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ORIGINAL ARTICLE

Exhibition of Total Retention, Elimination and Rate of Decline of Arsenic in *Rattus norvegicus*

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ABSTRACT

Heavy metals are the most toxic pollutants due to their miscellaneous effects are soluble in water and readily absorbed into the living organism. Metal ions of high toxicity cause harmful effects at blood and organ level. Industrial pollution has further aggravated environmental metal pollution, a serious problem and an alarm for future generations. In India arsenic contamination has involved nine districts of West Bengal, fifteen districts of Bihar, nine districts of Utter Pradesh and one district each of Chhattisgarh and Northeastern states. In nine districts of Utter Pradesh (Agra, Aligarh, Balia, Balrampur, Gouda, Gorakhpur, Lakhimpur-kheri, Mathura, and Moradabad), arsenic concentration was found more than WHO's presumed limit (0.05mg/l). Many common arsenic compounds can dissolve in water. Thus, arsenic can get into lakes, rivers, or underground water by dissolving in rain or snow or through the discharge of industrial wastes. Some of the arsenic will stick to particles in the water or sediment on the bottom of lakes or rivers, and some will be carried along by the water. Ultimately, most arsenic ends up in the soil or sediment. Although some fish and shellfish take in arsenic, which may build up in tissues, most of this arsenic is in an organic form called arsenobetaine. Keeping these points in view, the present study is undertaken to study the amount of arsenic in brain, liver and kidney of albino rats after acute and subacute treatments.

Key words: *Total Retention, Arsenic, Rattus norvegicus*

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INTRODUCTION

Heavy metals are the most toxic pollutants due to their miscellaneous effects, are soluble in water and readily absorbed into the living organism. Metal ions of high toxicity cause harmful effects at blood and organ level. Industrial pollution has further aggravated environmental metal pollution, a serious problem and an alarm for future generations. Recent, several kinds of products rat poison, ant poison, weed killer, some types of medicines used in the home exhibited arsenic in them. Today the quantity of arsenic released by human activities exceeds amounts released from natural sources at least threefold. The major sources of arsenic release to the environment are arsenic-treated lumber discarded in landfills, and coal fired power plants, currently, the most heavily exposed people in the United States are in those industries that use arsenic-containing compounds, including carpentry involving CCA pressure-treated lumber, and copper or lead smelting, electronics manufacturing industry and pesticide application (Rossman, 2007).

Arsenic contamination has become a major health problem in certain area of the world, especially part of Bangladesh, United State, Taiwan, Mexico, Japan, and India. In India

arsenic contamination has involved nine districts of West Bengal, fifteen districts of Bihar, nine districts of Utter Pradesh and one district each of Chhattisgarh and Northeastern states. In nine districts of Utter Pradesh (Agra, Aligarh, Balia, Balrampur, Gouda, Gorakhpur, Lakhimpur-kheri, Mathura, and Moradabad), arsenic concentration was found more than WHO's presumed limit (0.05mg/l). World Health Organization (WHO) has set a standard for arsenic in drinking water i.e. 10 µg/l (0.01 mg/l), but increased Arsenic (As) level in groundwater is a major health concern in Asia and the world now a days. The EPA is in the process of setting the new arsenic standard for drinking water at 10 ppb (µg/L) to protect humans against the effects of long-term, chronic exposure to arsenic in drinking water. Soil with arsenic levels below 40 mg/L is considered to be normal soil and distribution of arsenic in the soil is anthropogenic or natural further, in air, arsenic is present in particulate shape as inorganic form. In the industrial areas, urban and suburban areas methylated arsenic is a minor component in the air and the major inorganic portion is a variable mixture of the pentavalent and trivalent arsenic besides, most foods contain normally less than 0.25 mg/kg arsenic with exception to some kinds of seafood. Marine organism possesses high amounts of organo-arsenicals which are arsenic derivatives not acutely toxic due to their low biological reactivity and rapid excretion in urine. Arsenic thus exists in three major forms in on immediate environment, the first inorganic, the second organic and the third arsine gas. Arsenic cannot be destroyed in the environment. It can only change its form, or become attached to or separated from particles. It may change its form by reacting with oxygen or other molecules present in air, water, or soil, or by the action of bacteria that live in soil or sediment. Arsenic released from power plants and other combustion processes is usually attached to very small particles. Arsenic contained in wind-borne soil is generally found in larger particles. These particles settle to the ground or are washed out of the air by rain. Arsenic that is attached to very small particles may stay in the air for many days and travel long distances. Many common arsenic compounds can dissolve in water. Thus, arsenic can get into lakes, rivers, or underground water by dissolving in rain or snow or through the discharge of industrial wastes. Some of the arsenic will stick to particles in the water or sediment on the bottom of lakes or rivers, and some will be carried along by the water. Ultimately, most arsenic ends up in the soil or sediment. Although some fish and shellfish take in arsenic, which may build up in tissues, most of this arsenic is in an organic form called arsenobetaine.

MATERIALS AND METHODS

Collection of Peripheral Blood:

Three rats were sacrificed from each group at the end of the acclimatization period after 3 hrs, 6 hrs, 12 hrs, 1day, 7days, 14 days and 21days treatment. Blood was collected by sacrificing three rats after etherization. The peripheral Blood samples were collected from the ventricle of the heart with the help of hypodermic needle and preserved in EDTA vials. 2.0 ml of heparinized blood was taken in test tubes and 4.0 ml of nitric acid was then added and heated on a hot plate. Hot per chloric acid was then added drop wise till the solution become clear. It was then diluted with 20 ml of de-ionized water for arsenic analysis.

Preparation of Tissues (Brain, Liver, Kidney) Homogenate:

The tissues were dissected out and carefully collected, washed with physiological saline (pH 7.4), blotted off blood between filter papers, then tissues were taken in homogenizer tubes and (10% w/v) Tris-HCl (0.1 M, pH 7.4) was then added. The tissue was grinded completely with the help of homogenizer. The homogenate samples were taken in beaker for acid digestion. 4.0 ml of concentrated nitric acid and 1.0 ml of per chloric acid was lightly heated on hot plate, cooled and diluted with 20 ml of de-ionized water. It was than

filtered through whatsmann filter paper no. 1 and the supernatant was used for the ICP-AES analysis.

Detection of Arsenic:

As_2O_3 was detected and assayed by Inductively Coupled Plasma- Atomic Emission Spectrometry (ICP-AES) by ARCOS spectrophotometer obtained from M/s. Spectro, Germany.

Working Principle:

Inductively Coupled Plasma- Atomic Emission Spectrometry (ICP- AES) is an emission Spectro-photometric technique, exploiting the fact that excited electrons emit energy at a given wavelength as they return to ground state after excitation by high temperature Argon Plasma. The fundamental characteristic of this process is that each element emits energy at specific wavelengths peculiar to its atomic character. The energy transfer for electrons when they fall back to ground state is unique to each element as it depends upon the electronic configuration of the orbital. The energy transfer is inversely proportional to the wavelength of electromagnetic radiation,

$$E = hc/\lambda$$

(Where h is Planck's constant, c the velocity of light and λ is wavelength), and hence the wavelength of light emitted is also unique.

Although each element emits energy at multiple wavelengths, in the ICP-AES technique it is Most common to select a single wavelength (or a very few) for a given element, which in this case is 189.042 nm. The intensity of the energy emitted at the chosen wavelength is proportional to the amount (concentration) of that element (As) in the sample being analyzed. Thus, by determining which wavelengths are emitted by a sample and by determining their intensities, it become easier to analyze qualitatively and quantitatively to the elements, which in the present case is as per sample relative to a reference standard. The wavelengths used in AES ranges from the upper part of the vacuum ultraviolet (160 nm) to the limit of visible light (800 nm). As borosilicate glass absorbs light below 310 nm and oxygen in air absorbs light below 200 nm, optical lenses and prisms are generally fabricated from quartz glass and optical paths are evacuated or filled by a non absorbing gas such as Argon such as ICP-AES *vide infra*

RESULTS AND DISCUSSION

After absorption and distribution in the rat, As_2O_3 is excreted rapidly initially followed by slow pace in following time intervals. A generally accepted indicator of the rate of elimination of a toxicant is it "half-life" (t1/2), which is the time required to remove 50 % of it from the bloodstream. The toxicants are excreted as the parent chemicals, as their metabolites, and/or as conjugates of them. The principal route of excretion is the urine, but the liver and lungs are also important excretory organs. Approximate 99.99 % inorganic arsenic has been eliminated in acute (3hrs) studies, which has been found reduced with decline rate of 0.085%, 0.142, 0.05%, 0.336%, 1.17% and 1.71% after 6hrs, 12 hrs & 1d acute and 7ds, 14dds & 21 ds sub-acute treatments respectively.

It is thus evident that higher elimination is accompanied with low retention of arsenic and low elimination results in higher accumulation of arsenic in vitals and body tissues. (Fierro, *et al*., 1999) In addition approximately 70% of an oral dose is excreted by feces within a few days. Most of the absorbed ionic arsenic is excreted in urine. Smaller amounts are excreted in saliva, bile, sweat, exhalation and breast milk. As^{+2} induced serum biochemical changes get activated in liver, kidney, brain and blood in rats (Gehle, 2009). The absorbed arsenic can cause various digestive disturbances as it has inhibited the production of the digestive enzyme such as trypsin, chymotripsin, and pepsin resulting in abdominal pain, bowel disease, ulcers and bloody diarrhea (Liu and Waalkes, 2008).

Table 1 a: Exhibition of retention of arsenic through one compartment open model following seven doses of arsenic trioxide in male albino rats

Days of intoxication	Dose (ppm/100 gm rats)	Chemical Remaining (ppm)				Total Chemical Remaining (ppm)	Chemical Remaining % of dose
		Brain	Kidney	Liver	Peripheral blood		
3 hrs	343	0.028	0.071	0.423	0.5	0.752	0.22 %
6 hrs	343	0.022	0.035	0.118	0.662	0.837	0.244 %
12 hrs	343	0.034	0.057	0.269	0.393	0.979	0.27 %
1 d	343	0.028	0.097	0.227	1.045	1.03	0.3 %
7 ds	49	0.034	0.05	0.112	0.79	1.396	2.85 %
14 ds	25	0.03	0.092	0.633	1.82	2.566	10.27 %
21 ds	17	0.023	0.098	0.277	3.88	4.271	25.13 %

Table 1 b: Exhibition elimination of arsenic through one compartment open model and first-order kinetics following seven doses of arsenic trioxide in male albino rats

Days of intoxication	Dose (ppm/100 gm rats)	Chemical Remaining (ppm)				Total Chemical Eliminated (ppm)	Chemical Eliminated % of dose
		Brain	Kidney	Liver	Peripheral blood		
3 hrs	343	0.028	0.071	0.423	0.5	342.25	99.78 %
6 hrs	343	0.022	0.035	0.118	0.662	342.16	99.76 %
12 hrs	343	0.034	0.057	0.269	0.393	342.02	99.71 %
1 d	343	0.028	0.097	0.227	1.045	341.97	99.67 %
7 ds	49	0.034	0.05	0.112	0.79	47.6	97.15 %
14 ds	25	0.03	0.092	0.633	1.82	22.435	89.74 %
21 ds	17	0.023	0.098	0.277	3.88	12.73	74.88 %

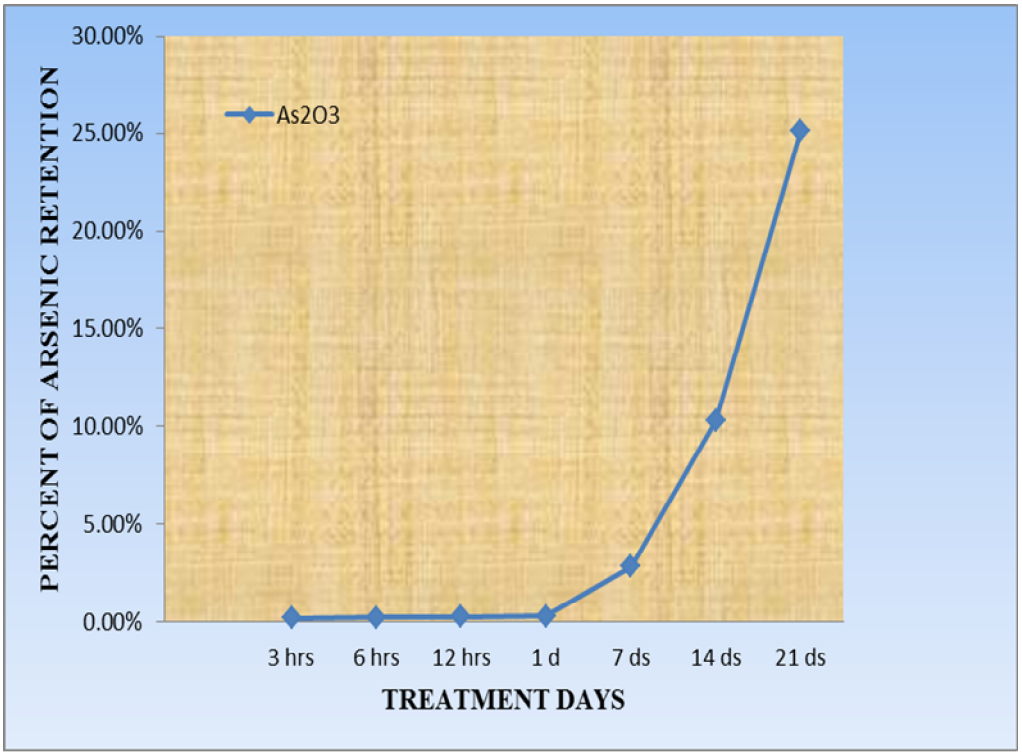

Fig. 1a: Depiction of total retention of arsenic in male albino rats

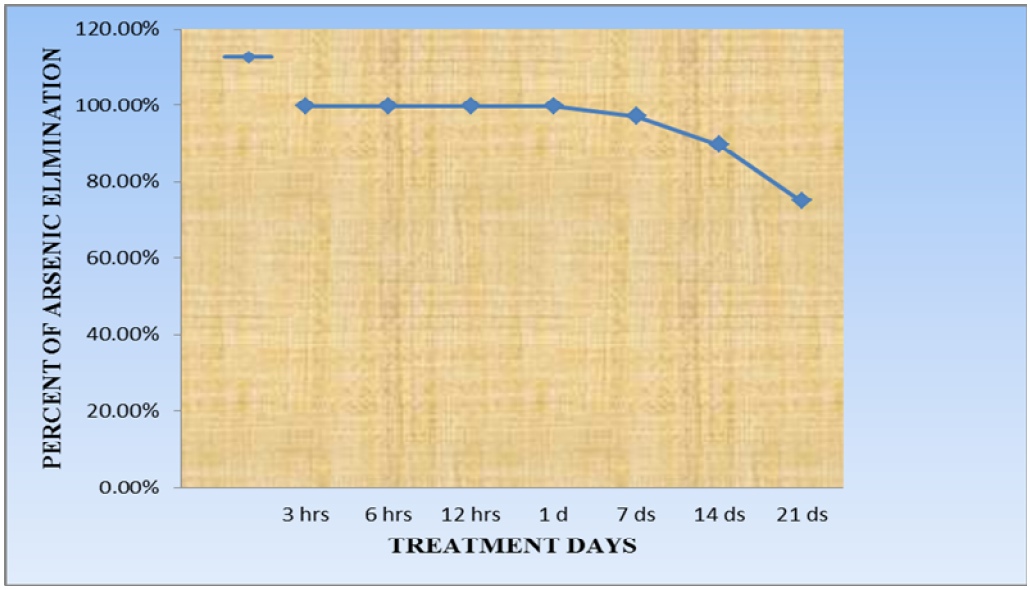

Fig. 1b: Depiction of total elimination of arsenic in male albino rats

Table 1 c: Exhibition of rate of decline of arsenic through one compartment open model and first-order kinetics following doses of arsenic trioxide in male albino rats

S.No.	DAYS OF INTOXICATION	RATE O DECLINE % (As_2O_3)
1.	3 hrs	-
2.	6 hrs	0.085
3.	12 hrs	0.142
4.	1 d	0.051
5.	7 ds	0.366
6.	14 ds	1.17
7.	21 ds	1.71

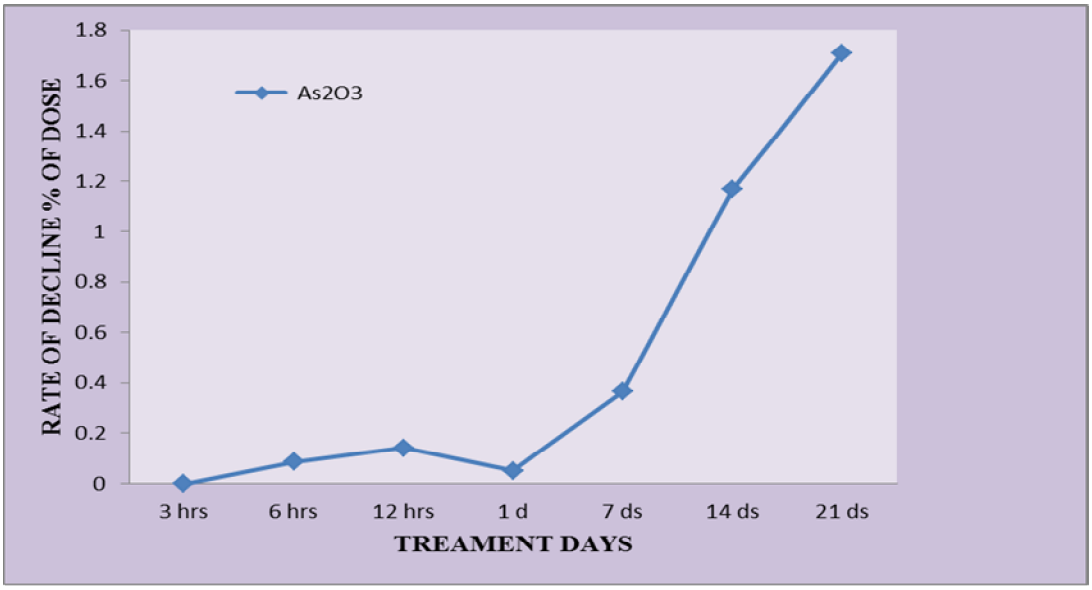

Fig. 1c: Depiction of rate of decline of arsenic in male albino rats

Absorption and distribution of arsenic trioxide has been observed to follow the first-order kinetics through one compartment open model and states that the rate of reaction is proportional to the amount of substance present. In addition accumulation of arsenic also follow the second-order kinetics which states that the rate of the reaction is directly proportional to the square of the concentration of one of the reactants, which was justified by the variance analysis incorporating one-way ANOVA and which further, indicates the rejection of null hypothesis showing dissimilarity in the respective mean amounts of arsenic in the vital organs and brings about several lethal damages even at low-dosages (sub-acute treatments); the only difference lies in the magnitude. Similar finding has earlier been reported by by Agarwal (2014) in albino rat following $HgCl_2$ intoxication. Pearson's correlation has been studied between blood and brain revealed negative correlation, whereas blood and kidney, blood and liver and liver and kidney exhibited positive correlations. Hence, if arsenic concentration decreases in brain, it increases in other vital organs and *vice versa.*

Absorption and distribution study highlighted the accumulation, total retention and elimination of arsenic in body tissues including brain, kidney, liver and peripheral blood which followed the first order reaction kinetics through the one compartment open model. In addition, accumulation of arsenic also follows the second order reaction kinetics. The retention of arsenic has been observed *vide infra:*

Blood > liver > kidney > brain

It is thus evident that arsenic is distributed in different tissues and organs creating metabolic disturbances leading to deleterious effects. The concentration of arsenic deposits is differential in vital organs and the extent of damage induced by arsenic depend on its concentration, as well as its acceptability by the organs.

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