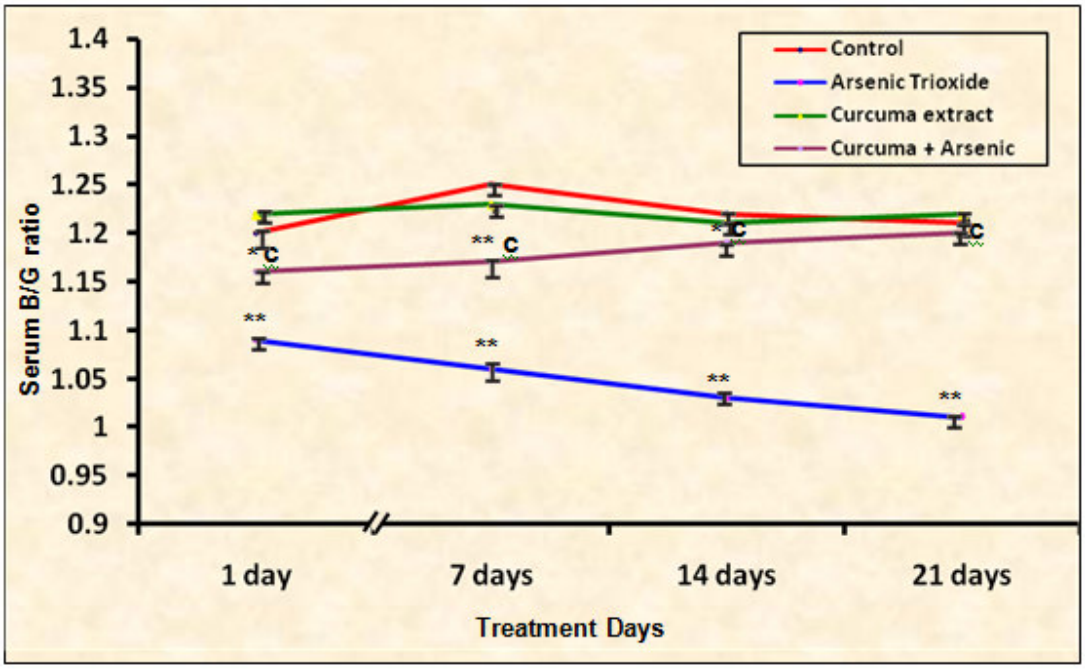

Table 1
B/G ratio in albino rat after treatment with Curcuma followed by arsenic trioxide

Treatment days	Control	Arsenic treated	Curcuma treated	Curcuma + arsenic treated	Significance of F
	Mean ± S.Em	Mean ± S.Em.	Mean ± S.Em.	Mean ± S.Em.	
1 day (acute)	1.20 ± 0.02	1.09 ± 0.01**	1.22 ± 0.01	1.16 ± 0.01*^{c}	p < 0.01
7 days (subacute)	1.25 ± 0.01	1.06 ± 0.02**	1.23 ± 0.01	1.17 ± 0.02**^{c}	p < 0.01
14 days (subacute)	1.22 ± 0.01	1.03 ± 0.01**	1.21 ± 0.01	1.19 ± 0.09*^{c}	p < 0.05
21 days (subacute)	1.21 ± 0.01	1.01 ± 0.01**	1.22 ± 0.01	1.20 ± 0.01^{c}	p < 0.01

Significance level * p < 0.05, ** p < 0.01 (SNK Test) Vs control
Significance level ^{a}p > 0.05, ^{b}p < 0.05, ^{c}p < 0.01 Vs arsenic

DISCUSSION

Arsenic is one of the most dangerous occupational and environmental toxins. Having been absorbed from the alimentary tract, metal forms durable combination with the protein thionein, forming metallothionein, which plays an important role in the further metabolism of this metal. The kidney and liver are considered to be the most susceptible organs for the metals, because these organs contain most of the metallothionein binding toxic metals[10]. Arsenic affects the mitochondrial enzymes, impairs the cellular respiration, and causes cellular toxicity[11]. Recent studies demonstrated that arsenic compounds during their metabolism generate excessive amount of ROS leading to oxidative stress impairing endogenous antioxidant defense mechanisms, enhance lipid peroxidation and simultaneously damaging the cellular macromolecules such as lipids, proteins and DNA, resulting in disruption of cell structure and functions[12,13]. These studies were indicated that the pathological changes in kidney tissues of rat exposed to arsenic may be related to the arsenic induced oxidative stress. Histopathological changes such as shrinkage of glomerulus, damage in bowman's capsule, fatty degeneration in PCT and DCT, necrosis and pyknosis suggest that the kidney may be a major target of arsenic toxicity[14]. Glomerulus seem to be more sensitive to arsenic induced nephrotoxicity, because it receives one quarter of cardiac output and are perfussed at the highest pressure of any capillary bed in the body and is an initial site of chemical exposure within the nephron, is vulnerable to injury by a number of toxicants[15]. Excessive production of oxygen free radicals due to arsenic trioxide, result in the onset of renal dysfunctioning and numerous diseases. Therefore, *Curcuma aromatica*

is used as natural antioxidant. The leaf extract of *Curcuma aromatica* contains many monoterpenes, these are members of a class of compounds called the terpenoids as β myrcene, linalool, limonene, γ terpinene, α phellandrene, p cymene, α terpinolene, α terpinene, 4 terpineol, borneol, para cymen-8-ol and 1,8-cineol. These compounds are also known as volatile isoprenoids (VIPs) and they show antioxidant activity[16] and may arrest oxidative stress at several different levels. Because these are lipophilic, may physically stabilize hydrophobic interactions in membranes, minimizing lipid peroxidation and reducing oxidative stress. VIPs may react with ROS to produce reactive electrophile species which are known to induce antioxidant and other defenses[17]. They inhibit the generation of reactive oxygen species and nitrite radicals in turn normalize the glomerular filtration rate (GFR), biochemical and histological changes in kidney.

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