ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

Arsenic Induced Perturbations in Cholinergic System and Energy Metabolism in Young and Adult Rat Brain: Reversal Effect of Vitamin-E

C. Bhuvaneswari Devi[1], K. Kiran Kumari[2], G. Indravathi[3]

Lecturer, Department of Zoology, S.P.W. Degree & PG College, Affiliated to S.V. University, Tirupati, Andhra Pradesh, India[1]

Research Scholar, Dept. of Zoology, S.P.W. Degree & PG College, Affiliated to S.V. University, Tirupati, Andhra Pradesh, India[2]

Research Scholar, Dept. of Zoology, S.P.W. Degree & PG College, Affiliated to S.V. University, Tirupati, Andhra Pradesh, India[3]

ABSTRACT: Arsenic (As) occurs naturally as an element, ranks as the 20th most occurring trace element in the earth's crust. Arsenic is a known human carcinogen which acts via a genotoxic mechanism. Chronic exposure to inorganic arsenic compounds may lead to peripheral and central neurotoxicity. The aim of this study was to examine the impact of As exposure on cholinergic system and energy metabolism. In the present study the three months albino rats were exposed to low dose (1.5 mg/kg body weight) and high dose (3 mg/kg body weight) of As through intraperitonial injection daily for a period of 3 weeks. After the period of dosage, the As exposed animals were divided in to two groups of which one group of both the doses were given Vitamin – E at a dose for a period of one week. The specific activity of enzyme AChE and ACh content were estimated in synaptosomal fractions of cerebral cortex, hippocampus and cerebellum of control and all As exposed rats. The specific activity of enzymes Mg^{2+} ATPase and $Na^{+}K^{+}$ATPase were determined in the mitochondrial fraction of cerebral cortex, hippocampus and cerebellum of control and all As exposed rats. It was observed that AChE, Mg^{2+} ATPase and $Na^{+}K^{+}$ATPase activities were decreased and ACh content was increased in cerebral cortex, hippocampus and cerebellum of As-exposed (both low and high dose) rats when compared to control rats. The effect of As was highly pronounced in high dose As exposed animals compared to the low dose exposed rats. However, AChE, Mg^{2+} ATPase and $Na^{+}K^{+}$ATPase activities were increased and ACh content was decreased in the rats supplemented with Vitamin-E along with low dose and high dose of As. Among the three brain regions the cerebral cortex was found to be more susceptible region towards As induced toxicity compared to the, hippocampus and cerebellum. This study demonstrates exposure to As provoked neuronal injury by inducing alterations in enzymes of cholinergic system and energy metabolism in dose dependent manner, where high dose exposure showed significant alterations compared to the low dose exposure. However, Vitamin-E treatment have partial ameliorative effects on these disturbance caused by As toxicity.

KEYWORDS: Acetylcholinesterase (AChE), Acetylcholine (ACh), Mg^{2+} ATPase and $Na^{+}K^{+}$ATPase, Brain regions, Arsenic, Vitamin-E

I. INTRODUCTION

Arsenic is the chemical element that has the symbol (As), atomic number 33 and relative atomic mass 74.92. Arsenic occurs in many minerals, mainly combined with sulfur and metals, and also naturally in the native (elemental) state. It was first documented by Albertus Magnus in 1250 [1]. Arsenic is a metalloid. It can exist in various allotropes, although only the grey form is industrially important. The main use of metallic arsenic is for strengthening alloys of copper and especially lead (for example, in automotive batteries). Arsenic is a common n-

DOI: 10.15680/IJIRSET.2014.0310062

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

type dopant in semiconductor electronic devices, and the optoelectronic compound gallium arsenide is the most common semiconductor in use after doped silicon.

A few species of bacteria are able to use arsenic compounds as respiratory metabolites, and are arsenic-tolerant. However, arsenic is notoriously poisonous to multicellular life, due to the interaction of arsenic ions with protein thiols. Arsenic and its compounds, especially the trioxide, are used in the production of pesticides (treated wood products), herbicides and insecticides. These applications are declining, however, as many of these compounds are in the process of being banned [2]. Meanwhile, arsenic poisoning as a result of the natural occurrence of arsenic compounds in drinking water remains a problem for many parts of the world including the United States.

Symptoms of acute intoxication usually occur within 30 minutes of ingestion but may be delayed if arsenic is taken with the food. Initially, a patient may have a metallic taste or notice a slight garlicky odor to the breath associated with a dry mouth and difficulty in swallowing. Early clinical symptoms at acute arsenic intoxication may be muscular pain, weakness with flusking skin. Severe nausea and vomiting, colicky abdominal pain, and profuse diarrhoea with rice-water stools abruptly ensure. Capillary damage leads to generalized vasodilation, transudation of plasma, and vasagenice shock. Arsenic's effect on the mucosal vascular supply, not a direct corrosissve action, leads to transudation of fluid in the bowel lumen, mucosal vesical formation, and sloughing of tissue fragments. The patient may complain of muscle cramps, numbness in hands and feet, reddish rashes in the body and intense thirst. In severe poisoning, the skin becomes cold and clammy, and some degree of circulatory collapse usually occurs along with kidney damage and decreased urine output. Drowsiness and confusion are often seen along with the development of a psychosis associated with paranoid delusions, hallucinations, and delirium. Finally, seizures, coma, and death, usually due to shock, may ensue.

Chronic arsenic poisoning is much more insidious in nature, often involving multiple hospital admissions before the correct diagnosis is made. Arsenical dermatosis was rarely picked up from the variety of so many dermatosis. The source of arsenic exposure is discovered in fewer than 50% of cases. The most prominent chronic manifestations involve the skin, lungs, liver and blood systems. This was first diagnosed in West Bengal and Bangladesh patient of Khulna in December, 1984, by Prof. K. C. Saha in July 1982 at School of Tropical Medicine, Calcutta [3].

The cutaneous changes are characteristic yet non-specific. An initial persistent erythematous flush slowly, over time, leads to melanosis, hyperkeratosis, and desquamation. The skin pigmentation is patchy and has been given the poetic description of "raindrops on a dusty road". The hyperkeratosis is frequently punctuate and occurs on the distal extremities. A diffuse desquamation of the palms and soles is also seen. Long-term cutaneous complications include the development of multicentric basal cell and squamous cell carcinomas [4]. One of us (KCS) found mostly squamous cell carcinoma and Bowen's disease both monocentric and multicentric but basal cell carcinoma was not found in skin out of 222 malignancies in arseni). Bowen's disease, a rare precancerous skin lesion, is associated cosis [3] with both arsenic and human papilloma virus (HPV).

Vitamin-E refers to a group of eight fat-soluble compounds that include both tocopherols and tocotrienols. There are many different forms of Vitamin-E, of which γ-tocopherol is the most common in the North American diet. γ-tocopherol can be found in corn oil, soybean oil, margarine and dressings. A-tocopherol, the most biologically active form of Vitamin E, is the second most common form of Vitamin E in the North American diet. This variant of Vitamin E can be found most abundantly in wheat germ oil, sunflower, and safflower oils. It is a fat-soluble antioxidant that stops the production of reactive oxygen species formed when fat undergoes oxidation.

II.MATERIALS AND METHODS

Procurement and maintenance of experimental animals: Young albino rats (Wistar) were purchased from IISc, Bangalore and maintained in the animal house of Watson Life Sciences, Tirupati. The animals were housed in clear plastic cages with hardwood bedding in a room maintained at $28^{o} \pm 2^{o}$ C and relative humidity 60 ± 10% with a 12 hour

DOI: 10.15680/IJIRSET.2014.0310062

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

light/day cycle. The animals were fed in the laboratory with standard pellet diet supplied by Sri Venkateswara Traders, Bangalore and water *ad libitum.*

Chemicals: Arsenic and Vitamin – E were selected as test chemicals. The chemicals used in this study namely Thiobarbutric acid, Glutathione oxidized, NADPH, DTNB, Reduced glutathione, Epinephrine were obtained from Sigma, USA. The remaining chemicals obtained from Qualigens, India.

Animal exposure to Arsenic and Vitamin- E: The young albino rats (3 months) were exposed to low dose of arsenic (1.5mg/kg body weight) and high dose of arsenic (3mg/kg body weight) through intraperitoneal injection daily for a period of 3 weeks. After the period of dosage, the arsenic exposed animals were divided into two groups of which one group of both the doses were given Vit E at a dose for a period of one week. After the period of dosage the animals were sacrificed through cervical dislocation and the tissues were stored at -80^{0}C for the further biochemical analysis.

BIOCHEMICAL STUDIES

Preparation of Crude Synaptosomal Fraction: Brain synaptosomes were prepared by homogenizing in 10 volumes (w/v) of 0.32 M sucrose buffer (0.32 M sucrose, 10 mM Tris-HCl, and 0.5 mM EDTA, pH 7.4).The homogenate was first Centrifuge at 1000*g* for 10 min at 4°C, and then the supernatant was centrifuged at 12,000*g* for 20 min. The buffy layer of pelleted synaptosomes was suspended in a low K_-HEPES buffer (125 mM NaCl, 5 mM KCl, 1.2 mM CaCl2, 1.2 mM Na2HPO4, 1.2 mM MgCl2, 5 mM NaHCO3, 10 mM HEPES, and 10mM glucose, pH 7.4).

Estimation of Acetylcholine (ACh): The acetylcholine content was estimated by the method of [5] as given by [6]. The synaptosomal fractions of cortex, hippocampus and cerebellum were placed in boiling water for 5 minutes to terminate the AChE activity and also to release the bound ACh. To the synaptosomal fractions 1 ml of alkaline hydroxylamine hydrochloride followed by 1 ml of 50% hydrochloric acid were added. The contents were mixed thoroughly and centrifuged. To the supernatant 0.5 ml 0.37M ferric chloride solution was added and the brown colour developed was read at 540nm against a reagent blank in a spectrophotometer.

Estimation of Acetylcholinesterase Activity (AChE): AChE specific activity was determined following the method of [7]. The reaction mixture contained 3.0ml of phosphate buffer (pH 8.0), 20 µl of 0.075M acetylthiocholine iodide (substrate) and 100µl of 0.01M DTNB (5, 5-Dithiobis-2-Nitrobenzoic acid). The reaction was initiated with the addition of 100µl of synaptosomal fraction. The contents were incubated for 30 min at room temperature and the color absorbance was measured at 412 nm in spectrophotometer (Hitachi, Model U-2000). For the determination of pseudo-cholinesterase activity, the substrate butyrylthiocholine iodide was added instead of acetylthiocholine iodide and the activity of butyrylcholinesterase (BuChE) was estimated and subtracted from the total cholinesterase activity to obtain the specific activity of AChE. The enzyme activity was expressed as µ moles of ACh hydrolyzed/mg protein/hr.

Estimation of Adenosine Triphosphatase (ATPase) activity (EC 3.6.1.3): Na^+K^+ and Mg^{2+}ATPase activities in the tissues were estimated following the method of [8]. 1% homogenates of the tissues were prepared in 0.25 M ice cold sucrose solution. Homogenates were divided into two parts. One part was centrifuged at 1400g and the supernatant thus obtained was used as an enzyme source for Mg^{2+}ATPase, while the other part of the homogenate was used for the estimation of the total ATPase.

Mg^{2+}ATPase: The reaction mixture for Mg^{2+}ATPase assay contained 0.5 ml of tris buffer (0.13 M; pH 7.4), 0.4 ml of substrate ATP, 0.5 ml of Magnesium chloride (0.05 M $MgCl_2$) and 0.2 ml of crude homogenate/ mitochondrial fraction (enzyme source). The contents were incubated at 37° C for 15 minutes and the reaction was stopped by the addition of 10% TCA. Zero time controls were maintained by adding TCA prior to the addition of homogenate/mitochondrial fraction. The contents were centrifuged at 1000g for 15 minutes and the inorganic phosphate was estimated in the supernatant fraction following the method of [9].

Na^+K^+ATPase: 1% (W/V) homogenate already set apart was used for the total ATPase assay. The reaction mixture in a final volume of 2.6 ml contained, 0.5 ml of Tris buffer (0.13 M; pH 7.4), 0.4 ml of substrate ATP, 0.5 ml $MgCl_2$ (0.05 M), 0.5 ml potassium chloride (KCl, 0.05 M), 0.5 ml of sodium chloride (NaCl, 0.05 M) and 0.2 ml of crude

DOI: 10.15680/IJIRSET.2014.0310062

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

homogenate/ mitochondrial fraction (enzyme source). The contents were incubated at 37° C for 15 minutes and the reaction was arrested by the addition of 1.5 ml of 10% TCA prior to the addition of homogenate. The contents were centrifuged and the inorganic phosphate was estimated in the supernatant fraction.

$$Na^+ K^+ ATPase = Total\ ATPase - Mg^{2+} ATPase$$

Estimation of protein content: Protein content of the kidney was estimated by the method of [10]. 1% (W/V) homogenation was prepared in 0.25 M ice cold sucrose solution. To 0.5ml of crude homogenate, 1ml of 10% TCA was added and the samples were centrifuged at 1000 g for 15min. The residue was resuspended in 0.5ml of 1N NaOH. And 4ml of alkaline copper reagent was added followed by 0.4ml of folin-phenol reagent (1:1 folin:H_2O). The color was measured at 600 nm in a UV- Vis spectrophotometer (Hitachi model U-2000) against blank. The protein standard graph was prepared using Bovine serum albumin. The protein content of the tissues was calculated using the standard graph.

Validity of experimental procedure:

Aliquots for assay

Aliquots were selected for the assay such that the initial rates were approximated as nearly as possible, yet providing sufficient product to fall in a convenient range for spectrophotometric measurement.

Enzyme units

The tissue soluble protein content in the homogenates/supernatants (enzyme source) was estimated using folin phenol reagent [10]. This was used for the expression of enzyme activity. Enzyme activities were expressed in standard units, i.e., µ moles of product formed or substrate cleaved per milligram protein/hr.s

Substrate requirements

All the enzyme activity levels were determined at saturating substrate concentrations i.e., in zero order.

Lambart Beer Law

Almost all the products of the reactions were measured by spectrophotometric methods, in which the optical densities (absorbance) of the resulting coloured complexes are proportional to the concentration of the reaction product. Standard graphs were prepared for each estimation between concentration of the substance (either product or substrate) and the optical density from which the activities of enzymes were calculated.

Statistical treatment of the data

The mean and standard deviation (SD), analysis of variance (ANOVA) and test of significance or students't' test was calculated using standard statistical software package.

III. RESULTS

AChE ACTIVITY:

In the present study, from fig 1, it was observed that the AChE activity in synaptosomal rat brain region of As-exposed (both low and high dose) rats was decreased when compared to control. However, the effect was highly pronounced in high dose exposed animals compared to the low dose exposed animals. AChE activity was found to be increased in the animals supplemented with Vit-E along with low and high dose As-exposed animals. Among the brain regions, CC was found to be more susceptible compared to the other brain regions.

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

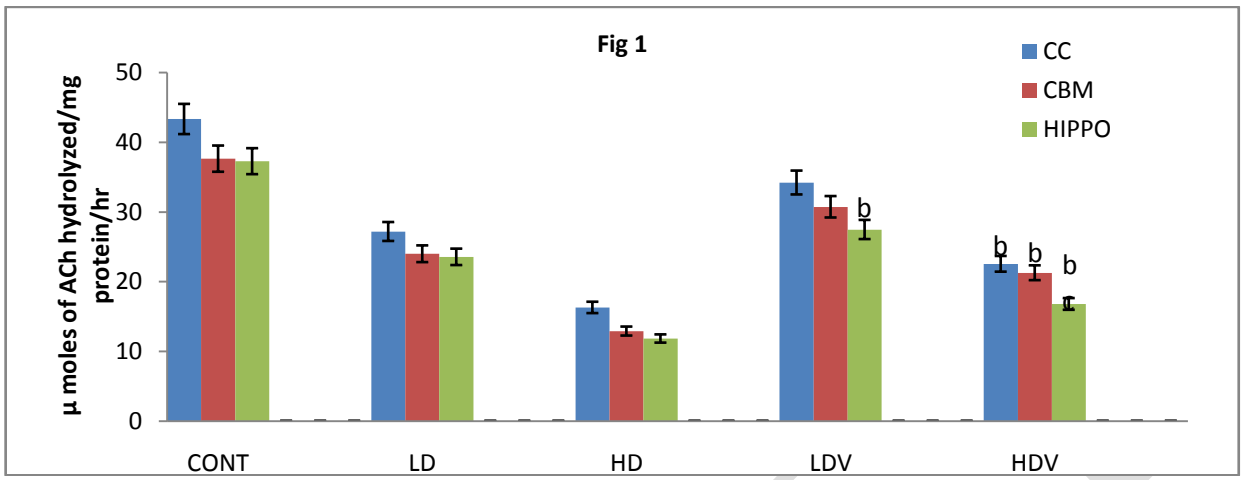


Fig 1: Protective effect of Vit-E against low dose As (1.5mg/Kg body weight) and high dose As (3mg/kg body weight) induced alterations in AChE activity levels in brain specific regions; CC (Cortex), CBM (Cerebellum) and Hp (Hippocampus). All values are extremely signification at p<0.0001, except the values (bare) marked with a: compared with control, b: compared with LD, c: compared with HD, d: compared with LDV.

ACh Content

In the present study, from fig 2, it was observed that the ACh content in synaptosomal of As-exposed (both low and high dose) rats was increased when compared to control. However, the effect was highly pronounced in high dose exposed animals compared to the low dose exposed animals. ACh content was found to be decreased in the animals supplemented with Vit-E along with low and high dose of As-exposed animals. Among the brain regions, CC was found to be more susceptible compared to the other brain regions.

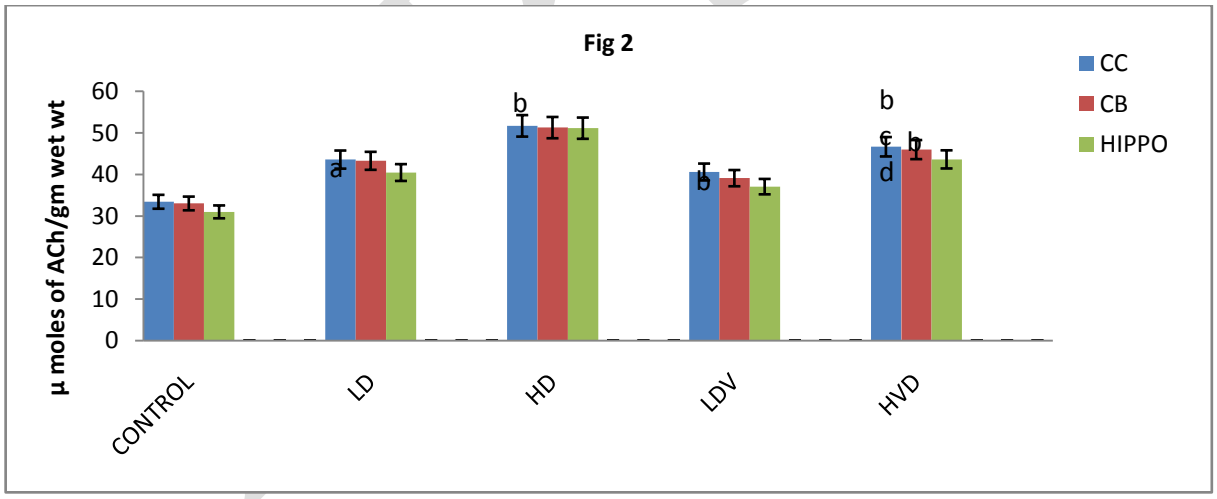


Fig 2: Protective effect of Vit-E against low dose As (1.5mg/Kg body weight) and high dose As (3mg/kg body weight) induced alterations in ACh activity levels in brain specific regions; CC (Cortex), CBM (Cerebellum) and Hp (Hippocampus). All values are extremely signification at p<0.0001, except the values (bare) marked with a: compared with control, b: compared with LD, c: compared with HD, d: compared with LDV.

DOI: 10.15680/IJIRSET.2014.0310062

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

Mg^{2+} ATPase activity:

From the data presented in fig.3, it was observed that the Mg^{2+} activity in synaptosomal of As-exposed (both low and high dose) rats was decreased when compared to control. However, the effect was highly pronounced in high dose exposed animals compared to the low dose exposed animals. Mg^{2+} activity was found to be increased in the animals supplemented with Vit-E along with low and high dose of As-exposed animals. Among the brain regions, CC was found to be more susceptible compared to the other brain regions.

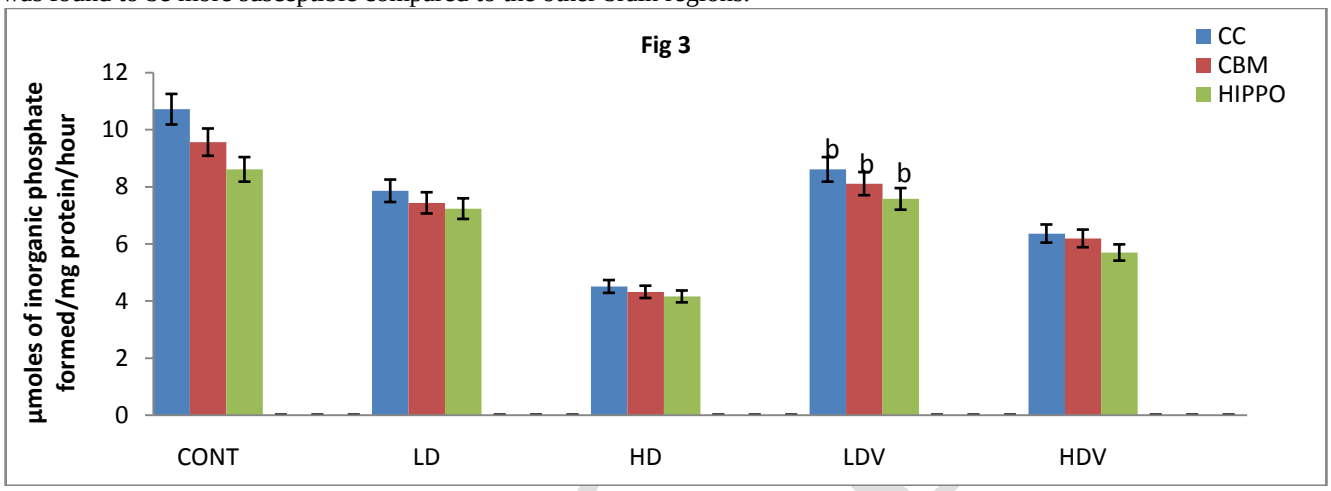
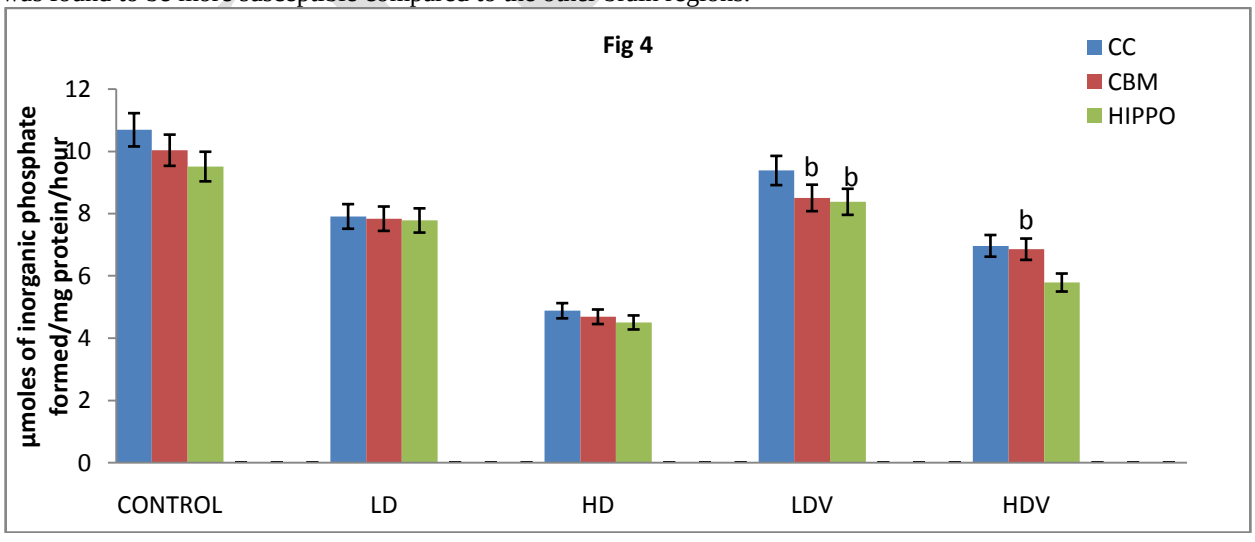


Fig3: Protective effect of Vit-E against low dose As (1.5mg/Kg body weight) and high dose As (3mg/kg body weight) induced alterations in Mg^{2+} ATP ase activity levels in brain specific regions; CC (Cortex), CBM (Cerebellum) and Hp (Hippocampus). All values are extremely signification at $p<0.0001$, except the values (bare) marked with a: compared with control, b: compared with LD, c: compared with HD, d: compared with LDV.

$Na^{+}K^{+}$ ATPase activity:

From the data presented in the fig.4, it was observed that the Na^{+}, K^{+} activity in synaptosomal of As-exposed (both low and high dose) rats was decreased when compared to control. However, the effect was highly pronounced in high dose exposed animals compared to the low dose exposed animals. Na^{+}, K^{+} activity was found to be increased in the animals supplemented with Vit-E along with low and high dose As-exposed animals. Among the brain regions, CC was found to be more susceptible compared to the other brain regions.



DOI: 10.15680/IJIRSET.2014.0310062

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

Fig.4: Protective effect of Vit-E against low dose As (1.5mg/Kg body weight) and high dose As (3mg/kg body weight) induced alterations in Na^+K^+ATp ase activity levels in brain specific regions; CC (Cortex), CBM (Cerebellum) and Hp (Hippocampus). All values are extremely signification at $p<0.0001$, except the values (bare) marked with a: compared with control, b: compared with LD, c: compared with HD, d: compared with LDV.

Protein content

From the data presented in the fig.5, it was observed that the protein content in synaptosomal fraction of As-exposed (both low and high dose) rats was decreased when compared to control. However, the effect was highly pronounced in high dose exposed animals compared to the low dose exposed animals. Protein content was found to be increased in the animals supplemented with Vit-E along with low and high dose of As-exposed animals. Among the brain regions, CC was found to be more susceptible compared to the other brain regions.

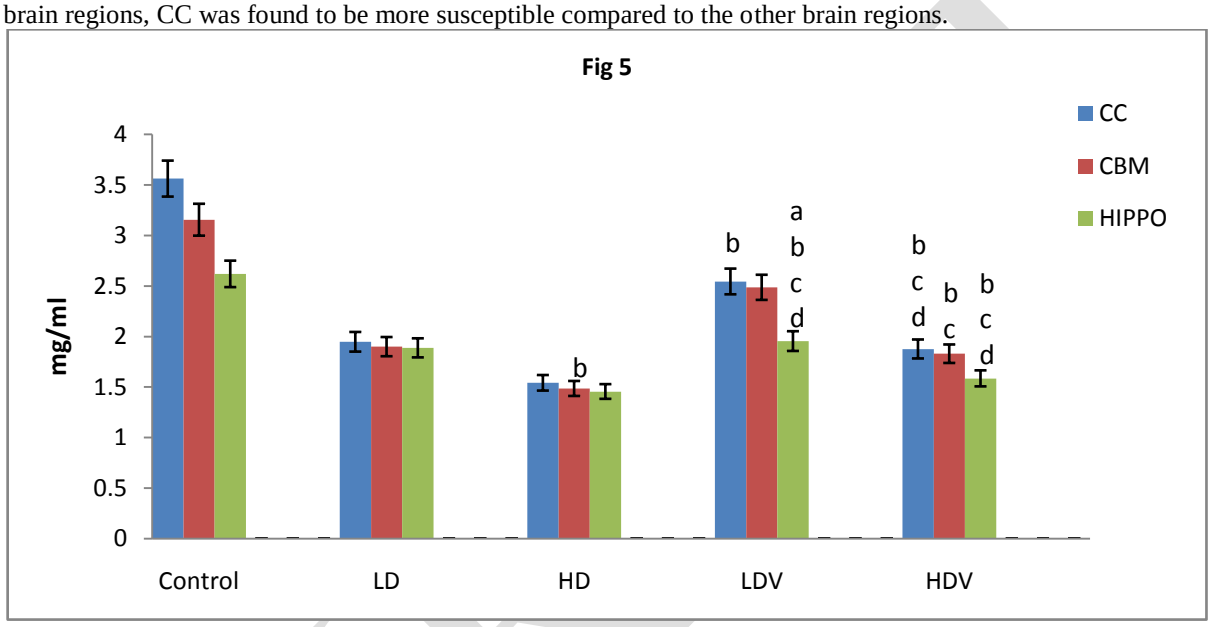


Fig5: Protective effect of Vit-E against low dose As (1.5mg/Kg body weight) and high dose As (3mg/kg body weight) induced alterations in protein content levels in brain specific regions; CC (Cortex), CBM (Cerebellum) and Hp (Hippocampus). All values are extremely signification at $p<0.0001$, except the values (bare) marked with a: compared with control, b: compared with LD, c: compared with HD, d: compared with LDV.

IV. DISCUSSION

As the cholinergic system and enzymes of energy metabolism such as Mg^{2+} ATPase and Na^+ K^+ ATPase have a relatively high sensitivity to metals such as As, Pb, Cd, the present study is designed to assess the dose dependent sensitivity of the enzymes of energy metabolism such as Mg^{2+} ATPase and Na^+ K^+ ATPase and cholinergic system towards these heavy metals.

Arsenic inhibited the activities of carbohydrate metabolism enzymes – Na^+K^+, Mg^{2+}ATPases. Such results have been reported by [11] after mice were exposed to arsenic trioxide. Arsenite inhibits SDH activity and also uncouples oxidative phosphorylation there by decreaseing ATP content in cell [12]. [13], have also reported a decrease in ATPases activity in fish brain, in support of our data.

In the present study rats treated with As showed a significant inhibition in the activities of membrane bound ATPases which may be due to the increased membrane lipid peroxidation. The activity of these enzymes was improved in Vit E-treated groups owing to the antioxidant potential. Similar reports of52 and 53 which showed the decreased

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

levels of membrane bound ATPase in the brain of cadmium intoxicated rats. Na^+K^+-ATPase is a key enzyme implicated in neural excitability, metabolic energy production.

The Na^+ K^+ ATPase is a highly-conserved integral membrane protein that is expressed in virtually all cells of higher organisms. The temporal change of the concentration of inorganic ortho-phosphate, which is produced as a result of the enzyme catalyzed ATP hydrolysis, serves as a measure of enzyme activity. The enzymes Na^+ K^+ ATPases and Mg^{2+}- ATPases have a relatively high sensitivity to certain classes of heavy metals and other pollutants and it has been shown that toxicosis from pollutants may develop primarily from ATPase inhibition. A number of studies have shown that ATPase activity was inhibited by heavy metals such as As [14], Cd^{2+} [15]. Heavy metal binding to sulfhydryl groups has often been implicated in Na^+ K^+ ATPase inhibition [16].

Mg^{2+}ATPase have a unique role in energy synthesis and are localized in the mitochondria and are presumed to be present in all types of cells. Mg^{2+}ATPase have been shownto be involved in Oxidative [17]. In most cases Mg^{2+}-ATPase is taken as an index of general ATPase activity because of its abundant distribution and dual localization in mitochondria and cytosol [18]. The role of Mg^{2+} ATPase is to maintain the high intracellular Mg^{2+} level in brain, changes of which can control the rate of protein synthesis and cell growth54. As transitional heavy metals compete with intra cellular Ca^{2+}, the alterations in Ca^{2+} level also leads to severe pathological lesions in brain [19].

As ATPases are membrane bound enzymes any damage to cellular organelles due to toxins, heavy metals or pesticides would certainly results in the decreased activity levels and same has been observed in albino rats treated with sublethal doses of an organophosphate compound Acephate. In the present study dose dependent and region specific inhibition of ATPase activity in different regions of the brain was observed. The decrease in the Mg^{2+} ATPase activity might be due to low operation of oxidative pathway, resulting in decreased formation of free energy and altered cellular energy metabolism [20]. [21] reported that the inhibition of Na^+ K^+ ATPase may be due to the flow of the Na^+ K^+ ions from the tissues to the blood. It is known that brain derived Na^+ K^+ ATPase is among the enzymes particularly affected by Lead (Pb) [22]. The decrease of Na^+ K^+ ATPase activity can change the gradients of Na^+ K^+ across the cell membrane and can be cause of the disturbances in neurotransmitters levels [23]. Recent studies show that brain Na^+ K^+ ATPase activity may be modified by certain neurotransmitters. The extent of Na^+ K^+ ATPase inhibition was dependent on the K^+ concentration, thus suggesting an interference with the K^+ site of the enzyme [24]. The inhibition of Mg^{2+} ATPases observed in the present study could lead to a reduction in ATP production which in turn would alter Na^+ K^+ pump activity, producing neuronal dysfunction. Changes in the metabolic balance of intact animals are due to shifts in the pattern of metabolism in individual tissues which are usually associated with changes in availability of metabolites or changes in activity of key enzymes. Several investigators have studies the action of metals on ATPase activities. Inhibition in Na^+ K^+ATPases in the nervous system was observed following pyrethroid exposure [25]. Inhibition of both Mg^{2+}ATPase s and Na^+ K^+ ATPases was also reported in cockroach ([26], [27]), rat [28]. Decrease in Mg^{2+}-ATPase activity in vertebrates and invertebrates was also reported by several workers [29].

An impairment of the cholinergic function was observed in As-treated animals, including alterations in ACh turnover rate, AChE activity59. AChE was inhibited by both low and high As concentrations and it was found that As is one of the metal inactivators of the enzyme (where Ca^{2+} is one of the activators) and is capable of inducing a conformational change in the protein, which leads to the formation of an ‘‘unreactive’’ enzyme [30].

The study of brain enzyme activities such as of AChE is essential in detecting the neurotoxic effects of certain heavy metals. Arsenic administration (5mg/kg) caused a significant decrease in brain AChE activity. This is in accordance with [31] who reported decreased rat brain AChE activity after oral Cd (5 mg/kg $CdCl_2$ for 1 month every other day) administration although [32] reported increase in AChE activity. It is likely that the way of administration and the quantity of Cd that reaches the brain and the enzyme differentiates the effect. Increases of AChE activity provoke enhanced acetylcholine (ACh) hydrolysis and choline reuptake [32]. Vitamin-E in particular increased rat brain AChE activity, a fact that is well related to total antioxidant status value increases compared to those induced by As treatment.

DOI: 10.15680/IJIRSET.2014.0310062

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

A decrease in the protein levels occurred following arsenic exposure, with a marked decrease especially in the high dose groups. Reduction in protein levels could be attributed to their damage by singlet oxygen, often due to oxidation of essential amino acids [33]. Aldehydes (MDA) formed during lipid peroxidation can react with –SH groups of proteins to damage them, thus inhibiting enzymes requiring –SH groups for their activities [34]. Thus, damage to proteins and total –SH occurs not only through ROS but also by binding of Arsenite to these molecules. Further, arsenic trioxide treatment also inhibited SDH and ATPases in our study effecting neural tissues metabolism. Arsenic effects mitochondrial enzymes and impairs tissue respiration, which seems to be related to cellular toxicity of arsenic. Thus, reduction in total protein is probably attributed to loss of growth due to low food intake as a result of As^{+3} poisoning.

V. CONCLUSION

Thus from the present study, it was concluded that, oral administration of arsenic leads to a decrease the brain AChE activity, and ATPases. However, when co-administered with As, Vit E maintained AChE activities in high levels, and prevent the cadmium-induced neurotoxicity. Vit E provokes an impressive enhancement in rat brain AChE activity and a significant increase in rat brain carbohydrate metabolic enzymes such as $Na^{+}K^{+}$ and Mg^{2+}ATPases. Thus toxic potential of Arsenic was cleanly illustrated by the increased inhibition or decreased activity levels of Mg^{2+}, Na^{+} K^{+} ATPase, AChE activity and increased ACh content in different brain regions of albino rats.

REFERENCES

[1]. Emsley, John, "Nature's Building Blocks". An A-Z Guide to the Elements. Oxford: Oxford University Press. pp. 43,513,529, 2001.
[2]. Sabina C. Grund, Kunibert Hanusch, Hans Uwe Wolf, "Arsenic and Arsenic Compounds", Ullmann' s Encyclopedia of Industrial Chemistry, Weinheim: Wiley-VCH, doi:10.1002/14356007, 2005.
[3]. Saha, K. C, "Chronic arsenical dermatoses from tube-well water in West Bengal during 1983-87". Indian J. Dermatol. 40(1), pp 1-12, 1995.
[4]. Pershagen, G. Braman, R. S., and Vahter, M, "In environmental Health Criteria 18: Arsenic, World Health Organ". Geneva, pp 76-146, 1981.
[5]. Metcalf, R.L, "In Methods of biochemical analysis". Vol 5. (Ed. Glick), Interscience Publishers Inc., N.Y. 1957. 5, 1-57, 1951.
[6]. Augustinssion, K.B, "Cholinesterase and anticholinesterase agents". (Ed. G.B. Koelle). Springer Verlag. Berlin. 5, 89-128,1963.
[7]. Ellman, G., Courtney, K., Andres, V., Feather-Stone, R, "A new and rapid colorimetric determination of acetylcholinesterase activity". Biochem Pharmacol. 7, 88–95, 1961.
[8]. Tirri, R., Lagerspetz, K.Y.H., Kohnen, J, "Temperature dependence of the ATPase activites in brain homogenates during the postnatal development of the rat". Comp. Biochem. Physiol. 44B, 473, 1973.
[9]. Fiske, C.H., Subba Row, Y., "The colorimetric determination of phosphates". *J. Biol. Chem.* 66, 375-400, 1925.
[10]. Lowry, O.H., Rosenbrough, N.J., Farr, A.L., Randall, R.J, "Protein measurement with Folin-phenol reagent". J. Biol. Chem, 193, 265-275, 1951.
[11]. Chinoy, N. J, "Studies on fluoride, aluminium and arsenic toxicity in mammals and amelioration by some antidotes". In Modern Trends in Environmental Biology (Tripathi, G., Ed.), CBS Publishers, New Delhi, Chapter 12, pp. 164-193, 2002.
[12]. Casarrett, L and Doull J, "Toxicology: the basic Science of Poisons". Mc Graw-Hill, New York, 2001.
Davis, Joseph R; Handbook Committee, ASM International (2001-08-01). "Dealloying". Copper and copper alloys. p. 390. ISBN 9780871707260
[13]. Shobha Rani, A. Sudharsan, R. Reddy, T. N, "Alterations in the levels of dehydrogenases in a fresh water fish, Tilapia mossambica (Peters) exposed to arsenite toxicity". Ind. J. Environ. Health, 42, 130-133, 2000.
[14]. Singerman, A, "Clinical signs Vs biochemical effects for toxic metals. In: Ed. Nordberg, G.F., Effects of Dose and Response Relationships for Toxic Metals". Elsevier. 207, 1976.
[15]. Valle, B. L. and Ulmer, D. D, "Biochemical effects of mercury, cadmium and lead". Ann. Rev. Biochem. 41: 91, 1972.
[16]. Carfagna, M. A., Ponsler, G. D. and Muhoberac, B. B, "Inhibition of ATPase activity in rat synaptic plasma membranes by simultaneous exposure to metals". Chem. Biol. Interact. 100, 53-65, 1996.
[17]. Skou, J. C, "Enzymatic basis of active transport of Na+ and K+ across cell membranes". Physiol. Rev. 45- 56, 1965.
[18]. Lehninger, L. and Albert, "The Molecular Basis of Cell Structure and Function". In: Biochemistry Edn., 2nd. Kalyani Publishers, Ludhiana, New Delhi, 1988.
[19]. Repetto M, "Toxicologia Fundamental". Diazide Santos Editions, Madrid, Spain, (1997).
[20]. Boyer, P, "In: The Enzymes". Academic press, NY. 3rd Edn. Vol. 13, 1977.
[21]. Bhaumik and Raychaudhuri, "Ind. J. Physiol". Allied. Sci. 29(4):111, 1976.
[22]. Fox, D. A., Rubinstein, S. D. and Hsu, P, "Developmental lead exposure inhibits adult rat retinal, but not kidney, Na+ K+ATPase". Toxicol. Appl. Pharmacol. 109, 482-493, 1991.
[23]. Struzynska, L., Dabrowska-Bouta, B., Lenart, J., Zborowski, J. and Rafalowska, U, "Some metabolic effects in rat brain synaptosomes after exposure to lead in vivo and in vitro". Bull. Pol. Acad. Sci. Biol. Sci. 42, 55-62, 1994.
[24]. Rodrigez, de Lores, Arnaiz, G. and Pena, C, "Characterization of synaptosomal membrane Na+ K+ATPase inhibitors". Neurochem. Int. 27, 319-32, 1995.
[25]. Desaiah, D., Cutkomp, L. K., Koch, R. B. and Jarvinen, A, DDT, "Effect of continuous exposure on ATPase activity in fish, pimephales promels". Arch. Env.Contam. Toxicol. 3, 132-141, 1973.

ISSN: 2319-8753

International Journal of Innovative Research in Science, Engineering and Technology

(An ISO 3297: 2007 Certified Organization)

Vol. 3, Issue 10, October 2014

[26]. Reddy, G. R., Madhusudhana, L., Shafeek, A. and Chetty, C. S, "Azadirachtin and cypermethrin induced alterations in electrophosiological properties of sensory and interneurons in the cockroach, periplaneta Americana". Bull. Eviorn. Contam. Toxicol.45, 416-418, 2001a.

[27]. Reddy, G. R., Shafeek, A., Chetty, C. S. and Sajwan, K. S, "Effects of cypermethrin and azadirachtin on neuronal AChE and ATPases in cockroach, periplaneta Americana". Paper presented at 9th International Congress of Toxicology, Brishane, Australia, 2001b.

[28]. Tang, C. Y, "Effectsof pyrethroids on rat brain synaptosomal ATPase activities". Chung-Huc Yu Fang Hsuch isa Chin. 23(2): 80-82, 1989.

[29]. Lakshmi Rajyam, C. H, "Evaluation of toxic effects of a synthetic pyrethroid fenvalerate in albino rat". Ph.D thesis, Sri Venkateswara University, Tirupati, India 1992.

[30]. Tomlinson G, Mutus B and McLennan I, "Activation and inactivation of acetylcholinesterase by metal ions". Canadian Journal of Biochemistry and Cell Biology, 59, 728–735, 1981.

[31]. El-Demerdash, F. M., M. I. Yousef, F. S. Kedwany & H. H. Baghdadi, "Cadmium-induced changes in lipid peroxidation, blood hepatology, biochemical parameters and semen quality of male rats: protective role of vitamin E and β-carotene". Food Chem. Toxicol. 42, 1563–1571, 2004.

[32]. Carageorgiou, H., V. Tzotzes, C. Pantos, C. Mourouzis, A. Zarros & S. Tsakiris, "In vivo and in vitro effects of cadmium on adult rat brain total antioxidant status, acetylcholinesterase, (Na^+,K^+)-ATPase and Mg^{2+}-ATPase activities". protection by L-cysteine. Basic & Clinical Pharmacology & Toxicology. 94, 112–118, 2004b.

[33]. Okuda, T., T. Haga, Y. Kanai, H. Endou, T. Ishihara & I. Katsura, "Identification and characterization of the high-affinity choline transporter". Nat. Neurosci. 3, 120–125, 2000.

[34]. Halliwell B, and Gutteridge J. M. C, "Free Radicals in Biology and Medicine", Clarendon Press, Oxford. 1985.