CSIR–Indian Institute of Toxicology Research, Lucknow

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CSIR–Indian Institute of Toxicology Research (IITR), Lucknow, established on 4 November 1965, is one of the pioneer institutions dealing with toxicology research in the Asia-Pacific region. IITR is the only laboratory in the CSIR family to be awarded the Good Laboratory Practice certification. The five decades of expertise and knowledge base in toxicology research have empowered the Institute to conduct studies in the following niche areas: (i) Food, drug and chemical toxicology, (ii) nanotherapeutics and nanomaterial toxicology, (iii) systems toxicology and health risk assessment, (iv) environmental toxicology, and (v) regulatory toxicology. The highlights of some of the scientific achievements for the year 2014 are described in this article.

Keywords: Health risk assessment, nanoparticles, nanotherapeutics, toxicology research.

THE Indian Institute of Toxicology Research (IITR), Lucknow was established on 4 November 1965 as Industrial Toxicology Research Centre. IITR is one of the pioneer institutions dealing with toxicology research in the Asia-Pacific region. Since 1965, IITR scientists have played a crucial role in assessment and prediction of toxic effects of chemicals to which industrial workers, miners, farmers and even the common man may get exposed. IITR is the only laboratory in the CSIR family to be awarded the Good Laboratory Practice (GLP) certification for carrying out toxicity and mutagenicity studies by the National GLP compliance Monitoring Authority, New Delhi.

The five decades of expertise and knowledge base in toxicology research have empowered the Institute to conduct studies in the following niche areas: (i) Food, drug and chemical toxicology; (ii) Nanotherapeutics and nanomaterial toxicology; (iii) Systems toxicology and health risk assessment; (iv) Environmental toxicology; (v) Regulatory toxicology.

The highlights of some of the scientific achievements for the year 2014 are described below.

Nanotherapeutics and nanomaterial toxicology

Curcumin, a natural polyphenolic compound obtained from the rhizome of the Indian spice turmeric (*Curcuma longa*), possesses pleiotropic, biological and pharmacological properties[1]. It provides neuroprotection in cellular and animal models of neurodegenerative and neurological disorders. However, the neuroprotective efficacy of curcumin is limited by its poor brain bioavailability due to poor absorption, rapid metabolism, systemic elimination, and limited blood brain barrier (BBB) permeability. Therefore, in order to achieve optimum curcumin-mediated biological effects and therapeutic benefits, it is imperative to enhance curcumin brain bioavailability. The studies performed at IITR provide evidence that curcumin-loaded poly(lactic-co-glycolic-acid) (PLGA) nanoparticles can easily cross the BBB, slowly and constantly releasing curcumin, thus enhancing the bioavailability of curcumin in a sustained and controlled manner in the brain. Nanoparticles may increase the neuroprotective efficacy of curcumin and reduce the dose required for bulk curcumin due to the smaller size, high ingression power along with longer stability as they readily transmigrate across the BBB.

Further studies suggest that curcumin-encapsulated PLGA nanoparticles (Cur-PLGA-NPs) potently induce neural stem cell (NSC) proliferation and neuronal differentiation *in vitro* and in the hippocampus and sub ventricular zone (SVZ) of adult rats, compared to uncoated bulk curcumin even at very low doses. A novel strategy was designed to differentiate NSCs into neurons using Cur-PLGA-NPs, which were able to release curcumin constantly and slowly. Cur-PLGA-NPs significantly increase the expression of genes involved in NSC proliferation and neuronal differentiation. Studies elucidated the molecular mechanisms underlying the canonical Wnt/β-catenin pathway activation and glycogen synthase kinase-3β (GSK-3β) inhibition by curcumin for the induction of neurogenesis. Curcumin nanoparticles increase neuronal differentiation by activating the Wnt/β-catenin pathway involved in the regulation of neurogenesis. These nanoparticles caused enhanced nuclear translocation of β-catenin, decreased GSK-3β levels, and increased promoter activity of the TCF/LEF and cyclin-D1. More interestingly, the role of curcumin in the enhancement of neurogenesis and its effects on behaviour were studied in an Aβ-induced rat model of learning and memory deficits and Alzheimer's disease (AD)-like phenotype. Studies showed that Cur-PLGA-NPs potently enhance NSC proliferation and neuronal differentiation

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and reverse Aβ-induced learning and memory deficits through activation of the canonical Wnt/β-catenin pathway. *In silico* studies indicated that curcumin interacts with the Wnt inhibitor factor (Wif-1), Dickkopf (Dkk-1), and GSK-3β. These results suggest that curcumin nanoparticles induce adult neurogenesis through activation of the canonical Wnt/β-catenin pathway and may offer a therapeutic approach in treating neurodegenerative diseases such as AD, by enhancing the brain self-repair mechanism.

Zinc oxide nanoparticles (ZnO NPs) with their unique physico-chemical properties conferred by various size formulations are extensively used in cosmetic products[2]. The enormous usage coupled with their release into the environment demands risk assessment of ZnO NPs on health and the environment. Toxicity of ZnO NPs is well understood in comparison to bulk ZnO. However, size-dependant toxicity of ZnO NPs is poorly understood. In this context, the adverse effects of different sizes (35, 50 and 100 nm) of ZnO NPs were examined in soil nematode, *Caenorhabditis elegans* and compared with bulk ZnO and $ZnCl_2$. Scientists at IITR have shown that growth, reproduction and behaviour of worms were adversely affected by ZnO NPs in a size-dependent manner. Further, exposure to ZnO NPs caused modulation of expression/function of genes associated with insulin/IGF-like signalling pathway and/or stress response pathway in a size-dependent manner in exposed worms. The expression of pro-apoptotic gene and suppression of anti-apoptotic genes, together with increased number of cell corpses in the germ line, indicated that apoptosis was also dependent on the size of the ZnO NPs. The findings suggest the inclusion of size as an additional measure for the cautious monitoring of disposal of ZnO NPs into the environment.

Neurotoxicology

Parkinson's disease (PD) is a prevalent and devastating neurodegenerative disorder having limited cure options and strong association with the loss of dopaminergic neurons in the substantia nigra region of the mid brain[3]. Etiology of PD includes both genetic and environmental factors. Paraquat (PQ), a widely used herbicide, is known to be associated with pathogenesis of PD. Scientists at IITR have reported that a mutation in *Drosophila methuselah* (*mth*(1)), which is associated with ageing, has a role in preventing dopaminergic neuronal cell death in PQ-exposed organism. Exposed *mth*(1) flies were shown to exhibit significant resistance against PQ-induced Parkinson's phenotypes and behaviour in terms of oxidative stress, dopaminergic neuronal degeneration, locomotor performance, dopamine content, phosphorylated JNK, pFOXO, Hid and cleaved caspase-3 levels. Conversely, over-expression of *mth* in dopaminergic neurons made the exposed organism more vulnerable to oxidative stress, neuronal cell death and behavioural deficit. The study suggests that lesser activation of JNK-mediated apoptosis in dopaminergic neurons of exposed *mth*(1) flies protects the organism from PQ-induced damage, which may be linked to a common mechanism for PQ-induced neurodegeneration.

Myelin is the functional implication of oligodendrocytes (OLs), which is involved in insulation of axons and promoting rapid propagation of action potential in the brain[4]. Defects in myelination process lead to the onset of several neurological and neurodegenerative disorders. Exposure to synthetic xenoestrogen bisphenol-A (BPA) causes cognitive dysfunction, impairs hippocampal neurogenesis and causes onset of neurodevelopmental disorders. However, the effects of bisphenol-A on oligodendrocyte progenitor cell (OPC) proliferation, differentiation and myelination, and associated cellular and molecular mechanism(s) in the hippocampus of rat brain are still largely unknown. The effects of bisphenol-A exposure during prenatal and post-natal periods on cellular and molecular alteration(s) in the myelination process in the hippocampus region of the rat brain at post-natal days 21 and 90 were observed. Bisphenol-A exposure both *in vitro* and *in vivo* altered proliferation and differentiation potential of OPCs and decreased the expression of genes and levels of proteins that are involved in myelination. These results suggest that bisphenol-A exposure both during prenatal and post-natal periods alters myelination in the hippocampus of the rat brain leading to cognitive deficits.

Differentiating neuronal cells derived from human umbilical cord blood stem cells have been used for the assessment of developmental neurotoxicity of monocrotophos (MCP), an organophosphate pesticide[5]. The differentiating cells were exposed to MCP during different stages of maturation, viz. day 2, 4 and 8, and changes in the markers of cell proliferation, neuronal differentiation, neuronal injuries and receptors were studied. There was significant upregulation in the different mitogen-activated protein kinases (MAPKs), apoptosis and neurogenesis markers and downregulation in the cell proliferation markers during neuronal differentiation. Scientists at IITR further identified significant upregulation in the expression of different MAPKs and proteins involved in oxidative stress, apoptosis and calpain pathways in the mid-differentiating cells exposed to MCP. The upregulated levels of these proteins seem to be the main cause of alteration during the differentiation process towards apoptosis, as fine-tuning of pro-apoptotic and anti-apoptotic proteins is desirable for the process of differentiation without apoptosis. The decreased acetylcholinesterase activity in dopaminergic and cholinergic receptors and increased acetylcholine levels in the differentiating neuronal cells indicate the vulnerability of these cells towards MCP-induced neurotoxicity. These results

suggest that differentiating neuronal cells derived from human umbilical cord stem cells could be used as a tool to assess the developmental neurotoxicity in human beings.

Cypermethrin induces the slow and progressive degeneration of the nigrostriatal dopaminergic neurons in rats[6]. Post-natal pre-exposure to low doses of cypermethrin is known to enhance the susceptibility of animals upon adulthood re-exposure. The study was undertaken to delineate the role of mitochondria in cypermethrin-induced neurodegeneration. Indexes of dopaminergic neurodegeneration, microglial activation, and mitochondrial dysfunction and its proteome profile were assessed in controls and cypermethrin-treated rats. Cypermethrin increased nigral dopaminergic neurodegeneration and microglial activation, while it reduced mitochondrial membrane potential and complex I activity. Cypermethrin treatment caused increase in c-Jun N-terminal kinase (JNK), caspase-3, tumour suppressor protein (p53), tumour necrosis factor-α (TNF-α), p38 mitogen-activated protein kinase (p38 MAPK) and heme oxygenase-1 (HO-1) expressions, and reduced B-cell lymphoma-2 protein (Bcl-2) expression. Syn-DOPA and minocycline prevented cypermethrin-induced microglial activation and reduction in mitochondrial membrane potential and complex-I activity, striatal dopamine content, and degeneration of nigral dopaminergic neurons. Furthermore, Syn-DOPA and minocycline reinstated the expressions of JNK, caspase-3, Bcl-2, p53, p38 MAPK, TNF-α and HO-1. The study demonstrates that cypermethrin induces mitochondrial dysfunction and alters mitochondrial proteome leading to oxidative stress and apoptosis, which regulate the nigrostriatal dopaminergic neurodegeneration.

General toxicology

RT-PCR-based TaqMan low-density array (TLDA) study was initiated to investigate similarities in the mRNA expression of target genes altered by exposure to diesel exhaust particles (DEPs) in freshly prepared peripheral blood mononuclear cells (PBMCs) and in the lungs[7]. Adult Wistar rats were treated transtracheally with a single dose of 7.5 or 15 or 30 mg/kg DEPs and sacrificed 24 h later. Diesel exhaust particles treatment induced similar patterns of increase in the expression of polycyclic aromatic hydrocarbon-responsive cytochrome P450s, the phase II enzymes and their associated transcription factors in both the lungs and PBMCs, at all doses. Similar to that seen in the lungs, a dose-dependent increase was observed in the expression of genes involved in inflammation, such as cytokines, chemokines and adhesion molecules, in PBMCs. The expression of various genes involved in DNA repair and apoptosis was also increased in a dose-dependent manner in PBMCs and the lungs. The present TLDA data indicating similarities in the responsiveness of candidate genes involved in the toxicity of DEPs between PBMCs and the lungs after exposure to these particles, demonstrate that expression profiles of genes in PBMCs could be used as a surrogate for monitoring the acute toxicity of fine and ultrafine particulate matter present in vehicular emissions.

Preventive toxicology

Mammalian FoxO proteins manipulate a plethora of genes modulating cellular functions, including cell cycle regulation, apoptosis, DNA damage repair and energy metabolism[8]. FoxO overexpression and nuclear accumulation have been reported to show correlation with hindered tumour growth *in vitro* and *in vivo*, while down-regulation of FoxOs via phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) pathway has been linked with tumour promotion. This study explores the intervention of berberine, a plant-derived isoquinoline alkaloid, with FoxO family proteins in hepatoma cells. Berberine significantly upregulated the mRNA expression of both FoxO1 and FoxO3a. Their phosphorylation-mediated cytoplasmic sequestration followed by degradation was prevented by berberine-induced downmodulation of the PI3K/Akt/mTOR pathway which promoted FoxO nuclear retention. *PTEN*, a tumour suppressor gene and negative regulator of the PI3K/Akt axis, was upregulated while phosphorylation of its Ser380 residue (possible mechanism of *PTEN* degradation) was significantly decreased in treated HepG2 cells. Exposure to berberine induced a significant increase in transcriptional activity of FoxO. Also, FoxO transcription factors effectively heightened BH3-only protein Bim expression, which in turn, being a direct activator of proapoptotic protein Bax, altered Bax/Bcl-2 ratio, culminating into mitochondrial dysfunction, caspases activation and DNA fragmentation. The pivotal role of Bim in berberine-mediated cytotoxicity was further corroborated by knockdown experiments where Bim-silencing partially restored HepG2 cell viability during berberine exposure. The findings suggest that the antiproliferative effect of berberine may in part be due to mitochondria-mediated apoptosis with Bim acting as a pivotal downstream factor of FoxO-induced transcriptional activation.

Societal activities

Recently, children with high mortality rate have been studied in the northern parts of India, including Saharanpur, for which the etiology is still not established, although a case control study has been linked to the consumption of *Cassia occidentalis* (CO) seeds[9]. In the present study toxicity of CO seeds (0.5, 1 and 2 w/w%) in the diet was investigated in Wistar rats. It was observed that CO seeds (after 28 days) caused significant increases in the serum markers, viz. transaminases, alkaline phosphatase and

lactate dehydrogenase along with histopathological lesions in hepatic tissue. CO consumption also showed decrease in grip strength, vacuolization and myopathy of skeletal muscles along with increases in serum creatinine and creatinine phosphokinase, suggesting muscular damage in the animals. Neuronal damage in CO-treated animals was evident by a marked increase in glial fibrilar acidic protein and decrease in β-tubulin III. The experimental findings of CO consumption showed liver, muscles and brain to be the target organs, which were similar to the clinical data of poisoning cases as observed in the present study. Overall, the study suggests that CO seed consumption is the main etiological factor in children population suffering from hepatomyoencephalopathy in India.

Several toxicological manifestations of deoxynivalenol (DON), a mycotoxin found in cereal food are well documented; however, dermal toxicity is not yet explored[10]. Single topical application of DON (84–672 nmol/mouse) significantly enhanced dermal hyperplasia and skin oedema. DON (336 and 672 nmol) caused significant enhancement in [(3)H]-thymidine uptake in DNA along with increased myeloperoxidase and ornithine decarboxylase activities, suggesting tissue inflammation and cell proliferation. Furthermore, DON (168 nmol) caused enhanced expression of RAS, and phosphorylation of PI3K/Akt, ERK, JNK and p38 MAPKs. DON exposure also showed activation of transcription factors, c-fos, c-jun and NF-κB along with phosphorylation of IkBα. Enhanced phosphorylation of NF-κB by DON caused over expression of target proteins, COX-2, cyclin D1 and iNOS in skin. Though a single topical application of DMBA followed by twice weekly application of DON (84 and 168 nmol) showed no tumourigenesis after 24 weeks, histopathological studies suggested hyperplasia of the epidermis and hypertrophy of hair follicles. These results suggest that DON-induced cell proliferation in mouse skin is through the activation of MAPK signalling pathway involving transcription factors NFκB and AP-1, further leading to transcriptional activation of downstream target proteins c-fos, c-jun, cyclin D1, iNOS and COX-2, which might be responsible for its inflammatory potential.

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