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RESEARCH ARTICLE

Effect of widely used industrial chemicals on cell signaling pathways and cytoskeletal integrity in mammalian system

Dipanwita Mukherjee[12], Aniruddha Mukhopadhyay[2] and Anindita Chakraborty[1]*

1. UGC-DAE-Consortium for Scientific Research, Kolkata Centre, 3/LB-8, Bidhannagar, Kolkata-700098

2. Department of Environmental Science, University of Calcutta, Kolkata-700019

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Corresponding Author

Anindita Chakraborty

Abstract

The paper described effects of 4-Dimethylaminoazobenzene (*p*-DAB) and Diethylnitrosamine (DENA), two well known hepatotoxicants, on cytoskeletal dynamics of hepatocytes and associated signaling pathways. Swiss albino mice exposed to *p*-DAB (165mg/kg bw/mouse/day, orally) and DENA (Single dose, i.p 200mg/kg bw) for six weeks manifested drastic distortion of microfilament and microtubules architecture in the hepatocytes in addition to remarkable increase in intracellular ROS generation. *p*-DAB and DENA induced dysregulation of signaling pathways involving Rho-GTPases was reflected in significant downregulation of expressions of RhoA, Rac1 and Cdc42 proteins. Data showed considerable changes in expression of regulatory kinases viz. LIMK1, LIMK2 and SAPK/JNK, and also highlighted the crosstalk between C-Raf and proapoptotic kinase MST2. The present study reported for the first time the potential of *p*-DAB and DENA to alter Rho signaling protein expressions and disrupt cytoskeletal architecture via involvement of intracellular free radicals. Our data provided a cue to consider such changes to be important benchmark for assessing carcinogenic prospective of *p*-DAB and DENA.

INTRODUCTION

Global epidemiologic studies have identified association of chemicals from environmental and occupational exposures with various diseases including cancer. Effect of occupational exposure to specific chemicals (benzene, benzidine, vinyl chloride etc) on the risk of renal cell carcinoma was assessed by group of workers (Hu et al., 2002). An elevated risk of lung cancer among workers in chromate production facilities has been reported by Gibb et al. 2000. However, even after identification of such disease causing chemicals, measurement of accumulated exposure of individuals in different environments and mechanisms or pathways of formation of diseases or syndromes remain an important challenge (Lawrence and Curtis, 2008). As such chemical pollutant induced tumorigenesis or carcinogenesis with the perspective of having molecular and cellular insights of the process has not been studied much.

Liver cancer has been established to have direct relation to exposure to chemicals. It has been reported that besides viral infection and other risk factors viz alcohol or aflatoxins, exposure to environmental pollutants share a major role in formation of hepatic carcinoma (Farazi1 and DePinho, 2006; Jemal et al., 2007). Hepatocellular carcinoma (HCC), which accounts for about 80%-90% of all liver cancer, is known to be the commonest cancer in many countries (Bosch *et al*., 1999) especially in Southeast Asia and sub-Saharan (Qian and Ling, 2004) ranking fifth as the cause of all cancer-related deaths .

Chemicals that are widely used in various industrial processes have significant toxic effect on human subjects. The azo-dye 4-Dimethylaminoazobenzene (*p*-DAB), used as a coloring agent for polishes, soap and also as a food additive, has been reported to have hepatotoxic effects in laboratory animals. Potential routes of human exposure to *p*-DAB are through inhalation, dermal contact and ingestion. When released in air, it primarily binds with particulate matter and, in surface water; it binds with sediment and/or gets bioaccumulated in aquatic organisms. Though its mobility is affected by soil pH, it is likely to bind to soil particles (Hazardous Substances Data Bank [HSDB], 2009). *p*-DAB is one of the 13 chemicals, considered as potential human carcinogen by the Occupational Safety and Health Administration (OSHA), and has been shown to be a slow acting carcinogen (Ling and Foster, 1980). It is designated as a Group-2B carcinogen by the International Agency for Research on Cancer (IARC) in 1987. Reactive metabolites produced from the aminoazodye N,N-dmethyl-4-aminoazobenzene(*p*-DAB) , bind covalently to proteins and nucleic acids and it has been suggested that such metabolites have a dominant role in mutagenicity and teratogenicity (Tsuda et al., 2001).

Diethylnitrosamine (DENA), a representative compound of the nitrosamine family, is another well-known toxicant to which human subjects are naturally exposed to. It is known to induce hepatotoxicity in experimental animal model (Ashwani et al., 2007, Pradeep et al., 2010). Nitrosamines, formed by the combination of amines and nitrates or nitrites are ubiquitous in the environment and human exposure occurs through food items (including meat, bacon, fish, and beer), cosmetics, drugs, smokeless or burned tobacco etc.). DENA has also been designated as a Group-2A carcinogen by the IARC in 1972. It is now known that DENA ethylates most bases of DNA depending on individual negative charges at each atom on DNA bases (Singer and Grunberger, 1983) and such ethylation may result in fragmentation of DNA (Saffhill et al., 1985). Although some earlier researchers have reported DENA induced renal toxicity in animal models, most of the earlier reports emphasized on genotoxic effect of DENA. DENA has also been known to induce damage in functioning of enzymes involved in DNA repair and is normally used to induce liver cancer in experimental animal models (Bhosale et al., 2002). Metabolic activation of DENA is reported to be responsible for the onset of the toxic effects (Zimmerman, 1993).

While several reports establish intermediate reactive compounds originating from the bioactivation of *p*-DAB and DENA form covalent bonds with important cell constituents, and induce onset of mutation, necrosis and cancer, (Fabiana et al., 2001, Schmitt et al., 1993) the precise mechanism showing early involvement of cellular integrity and cytoskeletal regulatory proteins associated with such toxic manifestation has not been probed yet.

In this perspective the present study has been designed to probe into the initial effect of exposure to *p*-DAB and DENA and reactive oxygen species (ROS) generation brings forth structural change in MTs and MFs and thereof some specific signal pathways viz. Rho-family of GTPases including RhoA, Rac1 and Cdc42, which regulate a numerous cellular processes including cytoskeletal dynamics . In addition the present study is carried out to examine the effect of *p*-DAB and DENA administration on LIM kinases (LIMK 1 & LIMK2) which are key mediators of translating RhoGTPase signals into biological effects on the actin cytoskeleton. Since alteration in actin cytoskeleton results in the activation of the c-Jun N-terminal kinase (JNK) stress-activated protein kinase (SAPK) pathway in a mammalian Ste20-like kinase (MST kinase) dependent manner, the present investigation also aims at assessing the effect of chemical carcinogen administration on JNK /SAPK pathway. Distinct role of C-Raf, another important candidate in this territory with its crosstalking nature with Rho signaling pathway as well as pro-apoptotic kinases (MST2) is also evaluated in the current research. In short the precise focus of the work is to evaluate role of *p*-DAB and DENA, potential chemical toxicant, on cytoskeletal integrity, per se.

Materials and Methods:

Chemicals

Alexa Flour 488 Phalloidin, Prestained protein ladder (Invitrogen, Molecular Probes), anti RhoA 119 (Ras homolog gene family member A), Rac1 , Cell division control protein 42 (Cdc42) and C-Raf, Santa Cruz Biotechnology, Santa cruz, CA, LIMK1,LIMK2, MST2 ,SAPK/JNK and β-Actin (Cell signaling technology, USA) are used. 4-dimethylaminoazobenzene (*p*-DAB), diethylnitrosamine (DENA), Mouse fluorescein isothiocyanate (FITC) conjugates monoclonal anti-β-Tubulin, 2, 7-dichlorodihydro-fluorescein diacetate (DCFH-DA) and all other required chemicals are purchased from Sigma Chemicals Company, St. Louis, USA. All the chemicals and reagents used are analytical grade.

Animals and treatments

Male Swiss Albino mice (*Mus musculus*), 2-3 weeks old , having body weights 18±20g were acclimatized under standard conditions of temperature and humidity with 12-h light/dark cycles for 6 weeks in well aerated, pathogen free conditions with standard mice feed (Hindustan Lever Ltd, Mumbai, MH, India) and water *ad libitum* prior to

carcinogen treatment. They were maintained in accordance with the guidelines of the rule of Instructional Animal Ethics Committee of University of Calcutta , Kolkata, India (constituted as per the Gazette of India " notification part II sec.3 (ii) 17 of the ministry of Environment and Forestry, Government of India, dated 8th September 1998 for the Prevention to cruelty to animal 1968"). The animals were divided into three groups (6 mice per group). First groups of mice were kept as normal control. Second groups of mice received *p*-DAB at a dose of 0.06%, administered orally, 165mg/kg bw/mouse/day via daily gavage for 6weeks and third groups of mice were subjected to DENA treatment (injected intraperitoneally, single dose, 200mg/kg). At the end of 6 weeks, the animals were sacrificed by asphyxiation.

Visualization of cytoskeletal morphology of hepatocyte:

Microfilaments (MFs) of isolated hepatocytes from the experimental mice were visualized by Fluorescence microscope following the protocol provided by the manufacturer (Molecular Probes) with certain modifications. Briefly, cells on cover slides were washed with phosphate-buffered saline (PBS) and then fixed with 3.7% paraformaldehyde in PBS for 30 min. The cells were permeabilized with 0.1% Triton x 100 in PBS for 15 min and then blocked with 1% bovine serum albumin in PBS for 30 min at room temperature. The MFs and nuclei on cover slides were then stained with Alexa Fluor 488 Phalloidin (1:40 dilution) and 10 pg/mL of Hoechst-33258, respectively and were incubated for 30 minutes. Microtubules (MTs) of isolated hepatocytes from the experimental mice were also visualized by Fluorescence microscope following the methods of Li and Chou (1992). In case of MTs cells were first washed with PBS and with a microtubule stabilizing buffer (PM2G) .The cells were fixed with 3.7% paraformaldehyde in PM2G for 30 min and washed with PBS once. The cells were then permeabilized with 0.5% NP-40 in PBS. MTs were labeled with mouse FITC conjugate monoclonal anti-β tubulin (1:25 dilution) for 1 hr at room temperature. Then the cover slides were mounted on glass slides with a drop of mounting solution (PBS: glycerol, 3:1) and visualized by fluorescence microscope.

Measurement of intracellular oxidative stress:

To determine *p*-DAB and DENA induced oxidative stress generation by measuring the level of intracellular reactive oxygen species (ROS), hepatocytes were incubated with 2,7-dichlorodihydro-fluorescein diacetate (DCFH-DA,10 mM) for 30 minutes and then the intensity of oxidized fluorescent product of DCFDA was monitored by flowcytometric analysis as described by Huang et al., (2008).

Assessment of RhoGTPases (RhoA, Rac1 and Cdc42) and C-Raf protein expression by Flow cytometry:

For this hepatocytes were isolated and prepared from all the experimental groups by two-step collagenase perfusion method. After mechanical disruption of liver capsule, the liver cells were collected in Hanks' balanced salt solution (HBSS) and serially filtered in 80, 60 and 40 micron mesh after two rounds of sedimentation. The cell viability was assessed by trypan blue and cell number was adjusted to $0.5–2\times10^6$ viable cells/ml. Cells collected by centrifugation were washed and then cells ($1* 10^6$) were resuspended in 1 ml of PBS fixed in 3.7% paraformaldehyde for 10 min at 37°C and then permeabilized in ice-cold methanol. Cells after permeabilization were suspended in incubation buffer and kept for 10 min at 37°C. Cells were then incubated with specific primary antibody for 1 h at 37°C. Cells were washed in incubation buffer and were incubated for 1 h with a FITC-conjugated secondary antibody at 37°C. Cells were then rinsed and resuspended in PBS. FITC fluorescence signals were detected with BD FACS caliber instrument (Becton-Dickinson, San Jose, CA) equipped with an argon laser that emits at 488 nm. The peak emission of FITC is at 525 nm, which was measured in the FL1 channel. For each sample, auto fluorescence signal of unstained hepatocytes were measured and used to adjust the fluorescence intensity of FITC stained hepatocytes. Mean Fluorescence intensity (MFI) was calculated by BD CellQuest software. Results were plotted as log fluorescence intensity versus relative cell number and at least 50 000 cells were analyzed.

Measurement of LIMK1, LIMK2 MST2 and SAPK/JNK expression in hepatic tissue by Western blot analysis:

Whole cell extract of liver tissue was prepared with the help of radioimmunoprecipitation assay (RIPA) buffer (0.1% sodium dodecyl sulfate, 0.5% deoxycholic acid, 1% Igepal, 150 mM NaCl and 50 mM Tris-HCl). To prepare the cytosolic fraction, liver was sonicated (Sonicator- Omni-Ruptor 4000) using tissue homogenization buffer (50mM Tris-HCl [pH 8], 10 mM KCl, 1 mM EDTA disodium salt, 0.2% nonyl phenoxylpolyethoxylethanol, 10% glycerol, and 1 µg/mL each of leupeptin and aprotinin in distilled water) and centrifuged using cold centrifuge (SORVALL RC6 PLUS) to separate cytosolic fraction. Protein concentration was determined by the method of Lowry *et al*., (1951). Equal amounts of protein (50 µg) in each lane were subjected to 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane. The membrane was blocked with 3% bovine serum albumin solution and kept overnight at 4°C. Immunoblotting was done using specific monoclonal

antibody. β-Actin was used as loading control for cytosolic extracts. Immunoblots picture were taken by Gel Doc (G: BOX, Syngene) and the images were analyzed using Quantity One software (Bio-Rad Laboratories, Hercules, CA, USA).

Statistical analysis

All numerical data were presented as means ± standard error of the mean (s.e.m). One-way analysis of variance (ANOVA) with Tukey's post hoc test was used for statistical evaluation of the data and for the determination of level of significance in various groups of animals. We had considered $p < 0.05$ statistically significant.

Results and Discussion

Changes in hepatocyte cytoskeletal morphology

Microscopic analysis of F-actin and β-tubulin of hepatocytes showed cytoskeletal distortion in both the carcinogen treated groups when compared to the normal counterparts. In contrast to the highly organized network of MFs and MTs distributed evenly in a wavy and curving pattern along with distinctly visible nucleus seen in the normal hepatocytes, drastic distortion of the MFs and MTs were noted in the carcinogen treated groups. (Figure1). The intensity of damage of the cytoskeletal components was observed to be different in case of the two different carcinogens. Distribution of F-actin in *p*-DAB treated group showed ring-like assembly along with noticeable reduction in number of nuclei was evident (Fig.1A). Microfilament of the DENA treated group showed loss of filamentous nature of the F-actin which seemed to have aggregated to form rosette like structure. Microtubules of DENA treated groups (Fig.1B) showed uneven structure of β-tubulin along with reduction of nucleus. In *p*-DAB treated groups (Fig.1B) also severe destruction of β-tubulin structure was evident.

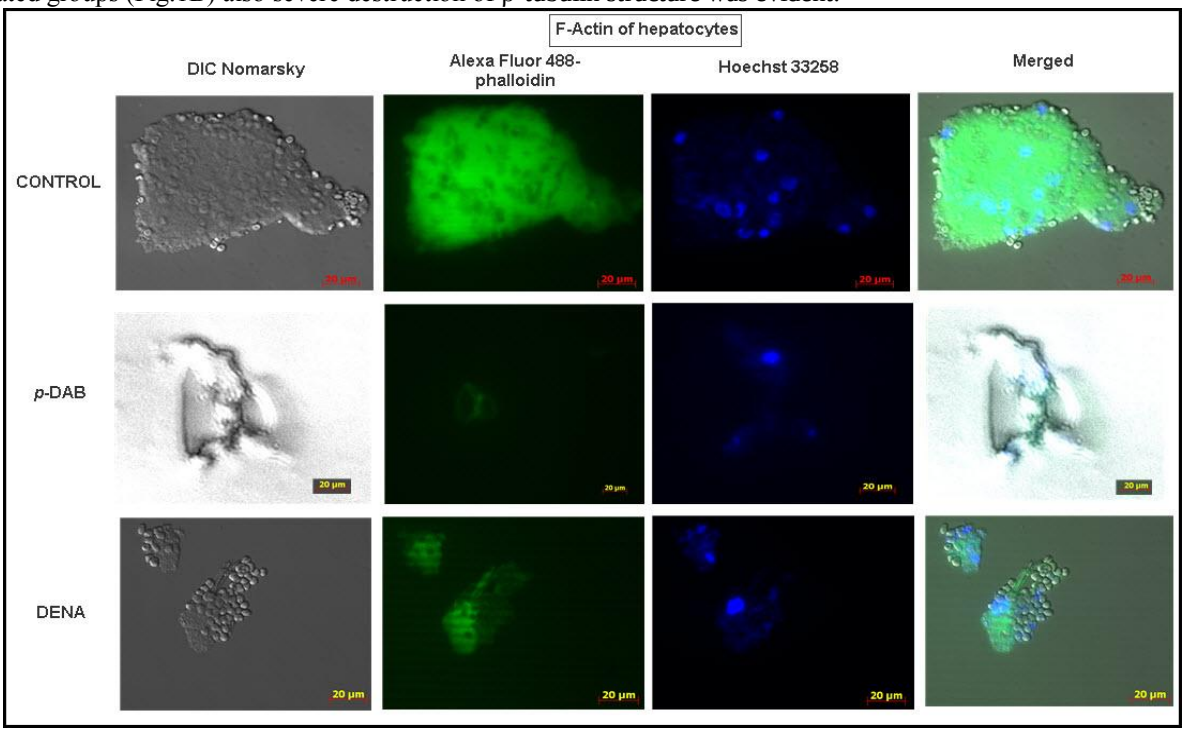

Fig. 1A: F-Actin of carcinogen treated hepatocytes of mice

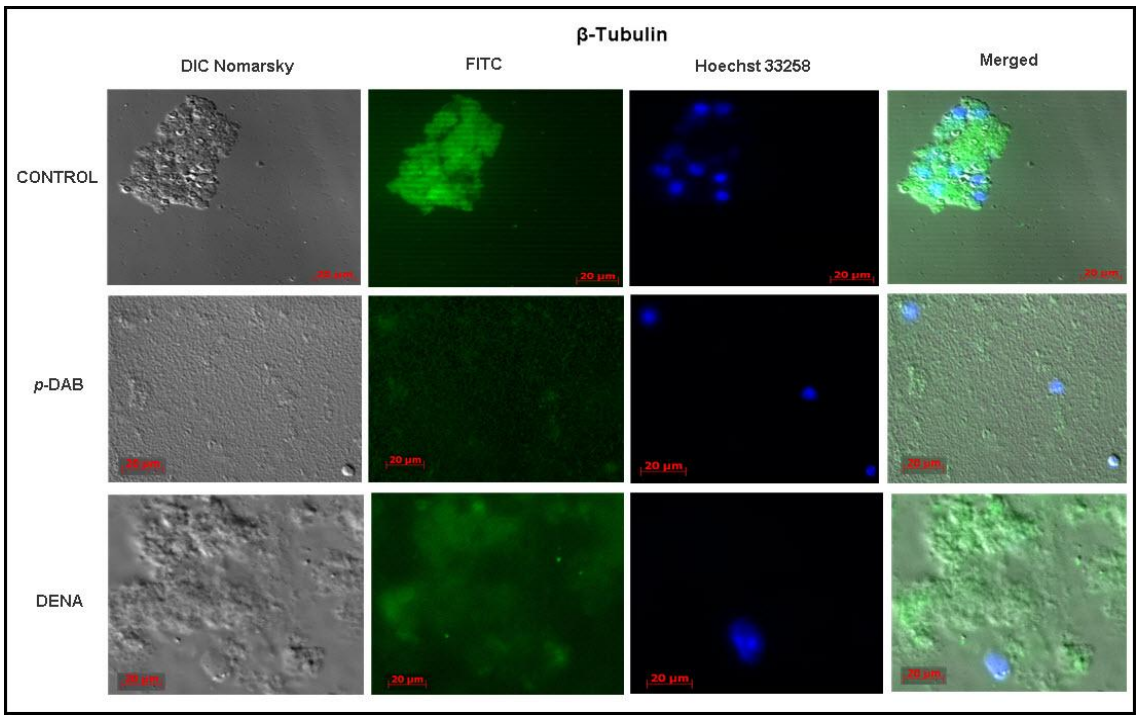

Fig. 1B: β-tubulin of carcinogen treated hepatocytes of mice

Figure 1: Fluorescence micrographs of mouse hepatocyte cytoskeleton (F-actin and β-tubulin). F-actin was stained with Alexa Fluor 488- labelled phalloidin and β-tubulin was stained with FITC conjugated monoclonal anti β-tubulin. Nucleus is stained with Hoechst 33258.

Intracellular ROS generation in hepatocytes

Data of the present study clearly establish *p*-DAB and DENA as a potential inducer of generating free radicals and reactive oxygen species (ROS) as evident from enhanced 2,7-DCFH-DA oxidation in hepatocytes of carcinogen treated groups as determined by significant increase in fluorescence intensity (61.02%) in *p*-DAB treated group and (169.40%) in DENA treated group than that of their control group.

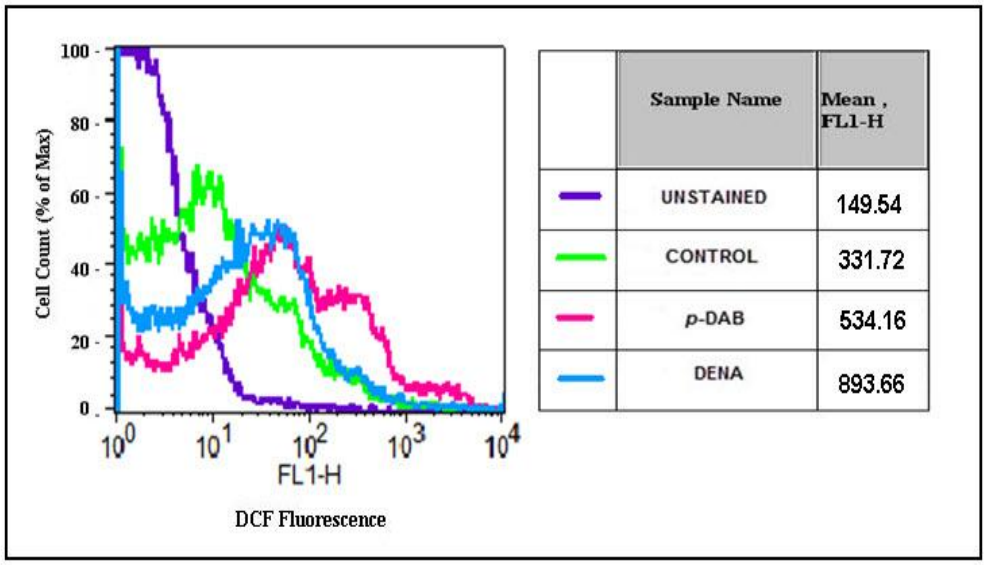

Fig.2 Flowcytometric analysis of intracellular ROS generation in mice hepatocytes in response to *p*-DAB and DENA treatment.

It has been established earlier that biotransformation of xenobiotic chemicals produces reactive oxygen species (Czekaj, 2004). Aminoazo-dyes have an exocyclic amino group that undergoes biochemical *N*-oxidation and further conversion to reactive electrophiles (Ohnishi et al., 2001). Biswas and Khuda-Bukhsh, (2005) have shown ROS induced by aromatic amines not only produce hepatotoxicity but also have role in carcinogenesis. *N*-hydroxy derivatives of *p*-DAB are known to interact with cellular components to form H_2O_2 which plays a crucial role in carcinogenic process (Kaneko et al., 1984). It has been suggested that ROS produced by toxic chemicals, might be involved in the generation of hepatic lesions (Gopal and Udayakumar, 2008). Hepatotoxic effect of DENA has been reported by a host of workers (Pradeep et al., 2010, Rezaie et al., 2013). Hence it can be presumed that DENA induced hepatotoxicity is caused by ROS generation. Oxidative stress has been reported to induce inhibition of actin monomer assembly followed by disruption of actin dynamics (Milzani et al., 1997). Our present result showing distortion of F-actin structure in the hepatocytes might be mediated through *p*-DAB and DENA induced ROS generation and oxidative modification of the cytoskeletal proteins. Distortion of F-Actin morphology due to exposure to toxic chemicals has been cited earlier by Allingham et al., (2006). Disassembly of actin filaments caused by exposure to toxic chemicals has been cited by a host of researchers Ruthel and Hollenbeck (2000). Uematsu et al., (2007). Chronic chemical stress induced oxidative insult has been reported to result in drastic alterations in tubulin cytoskeleton in rat liver (Miller et al., 2008). It has been shown by some earlier researchers that toxic effect of pesticides and /or insecticides are mediated via depolymerizing activity of MTs (Floor et al., 1995; Eisenhofer et al., 2004) and increase in oxidative stress associated with it (Ren et al.,2005). Diaz-Correles et al. (2005) documented toxicant induced aggregation of tubulin and MT destabilization. Xenobiotics induced ROS generation altered assembly of microtubules by oxidative modifications of proteins especially cysteine residues (Dinh et al., 2000). Therefore, it is possible that *p*-DAB and DENA, the promising environmental carcinogens used in this study may disrupt the cytoskeletal structure by directly blocking certain tubulin-SH groups or by oxidative modification the tubulin cysteine residues.

Differential signature of RhoGTPases (RhoA, Rac 1, Cdc42) and C-Raf:

Fig.3 highlighted down-regulation of, RhoA, Rac 1 and Cdc42 expression in hepatocytes of *p*-DAB and DENA treated groups when compared to their normal counterparts. Considerable negative induction was noted in Cdc42 expression of *p*-DAB treated groups (50.34%) with respect to that of the control. Expression patterns of RhoA and Rac 1 also exhibited similar trend showing appreciable down regulation of these two marker proteins (46.01% and 41.09% respectively) with respect to the normal counterparts. In DENA treated group significant depletion (43%) in Rac 1 expression was noted with respect to that of the control. Expression patterns of RhoA and Cdc42 also exhibited similar trend showing considerable down regulation of these two marker proteins (21.86% and 26.58% respectively) with respect to that of the normal counterparts.

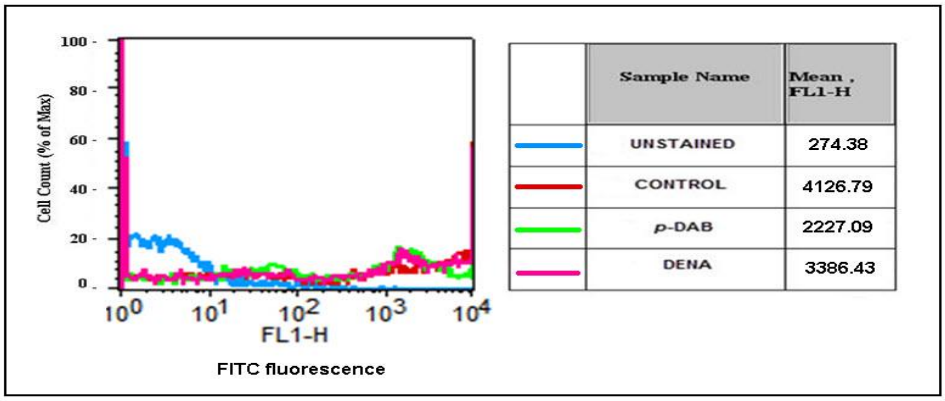

Fig. 3A Expression of RhoA in hepatocytes

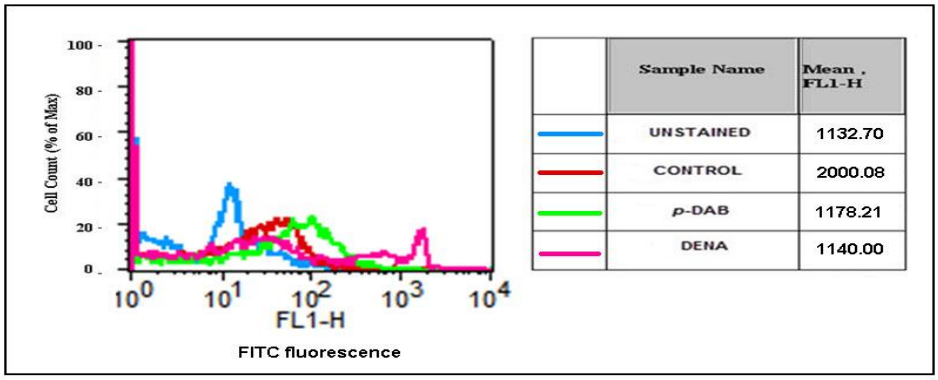

Fig. 3B Expression of Rac 1 in hepatocytes

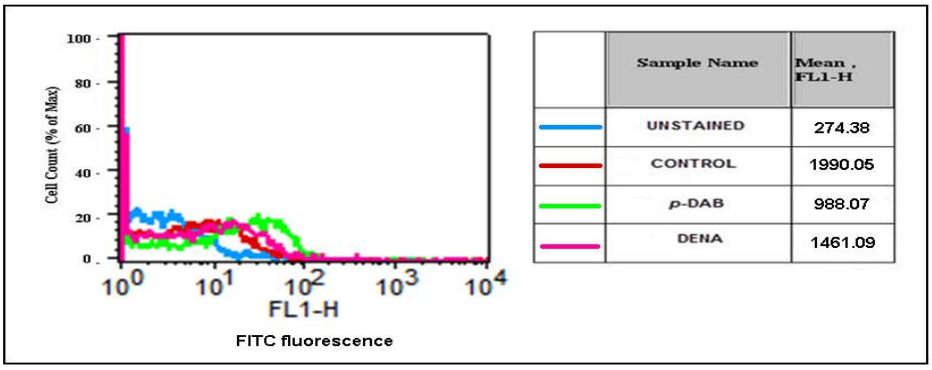

Fig. 3C Expression of Cdc42 in hepatocytes

Toxic chemical induced downregulation of RhoA, Rac1 and Cdc42 expression has been reported recently by Ravindran et al., (2013) in human cell line exposed to Cadmium (Cd). Similar downregulation of RhoA, Rac1 and Cdc42 expression was observed by Zhang et al., (2007) in phenylalanine-induced abnormalities in cultured cortical neurons. Inactivation of Rho A induced by severe oxidative stress has been shown by Heo et al (2006). However their group showed stimulation of Rac and Cdc42. Cytotoxins from *Clostridium sp.* are known to cause inactivation of RhoGTPases (Aktories et al., 2004). Rotenone, a neurotoxicant, has been shown to induce inhibition of actin dynamics through modifications of Rho -GTPase activity (Sanchez et al., 2008).Small GTPases of the Rho family are known to control cell growth, morphogenesis, cell motility, cytokinesis, trafficking and organization of cell cytoskeleton. They are also involved in oncogenesis (Jaffe and Hall, 2005). Yamazaki et al., (2005) reported that reorganization of cytoskeleton is a critical factor with respect to alterations in cell morphology, motility, adhesion and invasion. Changes in cell morphology is considered as one of the crucial adaptive strategy of the cell in response to a toxic chemical and toxicant induced alterations in cell morphology has been reported in various cell types (Espevik et al., 1982, Paksy et al., 1997). Aberrant regulations of Rho-GTPases and their effectors have been documented to alter cytoskeleton arrangement (Wong et al., 2010). Our result validates correlation of down regulation of Rac1and RhoA proteins with the deregulation of cytoskeletal arrangement as postulated earlier by Ravindran et al., (2013) while studying the effect of cadmium (Cd), a heavy metal toxicant, on *in vitro* cultured human lung adenocarcinoma.

C-Raf, one of the kinases modulating MEK and ERK pathways has distinct crosstalking abilities with Rho signaling (Ehrenreiter et al., 2005; Piazzolla et al., 2005 and Ehrenreiter et al., 2009). Relation of C-Raf with the two pro-apoptotic kinases ASK1 and MST2 has drawn serious attention of several researchers (Chen et al., 2001; Yamaguchi et al., 2004, O'Neill et al., 2004; Matallanas et al., 2007). These pathways have been shown to participate in liver carcinogenesis (Zender et al., 2006; Hui et al., 2008; Wong et al., 2008). Our data showing significant upregulation of C-Raf (77.41%) in *p*-DAB treated group with respect to their normal counterparts validate potential of *p*-DAB as a Group 2B carcinogen designated by the International Agency for Research on Cancer (IARC). However expression of C-Raf was less significant (20.76%) in DENA treated group with respect to control group. Over-expression of C-Raf is frequently observed in squamous cell carcinomas (Riva et al. 1995), lung adenocarcinomas (Cekanova et al. 2007) and with particularly high frequency in hepatocellular carcinomas (Huynh et al. 2003; Hwang et al. 2004; Hopfner et al. 2008.

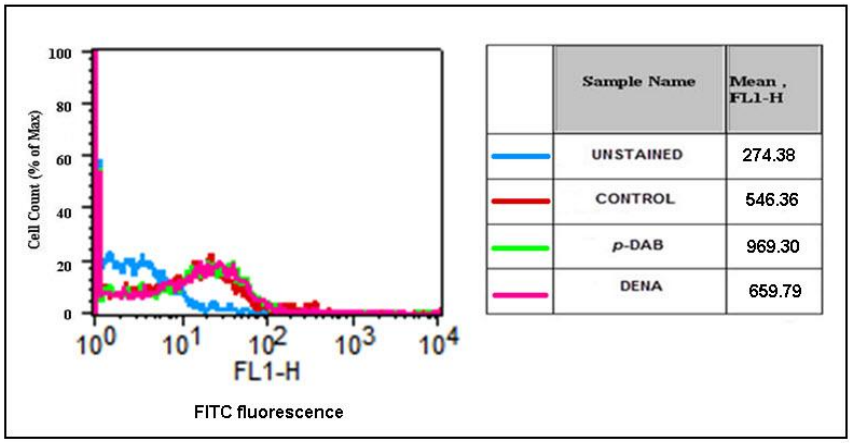

Fig. 3D Expression of C-Raf in hepatocytes of mice

Fig. 3 Flowcytometric expression of RhoGTPases(RhoA,Rac1,Cdc42) and C-Raf

In short, our data presents the cue for the first time that administration of *p*-DAB and DENA for 6 weeks induce marked alterations in cytoskeletal structure via involvement of Rho-GTPases mediated signal transduction pathways and ROS generation. Klaunig et al. (2010) conjectured that alterations in signal pathways are important links between xenobiotic exposure and tumorigenesis. Furthermore, ROS generation has been inferred to be directly associated with carcinogenesis (Hwang and Bowen 2007). In tune with these findings our data set forth the importance of considering cytoskeletal changes and associated alterations in Rho family proteins together with increased level of intracellular ROS, as a signal of onset of tumorigenic or carcinogenic process induced by exposure to *p*-DAB and DENA, which are designated as Group-2B and Group-2A carcinogens respectively by the International Agency for Research on Cancer (IARC), 1972. Reports from some recent studies have demonstrated down-regulation of RhoA expression in renal cell carcinoma (RCC) and it has been suggested that RhoA plays an important role in enhancing migration and invasion of pancreatic carcinoma cells and cytoskeleton disorganization in RCC (Dreissigacker et al., 2006).

Modulation of cytoskeletal regulatory kinases LIMK1, LIMK2, MST2 and SAPK/JNK expression in hepatic tissue

Fig 4 represented Densitometric analysis of the regulatory kinases in *p*-DAB and DENA treated mice as compared to the normal control counterparts. LIMK1 was overexpressed in both the carcinogen treated mice group when compared to their normal counterparts. In both DENA and *p*-DAB treated groups, LIMK1 was noted to be significantly upregulated showing near 1fold or even more enhancement in expression of the protein (109.32% and 72.17% respectively) as compared to their normal counterparts, In contrast to LIMK1, expression of LIMK2 manifested no remarkable change in *p*-DAB treated mice with respect to that of the control. While in DENA treated group an enhancement of LIMK2 expression was noted (95.78%). Densitometric analysis showed MST2 manifest 1.5 fold upregulation in DENA treated groups in contrast to little or no significant change in the expression of this marker protein in hepatic tissue of *p*-DAB treated group. However data showed activation of stress-activated protein kinase (SAPK/JNK) in both *p*-DAB and DENA treated groups (15.44% and 38.55% respectively).

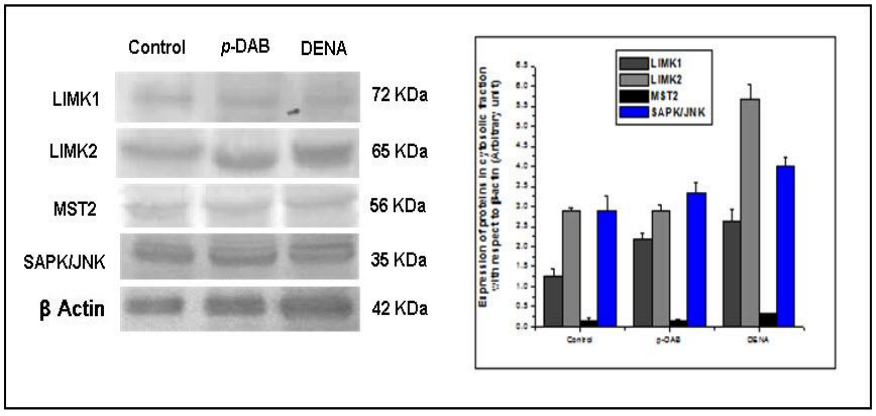

Fig. 4 Western blotting analysis together with quantitative measurement of labeling density of LIMK1,LIMK2,MST2 and SAPK/JNK expression.

Overexpression of LIMK1 as observed in the present investigation corroborates the involvement of LIMK and Rho signaling in modulating cytoskeletal organization as claimed earlier by Scott and Olson, (2007). Dysregulated expression of LIMK 1 has been associated with various pathobiological conditions including many types of cancer (Heredia et al., 2006, Chen et al., 2014). Since it has been shown earlier that expression of MST2 partially depends upon C-Raf activity (Rauch et al., 2011), overexpression of C-Raf in *p*-DAB treated group might have some effect on expression of MST2 in this experimental group. However activation of MST2 kinase by *p*-DAB and DENA exposure has been sufficient enough to induce stimulation of JNK/SAPK pathway. It may be surmised that in response to *p*-DAB and DENA induced disruption of cytoskeleton, a significant activation of MST2 kinase is manifested which in turns leads to the activation of JNK/SAPK pathway since MST kinases are known to monitor cytoskeletal integrity and couple via SAPK/JNK pathways to regulation of cell cycle regulatory proteins since MST kinases are known to monitor cytoskeletal integrity and couple via SAPK/JNK pathways to regulation of cell cycle regulatory proteins (Densham et al., 2009).

Conclusion:

Data presented here is the first of its kind that elucidates *p*-DAB and DENA induced dysregulation of cytoskeletal integrity and signaling proteins mediated through intracellular ROS generation. The up-regulated and down regulated proteins involved in the regulation of the cytoskeleton dynamics suggest a role of cellular plasticity in the mechanism of chemical carcinogenesis as claimed earlier by Landcocz et al (2011). Our study provides a signal to consider early disturbances in the expression of cytoskeleton related proteins as biomarkers predictive for early events in chemical carcinogenesis.

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