SIGNAL TRANSDUCTION PATHWAYS FOR CELL SURVIVAL OR APOPTOSIS BY TRI- AND PENTAVALENT ARSENIC IN RAT BRAIN

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Abstract

Arsenate and arsenite affect the brain tissue in a dose independent manner and follow different pathways to exert their toxic effects. Arsenate present in the drinking water is highly toxic at 0.1ppm concentration and causes an array of metabolic disturbances like increased LPO, decreased GSH, increased NOS activity in rat brain. At higher doses also arsenate has damaging effects on biological systems but might follow different mechanism and coadministration of an antioxidant as per normal requirement of rat can control the lipid peroxidation and glutathione level to some extent, but cannot give significant protection against increased nitric oxide synthase activity which eventually causes nuclear changes at DNA or protein level and alter a number of physiological processes in the rat brain. Both arsenate and arsenite significantly affect specific signal transduction molecules in the glial cell population in the brain that are involved in mediating cellular transformation or apoptosis, including MAPKs, NF-kB, PI3K, Akt etc. High concentration of arsenic exposure may lead to apoptosis, whereas a low concentration of arsenic exposure is carcinogenic and may result in aberrant cell accumulation.

Introduction

Source of arsenic in the drinking water are of two types –

- **GEOGENIC**: Arsenic is present in association with iron pyrites in aquifer sediments. Desorption and dissolution of iron pyrites under reducing condition releases arsenic in the drinking water. Entry of oxygen in the aquifer due to huge groundwater withdrawal for

irrigation favors oxidation of arsenic rich iron sulfide and mobilization of arsenic to the aquifer.

- **ANTHROPOGENIC**: Agricultural pesticides and herbicides, paints, wood preservatives etc. and mining, smelting and burning of fossil fuels mix arsenic with surface soil (1,2).

Toxic effect of arsenic are of various types depending upon cell type-Non-Cancer Effects such as Gastrointestinal, Neurological, Immunological Reproductive, Vascular, Blackfoot Disease, Hypertensive ,Heart Disease, Pulmonary, Hematological, Skin Diseases , Hyperpigmentation, Keratosis etc. and Cancer Effects like Bladder, Kidney, Lung, Liver, Skin cancer are already reported. Arsenic mediated neuropathy has also been reported. The predominant clinical features of peripheral neuropathy are:

a) Paresthesias , b) Numbness, c) Pain, particularly of the soles of the feet.

d) Encephalopathy , severe headache, personality disturbance, convulsions or coma,e) Mental retardation, poor concentration,f) Loss of memory etc.(3,4,5).

Inorganic Forms of Arsenic in Drinking Water are of two types :

- As^{+3} (trivalent arsenic),predominate form in reducing environments.

  And predominate form in groundwater.

- As^{+5} (pentavalent arsenic),predominate form in oxidizing environments

Very few reports are available till date on the *in vivo* effect of arsenic in brain cells.

Brain tissue is highly susceptible to oxidative damage by arsenic due to high polyunsaturated fatty acid content, low catalase and glutathione peroxidase activity and low GSH and vitamin E content.

In our previous studies we have reported that treatment of rats with sodium arsenate in water containing 0.1,0.3 and 3.0 ppm of As for forty days reduced the glutathione level, superoxide dismutase and glutathione reductase activities indicating free radical generation in treated animal brain (6).

Materials and methods:

Animals and treatment:

Male Charles Foster rats of 3-4 weeks age and average body weight 100gm. were kept in laboratory condition of 12 hr. light and 12 hr. dark cycle with food *ad libitum* and water. Rats were divided in nine groups and each group consisted of six rats as follows:

Group 1: was given regular tap water for drinking and was considered as control.

Group 2: was given 0.1ppm Na-arsenate with drinking water.

Group 3: was given 0.1ppm Na-arsenate dissolved in drinking water plus Vitamin E at a basal dose of 144μg/kg body wt/ day.

Group 4: was given 0.3 ppm Na-arsenate mixed in drinking water.

Group 5: was given 0.3 ppm Na-arsenate with drinking water along with oral feeding of Vitamin E at the above dose.

Group 6: was given 0.1ppm Na-arsenite with drinking water.

Group 7: was given 0.1ppm Na-arsenite along with drinking water with oral dose of Vitamin E at 144μg/kg body wt/day.

Group 8: was given 0.3 ppm Na-arsenite with drinking water.

Group 9: was given 0.3 ppm Na-arsenite with drinking water along with oral feeding of Vitamin E at the above dose.

Dose of Vitamin E was selected on the basis of our previous laboratory work and is equivalent to the basal dose required by human (144μg/kg body wt./ day). The duration of treatment was 40 days, as in our preliminary studies the toxic changes of arsenic begin to appear after 40 days time point at the level of enzyme studies.(Choudhuri AN et al, 1999). Following 40 days of treatment with arsenate and arsenite , the rats were sacrificed by decapitation following anesthesia.

Nitric oxide synthase activity was assayed spectrophotometrically following Nins et al .(7).

The rat brain tissues were sliced and fixed in formal saline solution (10 ml formaldehyde, 90 ml of 0.9% NaCl in water)for 24 to 48 hrs in glass container with two changes . The fixed tissues were then dehydrated through graded alcohol(50%,70%, 90%a nd absolute alcohol).

The paraffin sections were deparaffinized in xylene and hydrated through graded alcohols (absolute, 90%, 70%, 50%, 30%) . Then the slides were stained with haematoxylin stain dehydrated gradually through graded alcohol upto 90%.Then the slides were stained in 2% eosin stain, dehydrated through 95%and absolute alcohol , cleared in xylene and mounted with DPX following Pearse ,1980(8). Slides were viewed under microscope at 400 X magnification .

For the preparation of glial cell lysate the brain cortex was dissected out and minced in a petri dish containing 3 ml calcium PVP buffer (7.5% polyvinylpyrrolidone w/v,10mM $CaCl_2$ and 1%BSA) on ice. T cells were separated by nylon mesh and sucrose density gradient at 15000 rpm. The lower band of the two bands are glial cells . The photograph was taken in a fluorescent microscope using goat anti GFAP (Glial fibrillary acidic protein) antibody and FITC conjugated secondary antibody (9).

For Western Blot analysis the glial cells were lysed in MAPK extraction buffer (Tris 10mM pH 7.4, EGTA4.5mM,EDTA 4.5mM,$Na_3VO_4$1mM,PMSF1 mM, Aprotinin(10µg/ml)and Leupeptin(10µg/ml)and centrifuged at 10,000 rpm at 4oC for 20 mins. The protein concentration of the supernatant was determined by Lowry's method (10).

Each protein sample was electrophoresed on 10% SDs –PAGE gel for 3hrs and then transferred to a nitrocellulose membrane (11,12).The membrane was washed in Tris –buffered saline (TBS) then blocked overnight with 3% w/v BSA dissolved in TBS. Then the membrane was incubated in 2ml of first antibody (p-ERK 1:1000 dil./ p-JNK 1:1000dil./p-Akt 1:1000dil./ p-p38 1:1000dil) in TBS buffer with gentle shaking for 3hrs . The membrane was then removed from 1st antibody solution , washed with buffer for several times and then was incubated for 1hr in 1:2000 dil of AP tagged anti rabbit /anti mouse IgG secondary antibody . The blots were

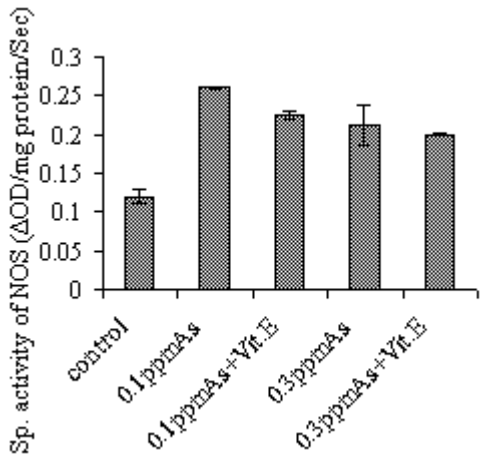
developed in presence of NBT-BCIP substrate , photographed and densitometric studies were done .

Result and Discussion

Fig1:

Dose-independent alteration in the total nitric oxide synthase (NOS) activity by arsenate (a) and arsenite (b) in the rat brain and role of Vit.E.

(a) (b)



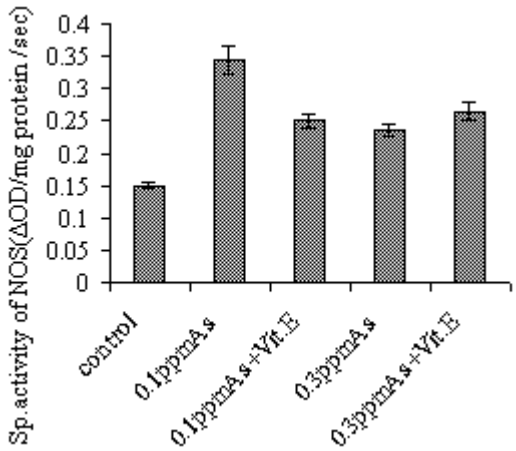
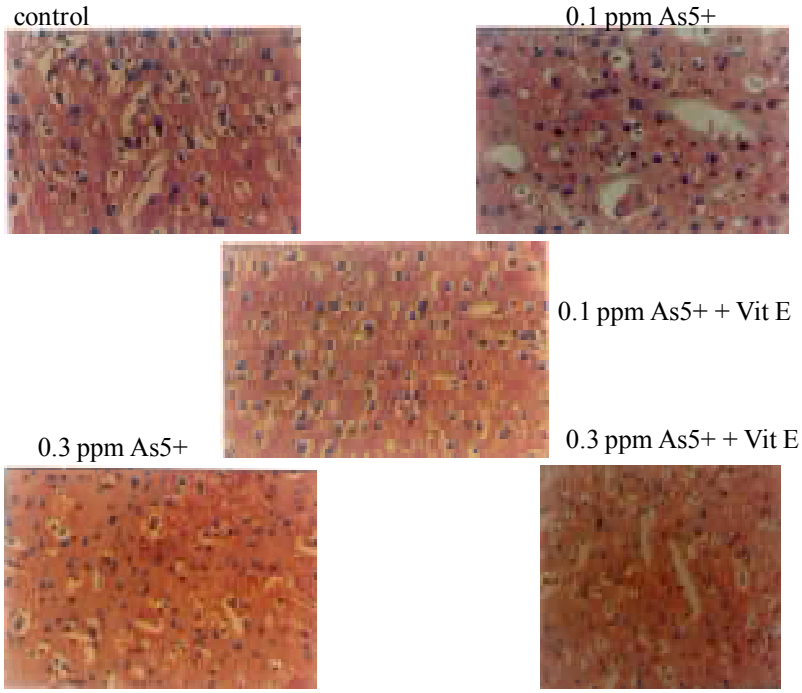
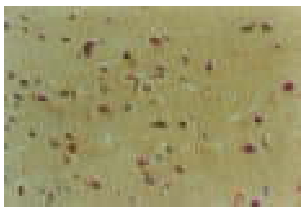


Fig 2:

Histological observation due to Arsenic treatment in rat brain is given below :



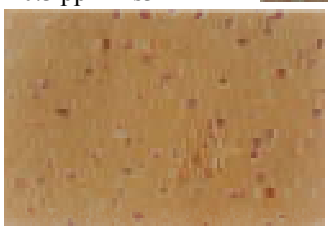
control



0.1ppm As 3+

0.1ppm As3+ + Vit E

0.3 ppm As3+



0.3 ppm As 3+ + Vit E

Fig. 3:

Isolated glial cells from rat brain :

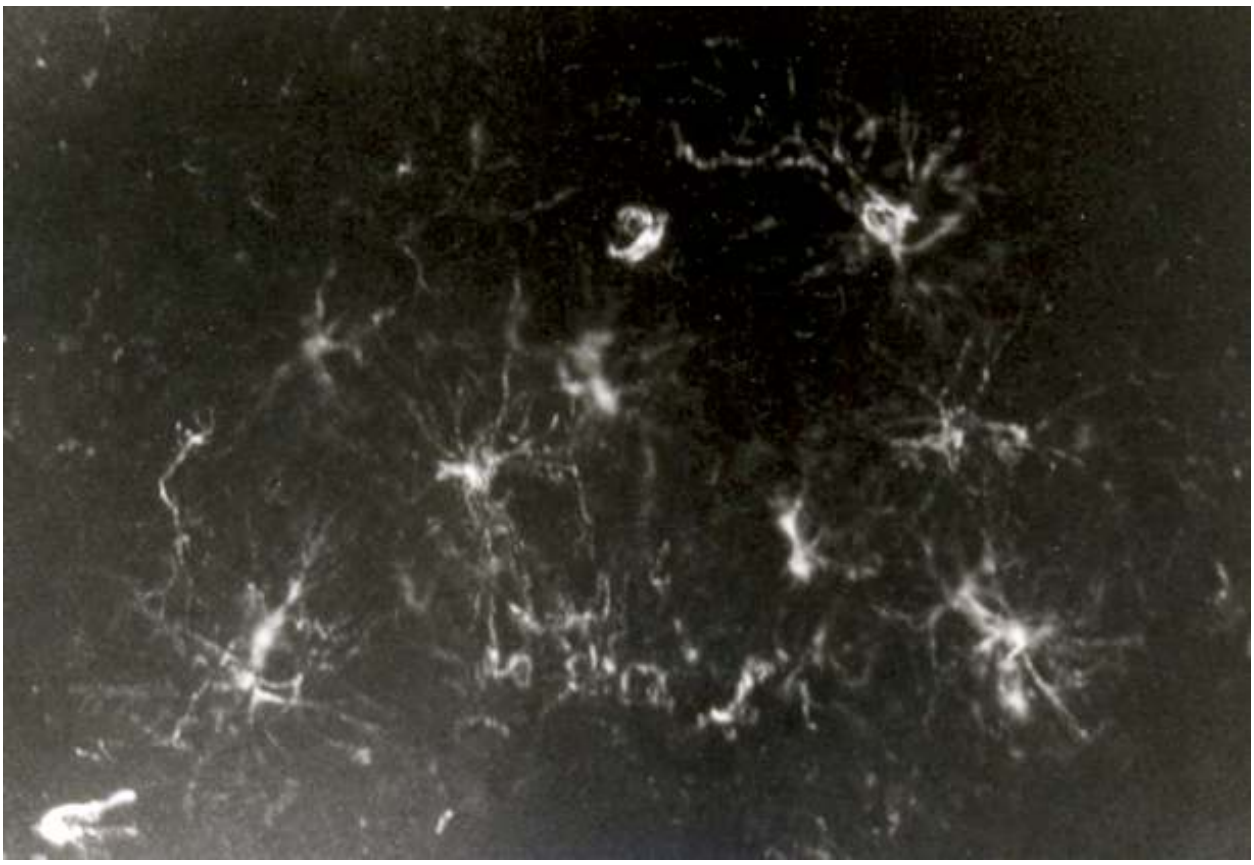


Fig.4:

Arsenate (a) and arsenite (b) mediated changes in the pPI3K expression in the rat brain glial cells in presence and absence of Vit.E**.**

(a) (b)

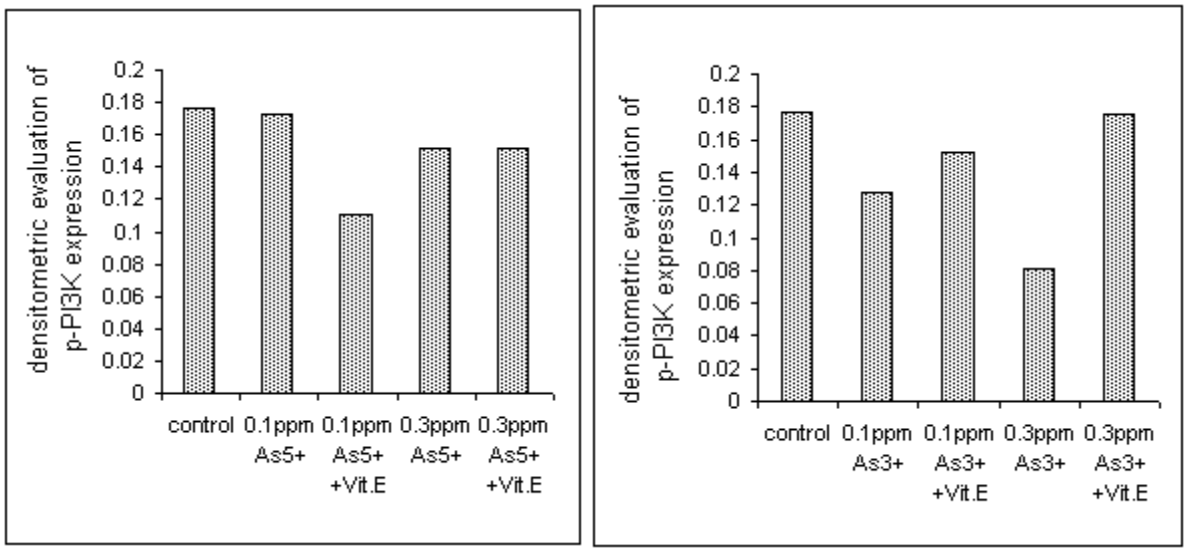


Fig.5:

Arsenate (a) and arsenite (b) mediated changes in the pAkt expression in the rat brain glial cells in presence and absence of Vit.E.

(a) (b)

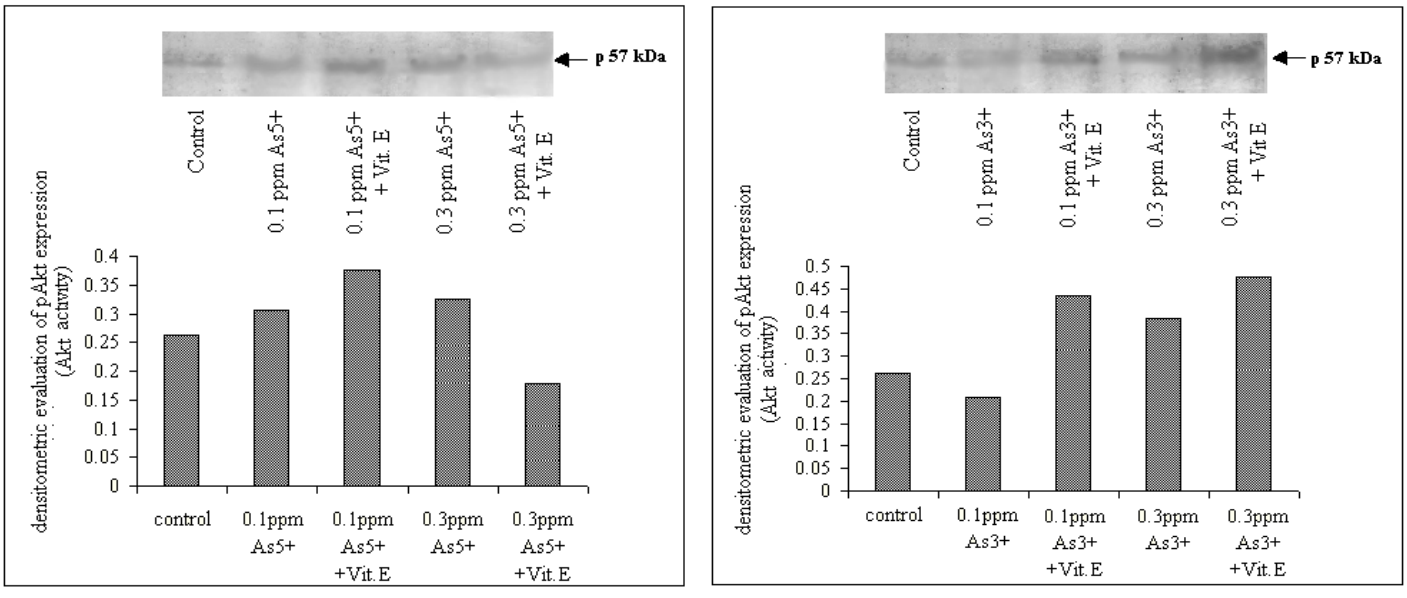


Fig.6:

Arsenate (a) and arsenite (b) mediated changes in the pERK expression in the rat brain glial cells in presence and absence of Vit.E.

(a) (b)

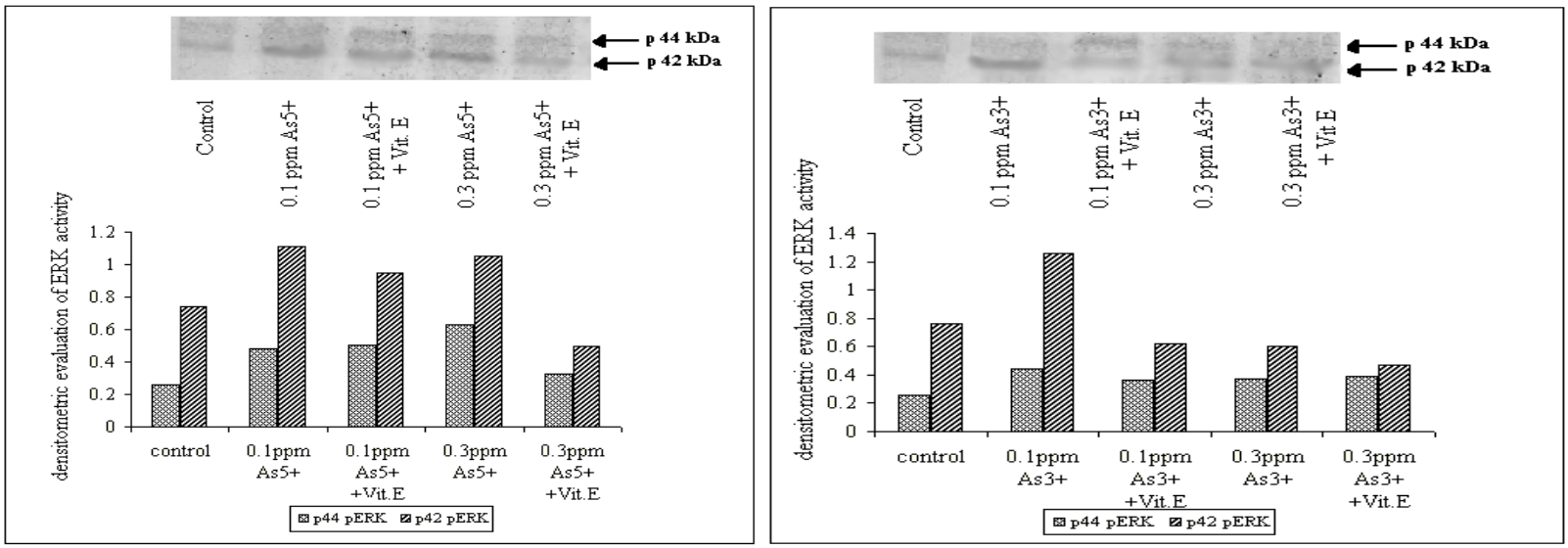


Fig.7:

Arsenate (a) and arsenite (b) mediated changes in the pJNK expression in the rat brain glial cells in presence and absence of Vit.E

(a) (b)

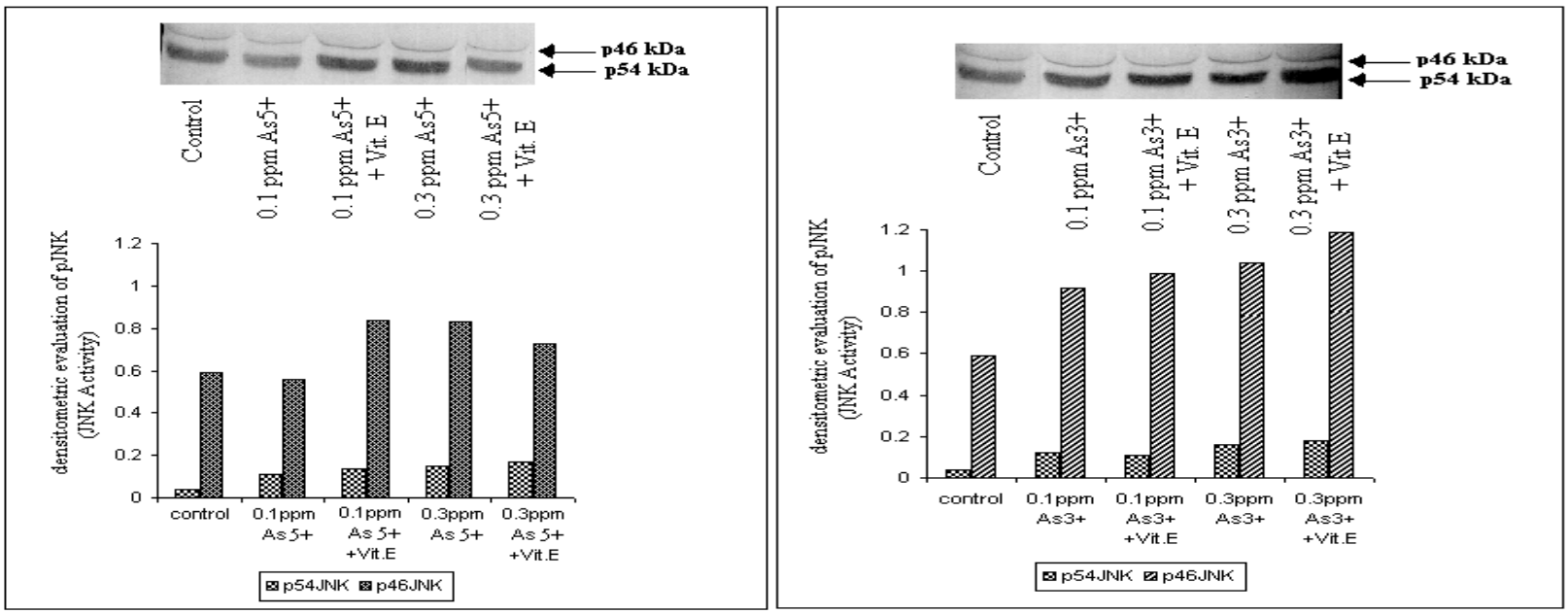
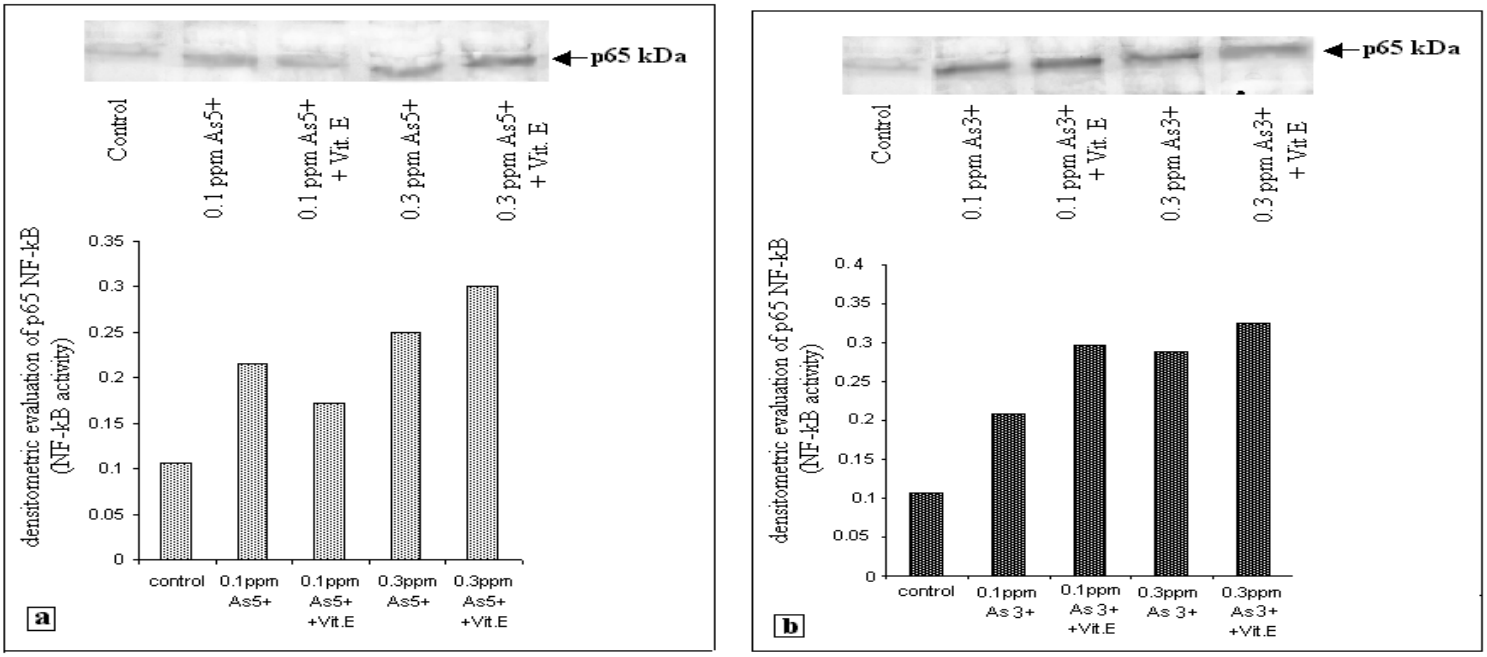


Fig 8:

Arsenate (a) and arsenite (b) mediated changes in the p65 NF-kB expression in the rat brain glial cells in presence and absence of Vit.E.

(a) (b)



Environmental exposure to aesenate and arsenite through drinking water is associated with an increased risk of skin , urinary bladder, liver , kidney lung cancers as well as neurodegenerative diseases (13).The histological slides of rat brain after arsenate treatment for 40 days showed damage in the lipid membrane and reduction in the nuclei size at 0.1 ppm dose in comparison to the normal and there was a recovery to normal level with simultaneous treatment with vitamin E . At higher dose ie., at 0.3ppm concentration the histology of rat brain showed marked lipid depletion from the membrane and remarkable shrinkage of the nuclei which was not recovered with vitamin E treatment . In contrast arsenite treatment showed no changes in lopid membrane but deformed nuclei were observed which were not rectified with vitamin E treatment .

In the rat brain the Ca dependent NOS is expressed constitutively (14). In our experiment its activity was markedly elevated at both the doses of both arsenate and arsenite but the effect of vit E was different in different cases . Therefore it can be implicated that arsenate and arsenite

mediated generation of reactive oxygen species differently turn on an array of biochemical changes in the rat brain cortex and manifest its pathological effect slowly which can be visualized in the histological slides .

The signal transduction pathways may be different in different cases . From our experiments with glial cells it was clear that arsenate in both the doses stimulates ERK activity and promotes cellular transformation and Vit E oppose such increase. But arsenite at lower dose enhances ERK activity but not at higher dose. Therefore apoptotic or cellular transformation may turn on or off due to different forms of arsenic in rat glial cells .

The c-Jun N terminal kinases (JNKs) are members of the MAPK family and like ERK1(p44) ans ERK2(p42) are activated through phosphorylation at conserved Thr and Tyr residues (15). The JNK pathway has been implicated in the regulation of apoptosis induced by various stimuli including arsenic (16).A recent study has shown that nuclear factor – kB(NF-kB) and JNK are reciprocal regulators for arsenite induced cell proliferation or apoptosis (17). We have shown that arsenate at 0.1 ppm dose doe not activate JNk but 0.3 ppm can do it in glial cells . This indicates that arsenate intake at low concentration may lead to cellular transformation while at higher concentration induces apoptotic changes . Arsenite on the other hand induces JNK at both the doses . Therefore arsenite intake even at small doses turn on the apoptotic machinery .

Under our experimental conditions , p38 activity showed marginal decrease at 0.1 ppm dose but enhanced significantly at 0.3 ppm dose of arsenate , while arsenite at both the doses increased p38 activity . These results suggested that arsenate at higher dose and arsenite at both the doses induce apoptotic changes .

In the PI 3K pathway p85 expression is not much altered in the glial cells of rat brain under in vivo condition by arsenate while arsenite caused dose dependent decrease in p85 expression . Of all the downstream effector of PI 3K Akt appears to be the most important to determine and direct the fate of the cells towards survival or apoptosis . In our study we have shown Akt activation is enhanced during arsenate treatment in both the doses whereas arsenite treatment only higher dose can enhance Akt activation .

Considering arsenate and arsenite's effect on the MAPK cascade as well as NF-kB activation in the glial cells it is clear that it is the balance between the cell survival pathway and the apoptotic

signaling pathway , that is disturbed due to arsenate and arsenite stress and resulted in the damaging but noncytotoxic changes at 0.1 ppm and 0.3 ppm doses in rat cortical glial cells .

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