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EFFECTS OF ARSENIC TRIOXIDE ON BRAIN BIOCHEMISTRY AND BEHAVIOR IN *RATTUS NORVEGICUS*

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Abstract

Toxic exposure to arsenic, which affects almost every organ system, including the brain, is a major health problem for many millions of individuals throughout the globe. This pervasive element may be found in the soil, water and atmosphere as well as creatures, rocks, volcanic emissions, and human activity. For the purpose of this research, arsenic trioxide was used to examine the biochemical effects on the brains of albino rats. Rats were placed into five equal groups, with three albino males in each group. We classified groups I and II as "controls," "acute," and "subacute," with durations ranging from seven days to fourteen days and twenty-one days, respectively. At a dosage of 3.43 mg/Kg b.w.t, rats of II, III, IV and V were given As_2O_3 orally during 1, 7, 14 and 21 days, respectively. GPx, Na^+-K^+ ATPase and Brain total protein concentrations were all shown to be lower after arsenic trioxide poisoning. These biochemical markers were also affected. An arsenic toxicity-induced neurotoxicity and free radical generation in the brain of albino rats led to DNA damage and cell death, according to the findings of this research.

Keywords: Arsenic trioxide, neurotoxicity, free radicals, glutathione peroxidase, total brain proteins, albino rats.

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Introduction

Earth's natural resources are a gift to mankind. One of the most serious threats to biodiversity and ecosystem processes is environmental pollution, which is currently threatening the delicate ecological balance between the many environmental components. There are a lot of heavy metals in our surroundings. The metallic characteristics of heavy metal are one of several. As a consequence of human activity, heavy metals are concentrated in the earth's crust and may harm human health. Humans are exposed to heavy metals from multiple sources, including polluted air, water, soil and food.

As one of these heavy metals, arsenic is one of the naturally occurring metalloids; its compounds are found in abundance in the natural world and are released into the atmosphere through both natural and man-made processes. Arsenic is readily available in the Earth's crust. More than 200 minerals contain arsenic.

One may calculate the arsenic atomic number by multiplying the atomic number by 33, which means that it has a total number of 33 electrons in each of its six orbitals. An orpiment known as "arsenic orpiment" is the source of the term "Arsenic". Many traditional medicines include arsenic as a contaminant. Arsenic was more widely employed in cosmetics than in agriculture as a pesticide to preserve crops. The most well-known colour of arsenic in the form of copper acetoarsenite was "Paris Green." It is used in the production of fungicides, pesticides, herbicides and cotton desiccants in industry. Murderers often use arsenic as a weapon. Greek doctors like Hippocrates and Galen promoted the use of arsenic as a therapeutic agent. Syphilis was treated with arsenic until World War II, when it was replaced with antibiotics. Syphilis, yaws, and several protozoan diseases were treated intravenously with arsphenamine (neoarsphenamine), a bright yellow substance containing 30% arsenic.

Arsenic is one of 129 prioritised contaminants recognised by the Environmental Protection Agencies. Arsenic is also included in the list of the 25 most dangerous chemicals to human health.

The safest level of arsenic in drinking water is 10 ng/l (10 ppb), while the maximum level of arsenic is 50 ng/l, according to the standards. For example, high arsenic concentrations have been found in the groundwater of several Asian nations like India and China as well as many other places of the world, including South America, the Caribbean, and Mexico.

Bangladesh and West Bengal, India, are the two worst-hit places in the world. Groundwater arsenic concentrations over the World Health Organization maximum allowed level are found in 42 districts in southern Bangladesh and nine districts in the neighbouring province of West Bengal. Arsenic contamination has been found in aquifers that provide water to more than a million tube wells in both regions. Some tube wells in West Bengal contain as much as 3400g/l of arsenic.

There are three main types of arsenic compounds: inorganic arsenic compound, organic arsenic compound, and arsine gas. Inorganic arsenic is generally more toxic than organic arsenic.

As a recognised cause of neurophysiological malfunction, developmental and cognitive impairment in the foetal brain at low and high dosages of inorganic arsenic.

Arsenic trioxide (As_2O_3), a dreaded toxic derivative, is known for its oxidative stress and free radical reaction, so

researchers have made an effort to understand how it affects the functional aspect of the brain, which is influenced by antioxidative enzymes (GPx) as well as energy-linked enzymes (ATPases, Na^+ and K^+) and proteins.

The purpose of this research is to examine the effects of arsenic trioxide on Glutathione peroxidase, ATPase enzyme, and Brain total proteins in the brain of albino rats following acute and subacute treatments.

Material and Methods

Experimental Animal

Researchers have chosen male albino rats (*Rattus norvegicus*) because of their susceptibility to foreign chemicals, such as xenobiotics (OECD, 420). The ethics committee of the zoology department of Dr. B.R. Ambedkar University, Agra, requested that the albino rats be raised in the animal house.

They were housed in polypropylene cages of 45 centimetres by 27 centimetres by 15 centimetres with a relative humidity of 27.5 percent and an illumination cycle of 12 hours for the 8-9-week-old, healthy, and adult albino rats. They were fed Golden feed pellets and water ad libitum, a normal pellet diet.

Experimental Chemical

Arsenic trioxide (As_2O_3) procured from (India Biologicals, Agra, India) was dissolved in distilled water.

Estimation of median lethal dose of arsenic trioxide using OECD guidelines

In all, there were 15 male albino rats split into five groups with a total of three rats each group. The test substance was dissolved in distilled water to make the solution. After conducting a sighting study, the starting dosage was determined (OECD, 420). A gavage tube was used to give the main trial's dosages of mg/kg b.wt. after the sighting study.

After 14 days, the number of dead and surviving rats was tallied. The log-dose probit regression line approach was used to examine the data (Finney, 1971). The regression line was constructed using log-dose and empirical probit on graph paper. It was used to calculate the predicted probit needed to estimate the median fatal dosage.

Experimental Protocol

The albino rats were divided into five groups, the first, control, the second, acute (1d) and the third sub-acute (7, 14 & 21 ds) with 3 rats each for determining the effect of As_2O_3 on the brain biochemistry.

Selection of Dose: The sub-lethal doses of As_2O_3 were estimated according to the following manner.

- **For acute (1d) treatment**:-

  34.3/10 = 3.43

- **For sub acute (7, 14 & 21 ds):-**

- **For 7ds:-**

  34.3/10 x 7 = 0.49

- **For 14ds**:-

  34.3/10 x 14 = 0.25

- **For 21ds**:-

  34.3/10 x 21 = 0.17

Behavioural Studies: The different behavioral parameters were recorded after acute and sub-acute treatment.

Morphometric Studies: The different morphological parameters measured after each treatment includes:

- **Estimation of body weight: -** The rats were weighted before and after acute (1 d) and sub-acute (7, 14 & 21 ds) treatments.
- **Estimation of brain weight:** - The brains were dissected out and put in physiological saline (pH 7.4) and blotted off between ash free filter paper. They were weighted at predetermined time intervals after acute (1d) and sub-acute (7, 14 &21 ds) treatments.
- **Estimation of brain/body weight ratio**: - The relative brain weight of acute (1d) and sub-acute (7,14 &21 ds) sets and of control were calculated *vide infra* :-

Biochemical Studies

The animals were etherized and brains were dissected out after dismantling the cranium. The brain was removed and washed in physiological saline (pH 7.4) and blotted between two filter papers and weighed.

The brain was homogenized by using Teflon glass homogeniezer in ice cold medium containing 0.2 M Tris-HCl and 300 mM sucrose; pH 7.4. The homogenate was centrifuged at 1000rpm for 10 min. at 0°c in cold centrifuge. The supernatant was separated and used for the assay of biochemical estimations.

- **Estimation of glutathione peroxidase activity**

The activity of GPx was assayed according to the method described by Paglia and Valentine (1967).

- **Estimation of Na^+/K^+ ATPase activity**

The Na^+/K^+ activity was measured by the method of Svoboda and Mosinger (1981).

- **Estimation of brain total proteins**

The brain total proteins was estimated by the method of Lowry *et al*. (1951) method.

- **Biometrical Indices**

Following formulae were used for statistical analysis after Fischer and Yates (1950).

Observations

Five doses of 16, 32, 48, 64 and 80 mg/kg b. wt. have been selected and administered orally with the help of gavage tube. The survival number and survival percentage for each doses have been calculated. The survival rate decreases with increasing dose of arsenic trioxide.

Median lethal dose has been calculated by log dose/probit mortality method (Finney, 1971) (Fig. 1).

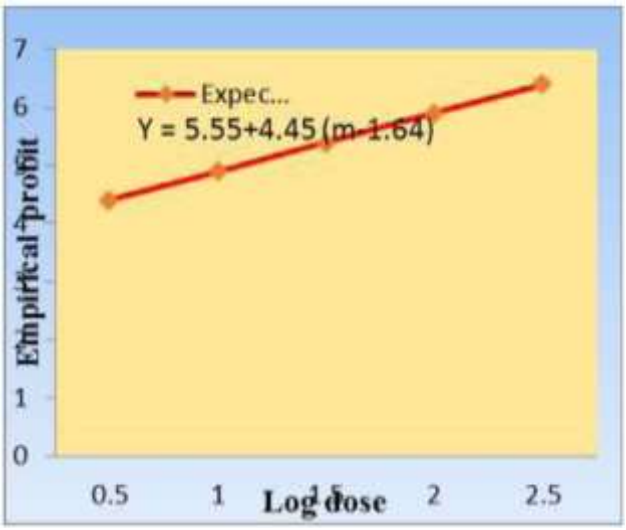

Fig.1. LD_{50} determination using regression equation.

Behavioural changes

No immediate changes have been seen in albino rats after arsenic trioxide intoxication. Letharginess, crowding and piling have been seen after 12 hours of arsenic trioxide intoxication.

Further piling, thirst, food avoidance and discolouration of fur has also been observed in sub-acutly treated rats (Table-I).

Table- I

Changes in behaviour	Acute		Subacute	
	Spontaneous Response	Delayed Response	Spontaneous Response	Delayed Response
Lethargyness	0	++	0	0
Piling	0	+	0	0
Crowding	0	++	0	++
Food avoidance	0	0	0	+
Thirst	0	0	0	++
Discolouration of fur	0	0	0	+

+ = low effect, ++ = Higher effect 0 = No effect

A non-significant increase ($p>0.05$) in mean value of body weight has been observed after acute (1d) As_2O_3 intoxication compared to control (Fig. 2).

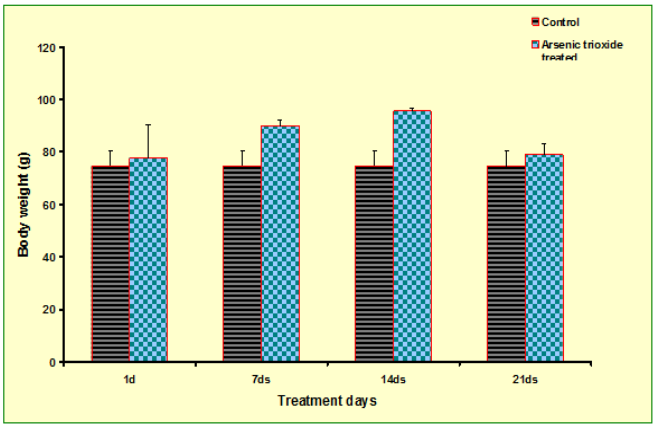

Fig. 2

A non-significant increase ($p>0.05$) in mean value of Brain weight has been observed after acute (1 d) As_2O_3 intoxication compared to control (Fig. 3).

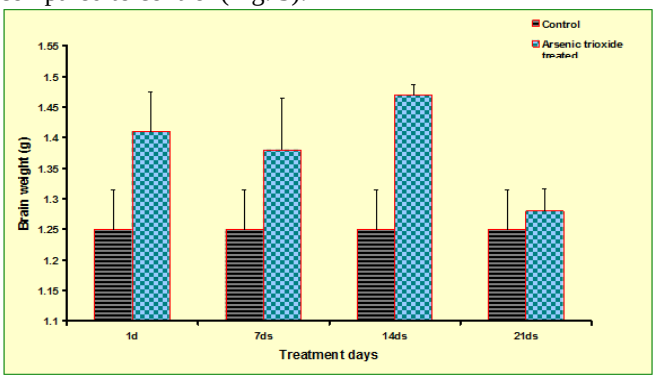

Fig. 3

A non-significant increase ($p>0.05$) in mean value of brain weight / body weight ratio has been observed after acute (1 d) and subacute As_2O_3 intoxication compared to control (Fig. 4).

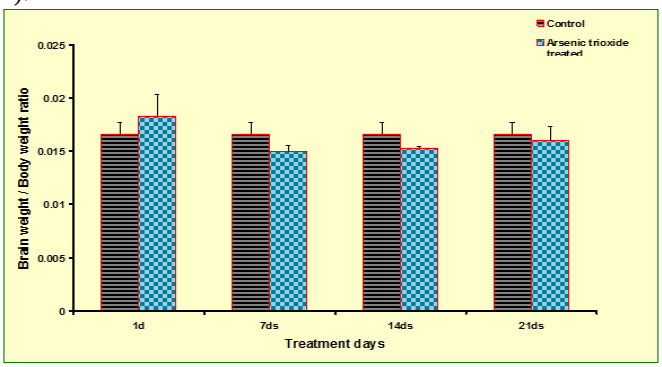

Fig. 4

A significant decrease ($p<0.05$) in mean value of glutathione peroxidase activity has been observed after acute (1 d) and subacute As_2O_3 intoxication compared to control (Fig. 5).

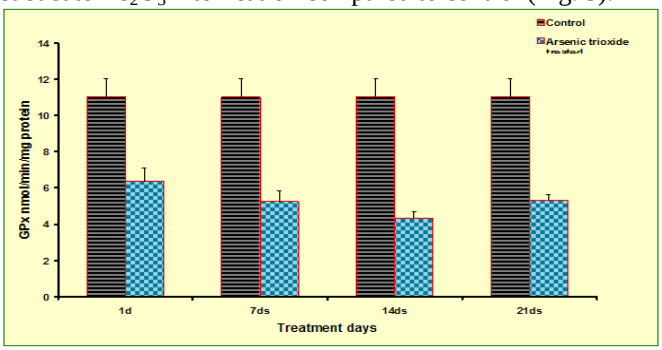

Fig. 5

A significant decrease ($p<0.05$) in mean value of Na^+ ATPase activity has been observed after acute (1 d) and subacute As_2O_3 intoxication compared to control (Fig. 6).

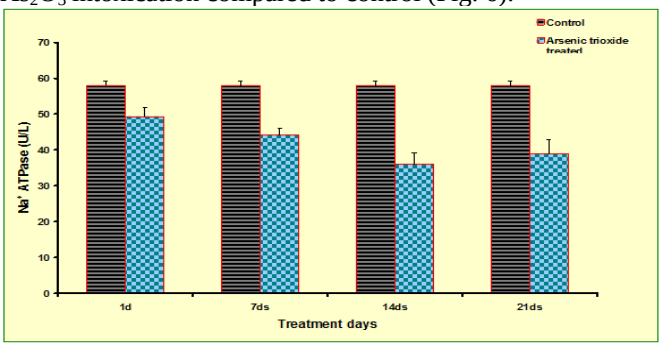

Fig. 6

A significant decrease ($p<0.05$) in mean value of K^+ ATPase activity has been observed after acute (1d) and subacute As_2O_3 intoxication compared to control (Fig. 7).

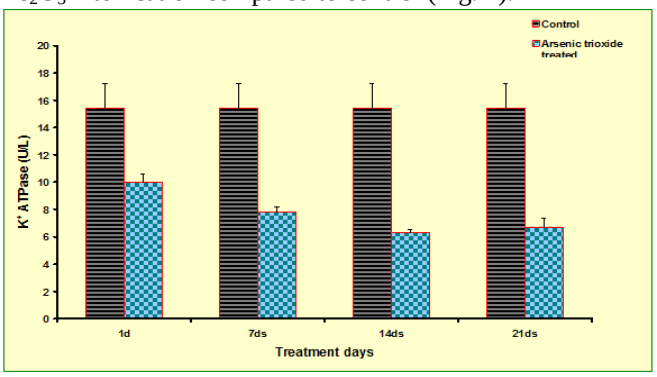

Fig. 7

A highly-significant decrease ($p<0.001$) in mean value of Brain Total Proteins has been observed after acute (1d) and subacute As_2O_3 intoxication compared to control (Fig. 8).

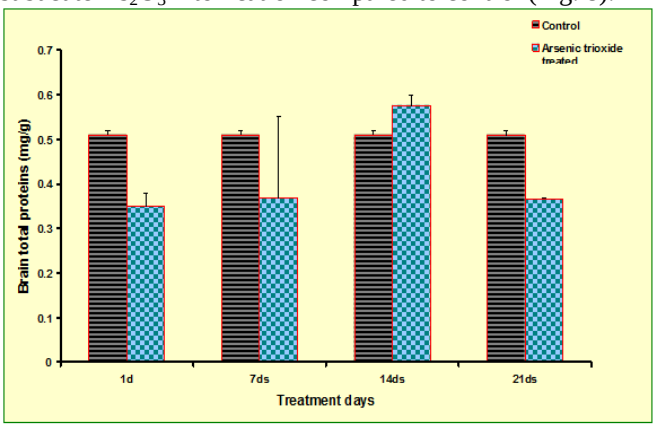

Fig. 8

Discussion

It is estimated that arsenic poisoning affects millions of people worldwide. Drinking water may be contaminated due to arsenic seeping into aquifers from natural geological sources or mining and other industrial operations (Ratnaike, 2015).

The dosage, frequency and length of exposure, the species and age and gender as well as individual susceptibilities to arsenic's toxicity are all factors that contribute to arsenic's lethal effects (Tchounwou *et al*., 2012).

Thiol-containing amino acids, peptides, and proteins interact with arsenic (arsenite). Sulfhydryl groups of important proteins and enzymes are bound by it, resulting in the inhibition of these enzymes and, in turn, the disruption of physiological and biochemical processes.

In addition to oxidative stress, arsenic has been shown to increase the generation of free radicals such as hydroxyl radicals, superoxide anions, dimethyl arsenic peroxy radical, dimethyl arsenic radical, and nitric oxide, as well as impairing the antioxidant system in the brain and other biological tissues (Lyn *et al*., 2000) (Flowchart-2).

Toxic effects of arsenic are mostly due to its ability to block roughly 200 enzymes involved in the production and repair of DNA and other cellular processes (Singh *et al*., 2011).

The neurotoxic nature of arsenic and its chemical derivatives has long been established (Vahidnia *et al*., 2007). As a result, arsenic is able to pass the blood-brain barrier and concentrate in the brain, where it exerts its neurotoxic impact. Research has shown that (Rodriguez *et al*., 2001; Jin *et al*., 2006 and Rosado *et al*., 2007).

An electromyographic result comparable to those seen in patients with Guillain-Barré syndrome is the most common finding (Ratnaike, 2015) and has been presented in the current study.

As a result of the brain's poor oxidative capacity, its high oxygen use (20% of the oxygen consumed by the body), high iron concentration, presence of unsaturated fatty acids and lower activities of detoxifying enzymes, the brain is more susceptible to oxidative stress than other organs. Like SOD, catalase, or GRE, for example (Rodrguez *et al*., 2005). As a first line of defence against oxidative damage, antioxidant enzymes are regarded (Herrera *et al*., 2013). To put it another way, antioxidants are molecules that prevent other molecules from being oxidised and maintain equilibrium between pro-oxidant and antioxidant molecules. In order to prevent harm to important components, antioxidants give up electrons to extremely unstable reactive species.

No toxic effects were seen in the experimental animals during the 12-hour observation period, but after acute treatment, behavioural changes were noted, including lethargy, piling, crowding, while subacute treatment resulted in increased thirst, food avoidance, and fur discoloration. No deaths were recorded at any of the sub-lethal doses of arsenic trioxide used in this study, as well, according to the findings.

After As_2O_3 injections, the control group weighed less than the As_2O_3-injected group at selected time points, including 1 day, 7 days, 14 days and 21 days. Findings in this study are in contradiction to those of Xi and Rodriguez, 2010 and 2002. The lower dose and short duration of treatment may be to blame for the observed metabolic disturbances.

GPx, a well-known enzyme for shielding cells from oxidative damage, plays an important part in this process. Selenomethionine reductase (GPx) converts reduced glutathione into oxidised glutathione and removes hydrogen peroxide (H_2O_2) from the body. By eliminating lipid hydroperoxides and H_2O_2 from the cell membrane, GPx may also put an end to the lipid peroxidation chain reaction. A well-known fact is that arsenic and selenium produce an insoluble and inactive arsenic-selenium complex, resulting in the suppression of enzyme function or affecting the expression and synthesis of selenoproteins such as GPx. In the presence of GSH, GPx plays a primary role in reducing organic hydroperoxides in membranes and lipoproteins (Muthumani and Prabu, 2012). Arsenic has been shown to impair the endogenous antioxidant enzyme defence system, such as GPx, in the brain. This research found a substantial drop in GPx level after one day, followed by a very significant decline after seven, 14 and 21 days. Oxidative stress causes the suppression of GPx levels, which disrupts the defence system's capacity to perform biological functions. In addition to their involvement in maintaining cellular homeostasis, Na^+/K^+ ATPase (Adenosine triphosphatase) play a key part in neurotransmission, the maintenance of ion gradient and the control of cell volume. The level of Na^+/K^+

ATPase was observed to be significantly lowered after acute and subacute treatments in the current studies.

Following the findings of Kumar and Reddy (2012), who reported that the inhibition of Na^+/K^+ ATPase activity can alter cell membrane Na^+/K^+ gradients and disrupt membrane and neurotransmitter functions, the significant decrease in the Na^+/K^+ ATPase level in the brain after one, seven, and 21 days of As_2O_3 intoxication is in agreement. Rats treated with As demonstrated suppression in the activities of membranebound Na^+/K^+ ATPase in Devi *et al*. (2014), which may be attributed to enhanced membrane lipid peroxidation; similar results have been obtained in the current work.

Structural proteins, such as ribonucleic acid (RNA), are essential for cell function, as well as the transmission and reception of information inside and between cells. It is therefore possible to use protein quantity as a biochemical and physiological indicator of organ health. In this study, the total protein content of the brain decreases significantly after only one day of intoxication with As_2O_3, whereas the total protein content of the brain decreases significantly after seven and fourteen days of intoxication with As_2O_3.

Oxidative damage to DNA is caused by a drop in brain total proteins, resulting in a halt in translation and subsequent protein synthesis. In 2006, Chunxiang *et al*. found that the brain protein content of rats treated for 10 and 30 days with the combination of HiF and HiAs decreased significantly. After As_2O_3 poisoning, Dubey *et al*. (2008) also studied the drop in protein levels in the rat brain.

Conclusion

The present study reveals that As_2O_3 alters the brain biochemistry of albino rats due to formation of ROS, high level of LPO and the decrease of antioxidant enzymes such as GPx, Na^+/K^+ ATPase and protein level as well as reduction of nonenzymatic antioxidant such as, GSH, which govern important role of detoxifying agents. Further changes in behaviour and morphology are suggestive of its (brain) supremacy that control all the activities of cell. It is with this reason that alterations in biochemistry of brain, leads to behavioural and morphological changes.

Conflicts of interest

There are no conflicts of interest or personal ties that could have influenced the research disclosed in this study, the authors said.

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