Review of literature
Chemical induced impaired neurogenesis and subsequent neurodegeneration in Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), epilepsy, Huntington's disease (HD), etc. is well reported. Last couple of decades has witnessed to make extensive efforts to unravel the mechanistic understanding on the issue. It has been documented now that the rate of neurogenesis is regulated not only by selective expression and repression of a series of genes in neuronal progenitor cells at specific stages of development, but is also affected by variety of factors like age, growth factors, hormones, environmental or pharmacological stimuli and intercellular communications. Reports are showing that the endogenous production of NGF gradually decreases with aging in humans and its reduction may participate in neuron degenerative diseases. Therefore, it is important to search for biological modulators which can improve the age-dependent decrease in NGF in human brains. It appears that alteration in the rate of neurogenesis may have important function and therapeutic implication. Although many aspects of this formidable neuron developmental process have been elucidated, the cellular and molecular bases of the neurogenesis are still largely unknown and need to be dugout for better corrective advance research. However, the pace of work has hampered largely due to limitations like low sensitivity with less predictive values, high work-load, cost and ethical dubious in animal model based studies. The complex regional specificity of brain and non availability of human developing brain tissues due to ethical dubious are other limiting factors. The use of cell culture has proven to be a powerful approach to study and elucidate the mechanisms involved in the organ specific toxicity. PC12 cells, rat pheochromocytoma cells have been used as a model system for the differentiation of neuronal cell since external exposure of nerve growth factor (NGF) induces neuronal
morphology with physiological characteristics like, electrical excitability, expression and activity of many neurotransmitter receptors, formation of synaptic-like vesicles, etc. in PC12 cells. In the search of potential agent having neuronal differentiation capabilities, Resveratrol (RV) was found to be more promising than the other molecules tested in different models. However, RV mediated neuronal differentiation did not show any link with MAP kinases activation, known key regulators for switching on/off the neuronal differentiation in both in vitro and in vivo models. Thus, many questions are remained to be answered such as, which cascade of events and signaling pathways are involved in RV mediated neuronal differentiation? Whether, RV synergies or antagonizes the activity and expression of NGF? RV follows which pathway(s) other than the known pathways for neuronal differentiation?

**Neurogenesis:** Neurogenesis is a process where generation of functional neural cell lineages takes place from neural precursor cells. Earlier it was known that neurogenesis occurs only during embryogenesis and pre-natal development however after discovery of neuronal stem cells (NSCs) in adult brain it has been proved that neurogenesis also occurs in adult brain (Ming and Song, 2005). During development, NSCs are derived from radial glia and neuroepithelial cells lining of the neural tubes. Neurogenesis is highly systematic process where subsets of neurons are generated first, followed by astrocytes and then oligodendrocytes thus it keep providing new brain cells in adulthood as well as developing brain (Okano and Temple, 2009).

**Neurogenesis in adult brain:** Until first report of adult neurogenesis in mammalian brain was published 48 years ago, neuron cell recovery from injury, disease and ageing was thought to be impossible as neurogenesis in adult brain was not accepted. By using
3H-thymidine autoradiography Altman et al. for the first time detected construction of new cells with morphological characteristics of neurons in olfactory bulb and dentate gyrus of adult rat (Altman, et al., 1965). Discovery of neural stem cell existence in brain and other refinements of our notion of the brain biology have converged to help make the concept of neurogenesis in adult brain more easily acceptable. Neural stem cells exist throughout life in the adult brain and can renew and give rise to new neurons, astrocytes, and oligodendrocytes, just as in the developing brain thus also overcome the mechanical problem of accommodating the dividing neuron. This has been shown from the subventricular zone (Reynolds, et al., 1992) and then in the dentate gyrus of the hippocampus (Gage, et al., 1995) and in most structures of the brain examined till now (Palmer, et al., 1995) (Kondo, et al. 2000). The recognition of adult neurogenesis was further enhanced by the compelling evidence that fetal tissue could be grafted in the adult intact brain. Even more convincing was the fact that the damaged adult brain and spinal cord allowed these newly grafted cells to survive and differentiate (Bjorklund, et al., 1985). The grafted cells could also receive and send signals and release transmitter in a behavior- dependent manner in the adult damaged brain (Dunnett, et al., 1994). Adult neurogenesis is the similitude of structural plasticity in the mature CNS environment. An advance in our understanding of principles of adult plasticity is not only shedding light on the basic principles of adult neurogenesis, but may lead to develop the strategies for cell replacement therapy after injury or degenerative neurological diseases as well (Ming, et al., 2005). Adult neural stem/progenitor cells inhabit in at least three main areas of the brain, in the anterior part of the subventricular zone (SVZ) along the walls of the lateral ventricles (Taupin, et al., 2002; Peterson DA, 2002; Temple, et al. 1999), in the
hippocampus in the subgranular zone (SGZ) of the dentate gyrus and along the posterior periventricular area (pPV), an extension of the SVZ (Lai, et al., 2003).

**Implications of Adult Neurogenesis in Pathological Conditions:** An alteration in adult neurogenesis in brain injuries and several neurological diseases and disorders, including neurodegenerative diseases such as Alzheimer’s, Huntington’s, and Parkinson’s diseases, traumatic brain injury and ischemic stroke (Abdipranoto et al., 2008); epilepsy and seizures; and psychiatric disorders such as depression, demyelinating diseases such as multiple sclerosis; and schizophrenia has been shown elsewhere. Thus understanding the cause of neural disease and its underlying mechanism is always being an utmost priority.

**Adult Neurogenesis in Brain Injury:** Traumatic brain injury (TBI) happens when sudden trauma causes damage to the brain. TBI typically causes neuronal loss especially in hippocampus-dependent cognitive functions. Recovery after TBI depends on the balance between neuronal injury and neuroregeneration. Accumulating evidence from animal studies of both focal and diffuse TBI suggests that neurogenesis is increased after TBI in both the SGZ of the dentate gyrus (Kernie et al., 2001; Yu et al., 2008; Zheng et al., 2011) and the SVZ of the lateral ventricles (Bye et al., 2011). However, it remains to be demonstrated positively whether neurogenesis contributes to recovery after TBI.

Other than TBI, Stroke is the leading cause of disability and death in humans and results from ischemia, blockage, or hemorrhage to the brain. Numerous studies in adult rodents (Liu et al., 1998; Jin et al., 2001; Kee et al., 2001) and monkeys (Tonchev et al., 2005; Koketsu et al., 2006) indicates that focal or global ischemia potently stimulate neurogenesis. In contrast, subarachnoid hemorrhage has been shown to be associated with a decrease in cell proliferation in the SGZ and SVZ (Mino et al., 2003). Although
functional neurogenesis has been demonstrated in the hippocampus (Van Praag et al., 2002) and newly born neurons in the SVZ have been shown to migrate to the damaged cortex (Arvidsson et al., 2002), however the role of ischemia-induced neurogenesis in recovery after an ischemic insult remains unclear.

**Adult Neurogenesis in Neurodegenerative Diseases:**

**Alzheimer’s Disease:** Alzheimer’s disease (AD) is a progressive neurodegenerative disease and the most common cause of dementia in older people. It is characterized by the accumulation of plaques and tangles in the brain, particularly in the hippocampus. The plaques contain insoluble deposits of amyloid peptide, whereas the neurofibrillary tangles are composed of aggregates of the hyperphosphorylated microtubule-associated protein (Selkoe, 2003; Zhang et al., 2012). The role of neurogenesis in AD is unclear. However some animal models of AD showed the increased hippocampal neurogenesis including the Sorl1 KO mouse (Rohe et al., 2008) and mutated APP23 mouse model (Mirochnic et al., 2009, Jin et al., 2004a). In contrast, adult neurogenesis has been found to reduce in double transgenic mice for mutated APP and presenilin-1 (Niidome et al., 2008) and triple transgenic mice for APP, presilin-1 (Rodríguez et al., 2008). Furthermore, hippocampal volume is reduced in people with AD (Roh et al., 2011). Conversely, treatment with cholinergic drugs, which increase cholinergic signaling, has been shown to stimulate neurogenesis (Mohapel et al., 2005; Kotani et al., 2006).

**Huntington’s Disease:** Huntington’s disease (HD) is another progressive neurodegenerative genetic disorder of the CNS. Initially, HD affects muscle coordination; eventually, it leads to cognitive decline and dementia. It is caused by an autosomal-dominant mutation on the Huntingtin (HTT) gene, resulting in an expansion of a CAG
triplet repeat stretch and alteration in the subsequent Htt protein. The altered Htt protein causes gradual pathological changes in the brain, giving rise to a wide spectrum of movement, cognitive, and psychiatric signs and symptoms. In genetic animal models of HD, such as the R6 transgenic mouse, adult neurogenesis is reduced (Lazic et al., 2004; Gil et al., 2005). In contrast, increased cell proliferation and neurogenesis is observed in postmortem HD human brain tissue (Curtis et al., 2003).

Parkinson’s Disease: Parkinson’s disease is also a degenerative disorder of the CNS that affects movement that results from the death of dopaminergic neurons in the substantia nigra. Signs and symptoms of PD include tremor, bradykinesia, muscle rigidity, impaired posture, speech changes, and eventually dementia. PD is characterized by the accumulation of α-synuclein aggregates in the form of Lewy bodies. There is little evidence supporting active neurogenesis in the adult substantia nigra. Furthermore, there does not seem to be reactive neurogenesis in either the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or 6-OHDA animal models of PD. One study reported the increased BrdU labeling in the substantia nigra after MPTP treatment as well as data supporting neurogenesis in the normal adult substantia nigra (Zhao et al., 2003), whereas another study found no new neurons in the substantia nigra of rodents injected with 6-OHDA (Frielingsdorf et al., 2004). However, after dopaminergic lesions with 6-OHDA or MPTP, the number of intrinsic tyrosine hydroxylase-positive (dopamine, NE) neurons increases in the striatum, and there is a transient elevation of adult neurogenesis in the hippocampus (Winner et al., 2009; Park and Enikolopov, 2010). Recent studies suggest that upon injury, reactive astrocytes promote adult DA neurogenesis through the Wnt/β-
catenin signaling pathway (L’Episcopo et al., 2011, 2012). In contrast, decreased neurogenesis has been shown in both the SGZ and SVZ in PD (Hoglinger et al., 2004).

**Amyotrophic Lateral Sclerosis:** Amyotrophic lateral sclerosis (ALS) is a fatal disease characterized by rapidly progressive muscle weakness, disability, and death. It is caused by the degeneration of lower motor neurons located in the spinal cord and the upper motor/cortical neurons in the brainstem and cortex. In mice carrying a mutation of superoxide dismutase 1, a mutation found only in rare familial patients with ALS, increased progenitor cell proliferation, migration, and neurogenesis has been reported (Chi et al., 2006). Adult neurogenesis and neural plasticity is modulated in several neurodegenerative diseases. It is noteworthy that genes often involved in neurodegenerative diseases such as PD (β-synuclein), AD (presenilin-1, τ), and HD (huntingtin) also seem to play important roles in regulating adult neurogenesis (Winner et al., 2011). Multiple sclerosis (MS) is an inflammatory autoimmune disease characterized by demyelination and axonal scarring. This leads to less effective communication between nerve cells in the brain and spinal cord. MS occurs most often in young adults and women (Compston and Coles, 2008).

**Adult Neurogenesis in Demyelinating Disease:** Multiple sclerosis (MS) is an autoimmune disease characterized by demyelination and axonal scarring and cause less effective communication between nerve cells in the brain and spinal cord. MS occurs most often in young adults (Compston and Coles, 2008) and is often associated with periods of worsening (relapsing) and improvement (remitting), as some remyelination takes place. Unfortunately, oligodendrocytes, the cells responsible for the making and maintaining of myelin in the CNS, cannot completely rebuild the sheath, because MS...
destroys oligodendrocytes. Normally, few new oligodendrocytes are generated in the adult brain (McCarthy and Leblond, 1988; Ehninger and Kempermann, 2003); however, after demyelination injury, some neuron-glial antigen 2 cells will give rise to oligodendrocytes (Redwine and Armstrong, 1998; Nait-Oumesmar et al., 1999; Levine et al., 2001). Furthermore, increased neurogenesis is observed in long-term lesions of MS (Chang et al., 2008). As the disease advances, the remyelination becomes less effective (Wolswijk, 1998), and neurologic function progressively deteriorates.

**Adult Neurogenesis in Epilepsy and Seizures:** Epilepsy, characterized by recurrent seizures, is the third most common neurological disorder after stroke and AD. Epileptic seizures result from abnormal synchronous neuronal activity in the brain (Fisher et al., 2005). Temporal lobe epilepsy (TLE) is the most common form of epilepsy in humans and is often associated with hippocampal lesions. In animal models of epilepsy, adult neurogenesis is profoundly enhanced in both the SGZ and SVZ (Parent et al., 1998; Ferland et al., 2002; Jessberger et al., 2007a). Precursor cell proliferation and adult neurogenesis may increase as much as 10-fold after short-term seizures. Evidence of increased cell proliferation has also been found in tissue from children with hippocampal sclerosis and seizures (Takei et al., 2007). However, chronic TLE is associated with decreased adult hippocampal neurogenesis (Hattiangady et al., 2004). Seizures, in addition to enhancing neurogenesis, seem to abnormally affect neuronal development, polarity, migration and integration (Jessberger et al., 2007b; Kraev et al., Parent, 2007; Hattiangady et al., 2008; Zhao and Overstreet, 2008).

**MAP Kinase pathways and signaling:** Mitogen activated protein kinases (MAP kinases) are serine-threonine protein kinases that are expressed in all eukaryotic cells.
MAPK constitute a large kinase network that regulates a variety of physiological processes such as cell growth, differentiation, and apoptotic cell death. MAP kinases are activated by diverse stimuli ranging from cytokines, growth factors, neurotransmitters, hormones, cellular stress, and cell adherence (Daoud, et al., 2005; Martin, et al., 2009; Lewis, et al., 1998). However, disturbances in MAP kinases cause tumorigenesis, neuron-inflammation and genetic and cellular alterations (Dolado, et al., 2007). More than a dozen mammalian MAP kinase family members have been discovered. MAP kinases lie within protein kinase cascades. Cascades convey information to effectors, coordinates incoming information from other signaling pathways, amplify signals, and allow for a variety of response patterns.

Cells recognize and respond to extracellular stimuli by engaging specific intracellular programs such as the signaling cascade that leads to activation of MAP Kinases (Roux, et al., 2004). All eukaryotic cells possess multiple MAPK pathways which regulate diverse cellular programs including embryogenesis, proliferation, differentiation and apoptosis based on cues derived from the cell surface, metabolic state, and environment of the cell. MAP kinases consist of a family of protein kinases, which are considered to play a crucial role in signal transduction pathways in mammalian cells leading mitogenic signals to their intracellular targets (Junttila, et al., 2008; Ravni, et al., 2006; Daoud, et al., 2005; Roux, et al., 2004). MAP kinase activity is regulated through three-tiered cascades composed of a MAP kinase, MAP kinase kinase (MAPKK, MKK or MEK) and a MAPKK kinase or MEK kinase (MAPKKK or MEKK). Where MAPKK is the kinase of MAPK and MAPKKK is the kinase of MAPKK (Roux, et al., 2004). In most cases, the MAPKKK is activated by small G proteins such as Ras, Rac, and Rap1 (Dan, et al.,
In mammals, three major MAPK pathways have been identified: MAPK/ERK, SAPK/JNK, and P38 MAPK. The ERK pathway is activated by a large variety of mitogens and by phorbol esters, whereas the P38 and c-Jun NH2-terminal kinase (JNK) / stress-activated protein kinase (SAPK) pathways are stimulated mainly by environmental stress and inflammatory cytokines (Ramen, et al., 2007).

**Role of MAP kinases in neural differentiation:** It has been demonstrated that external stimuli lead to the activation of different protein kinases by stimulating the activity of a number of serine/threonine kinases including the mitogen activated protein MAP kinase in PC12 cells (Martin, et al., 2009). Among the numerous differentiation agents, NGF and EGF are most widely studied (Kriegsheim, et al., 2009). NGF primarily promotes the differentiation through receptor tyrosine kinase, RTK (Vaudry, et al., 2002). NGF induced neuronal differentiation has been found involving the signaling cascade of Ras and Src (Mckay, et al., 2007). The closely related RTK activated by EGF stimulates proliferation rather than differentiation of PC12 cells (Lazaronci, et al., 1997). The responses to both NGF and EGF require ERK, a mitogen-activated protein kinase (MAPK) (Kriegsheim, et al., 2009). Neurite outgrowth stimulated by PACAP, an adrenomedullary neurotransmitter, also occur through ERK activation, in a process similar to but distinct from NGF signaling (Ravni, et al., 2006). These studies put into focus a fundamental question of signal transduction that how MAPK kinase are integrated with growth factors, hormones, neutrophins and differentiation process and signaling network. Literature says that the duration of signaling through ERKs may hold the key to very different outcomes of EGF and NGF stimulation. EGF induces rapid and transient Ras-, Raf- and Rap1-dependent ERK phosphorylation, whereas NGF
stimulation of ERK is both rapid and sustained, with sustained activation dependent on signaling to ERK through Rap1 (Kriegsheim, et al., 2009). Differential recruitment of phosphatidylinositol 3-kinase (PI3K) and scaffolding components (such as the adaptor FRS2) to activated TrkA, but not to EGF receptor complex, may be the explanation for sustained Rap1-mediated B-Raf activation by TrkA, but not by EGF receptor (Jourdi, et al., 2010; York, et al., 2002). PI3K also activates the c-Jun NH2-terminal kinases (JNKs), which through activation of c-Jun, can promote differentiation or apoptosis, depending on the exposure of cells to NGF (Leppa, et al., 2001). Thus differentiation, survival, and proliferation have been suggested to involve a balance among MAPK signaling pathways that depends on the combination of neurotrophins and other first messengers present in the cellular milieu (Juntila, et al., 2008; Roux, et al., 2004; Vaudry, et al., 2002; Takeda et al., 2002).

ERK activation is required for induced neurite formation in PC12 cells, as inhibition of ERK activation using specific inhibitors blocked neurite extension induced by NGF and EGF (Kriegsheim, et al., 2009). Conversely, in primary neural cultures ERKs are not required for neurite outgrowth or survival after growth factor withdrawal (Aglah, et al., 2008; Gunn-Moore, et al., 1997), suggesting that the requirement varies among different neural cell types. ERKs have been linked to long-term potentiation (LTP), both directly through the induction of ERK nuclear translocation by glutamate and by inference from the deficiency of an animal lacking the calcium-sensitive Ras exchange factor in acquisition of long-term memory (Rossomando, et al., 1989; Martin, et al., 1997). In addition to ERK, P38 MAP kinase has also been reported to participate in neural differentiation. Apoptosis signal-regulating kinase 1 (ASK1, a MEKK) - induced neurite
outgrowth effect was inhibited by a p38 inhibitor suggesting that the activation of P38 plays crucial roles in PC12 cell differentiation induced by ASK1 (Takeda, et al., 2000). Forskolin - induced differentiation of PC12 cells was found to be mediated through p38 MAP kinase, and in combination with nerve growth factor (NGF) a marked increase in neurite outgrowth was observed (Hansen, et al., 2000). In addition, BMP-2, bone morphogenetic proteins can also induce neurite out growth through p38 MAP kinase activation (Iwasaki, et al., 1999).

JNK MAP Kinase has also been suggested as one other important pathway showing promising effect on the neural differentiation (Kamata, et al., 2007; Takeda, et al., 2002). These JNKs directly activates c-jun, which ultimately targets to downstream effector molecules (ERK) for neural differentiation (Eom, et al., 2005; Leppa, et al., 1998). However, contrary to this, pretreatment of SH-SY5Y cell line with various inhibitors of P38 and ERK has shown to inhibit the retinoic acid induced neural differentiation, whereas JNK inhibitor had no effect to neural differentiation induced by retinoic acid in SH-SY5Y cell line (Singh, et al., 2003: Young-Mi, et al., 2003). The evidences of growth factors induced neuronal differentiation by activating MAP kinases are listed in table- 1.

<table>
<thead>
<tr>
<th>Cell types</th>
<th>Differentiating agent</th>
<th>MAPKs activated</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC12 Cells</td>
<td>IL-6</td>
<td>MEK</td>
<td>Sayoko Ihara (1997)</td>
</tr>
<tr>
<td>PC12 Cells</td>
<td>NGF &amp; EGF</td>
<td>ERK-MAPK</td>
<td>A. Kriegsheim (2009)</td>
</tr>
<tr>
<td>PC12 Cells</td>
<td>BMP-2</td>
<td>P38-MAPK</td>
<td>Shoji Iwasaki (1999)</td>
</tr>
<tr>
<td>Stem Cells</td>
<td>BMP-4</td>
<td>ERK-MAPK</td>
<td>Byoung-San Moon (2009)</td>
</tr>
<tr>
<td>PC12 Cells</td>
<td>Secretin</td>
<td>ERK-MAPK</td>
<td>Hyeon Soo Kim (2006)</td>
</tr>
<tr>
<td>Cells</td>
<td>Treatment</td>
<td>Signaling Pathways</td>
<td>Reference</td>
</tr>
<tr>
<td>------------</td>
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</tr>
<tr>
<td>PC12 Cells</td>
<td>Medium chain fatty acid</td>
<td>ERK-MAPK &amp; p38-MAPK</td>
<td>Y. Kamata (2007)</td>
</tr>
<tr>
<td>H19-7 Cells</td>
<td>bFGF</td>
<td>ERK-MAPK, Ras, MEK &amp; Src</td>
<td>Wen-Liang Kuo (1997)</td>
</tr>
<tr>
<td>PC12 Cells</td>
<td>Retinoic acid</td>
<td>ERK-MAPK</td>
<td>Estela Canon (2004)</td>
</tr>
<tr>
<td>PC12 Cells</td>
<td>Ascorbic acid</td>
<td>ERK-MAPK</td>
<td>Mari Haramoto (2008)</td>
</tr>
<tr>
<td>PC12 Cells</td>
<td>NGF &amp; EGF</td>
<td>ERK-MAPK &amp; p38-MAPK</td>
<td>Takaya Morooka (1998)</td>
</tr>
<tr>
<td>PC12 Cells</td>
<td>FGF-2 &amp; NGF</td>
<td>MAPK-ERK and hSef</td>
<td>Shiqin Xiong (2003)</td>
</tr>
<tr>
<td>SH-SY5Y</td>
<td>Retinoic acid</td>
<td>ERK-MAPK &amp; p38-MAPK</td>
<td>Ugra S. Singh 2003</td>
</tr>
<tr>
<td>Stem Cells</td>
<td>Retinoic acid</td>
<td>ERK-MAPK</td>
<td>Zegui Li (2006)</td>
</tr>
</tbody>
</table>

**cAMP pathway/PKA pathway:** Adenylyl cyclase (AC) belongs to a large family of proteins that are regulated by trimeric G-proteins linked to G-protein coupled receptors, is work as the effector molecule of one of the most widely utilized signal transduction pathways. It occurs in both soluble and transmembrane form. It uses ATP to generate its product, cAMP. cAMP, a secondary messenger, modulates cell growth and differentiation in organisms from bacteria to higher eukaryotes. Soluble AC (sAC) has been shown to be present in developing neurons, where, depending on the origin of the neuron, it was located in cell bodies, dendrites, axons and/or growth cones. One
anticipated role for sAC in developing neurons is to regulate growth of cones and promote axonal growth (Wu et al., 2006.).

NGF stimulates axon generation via stimulation of the small G protein Rap1. The confirmation that PC12 cells express sAC and the observation that sAC inhibition by small molecules or siRNA blocked NGF induced activation of Rap1 suggested that sAC is also involved in axon growth in response to NGF. (Stessin et al., 2006). The initial molecule purified to bind cAMP and probably the major target of cAMP was protein kinase A (PKA) or cAMP dependent protein kinase (Beavo et al., 2002). PKA is a serine/threonine kinase that in its inactive form consists as a tetramer of two regulatory subunits (R) and two catalytic subunits (C). Activation of the catalytic subunits further initiates a cascade of events ultimately activating the MEK-ERK signalling pathway via Rap1. Other cAMP effectors include some cyclic nucleotide-gated channels and the Epac (exchange proteins directly activated by cAMP) 1 and 2 proteins, also known as cAMP-regulated guanine nucleotide exchange factors 1 and 2 (cAMP-GEFI and cAMP-GEFII). These guanine-exchange factors facilitate the GDP→GTP exchange for the small GTPase proteins Rap1 and Rap2. Rap1 interacts with the serine/threonine kinases RAF1 and B-RAF, establishing a link between cAMP and MAPK signaling (Stessin et al., 2006).

**Role of cAMP pathway/PKA pathway in neuronal differentiation:** Pituitary adenylate cyclase-activating polypeptide (PACAP), initially isolated from ovine hypothalamic extracts, is involved in the regulation of major bain’s biological functions (Vaudry et al., 2000b). Indeed, PACAP is widely distributed in the CNS and peripheral tissues, and exerts pleiotropic effects on the brain as well as endocrine glands, cardiovascular system, gastrointestinal and respiratory tracts, gonads, immune cells, and tumours (Vaudry et al.
2000b). In the developing CNS, PACAP decreases the proportion of mitotic cells and promotes neuroblast differentiation (Lu and DiCicco-Bloom 1997). In the adult brain, PACAP modulates neurotransmitter release and inhibits apoptotic cell death (Uchida et al., 1996). The neurotrophic actions of PACAP were first observed in PC12 cells (Deutsch and Sun 1992). Since then, this cell line has been extensively used to investigate the signalling pathways involved in PACAP-induced cell differentiation and survival, and some of the genes that could encode the proteins acting in these processes have been identified. Interest in the molecular mechanisms of PACAP-induced neuronal differentiation is especially intense because it represents the prototype for G-protein-coupled receptor-mediated neurotrophin (Waschek et al., 1998; Beaudet et al., 2000; Vaudry et al., 2000b; Nicotet et al., 2002) just like nerve growth factor (NGF) which has extensively been used as a model to investigate the function of tyrosine kinase receptors in the nervous system.

Several studies showed that treatment of PC12 cells with PACAP induces neurite outgrowth (Deutsch and Sun 1992; Hernandez et al., 1995) whereas inhibits cell proliferation (Vaudry et al. 2002b). The action of PACAP on PC12 cells involves the PAC1-R which is known to be positively coupled to the adenylate cyclase, phospholipase C and calcium pathways. As it is possible to stimulate neurite outgrowth by increasing cAMP levels in PC12 cells (Gunning et al. 1981a), however this effect has been reported to be protein kinase A (PKA) independent. In addition to its consequence on neuritogenesis, PACAP, like NGF, increases the density of sodium and calcium channels necessary for the attainment of electrical excitability (Grumolato et al. 2003a) and stimulates the expression of the VACHT gene, which is a marker of cholinergic
neurons (Grumolato et al. 2003b). These observations signify that PACAP not only affects PC12 cell morphology but also plays an important role for the advancement of a neuronal phenotype.

**Mechanisms of cAMP/PKA and MEK/ERK cross-talk:** The well-synchronised functions in living beings are mediated by the action of signal transduction pathways, which are responsible for receiving a signal, transmitting and amplifying it, and implying the appropriate cellular responses. Mammalian cells possess numerous signal transduction pathways that, rather than acting in seclusion, interconnect with each other, a phenomenon referred to as cross-talk. This allows cells to regulate the distribution, duration, intensity and specificity of the response. The cAMP/cAMP-dependent protein kinase (PKA) pathway and the mitogen-activated protein kinase (MAPK) cascades modulate common processes in the cell and multiple levels of cross-talk between these signalling pathways elicits a particular cellular response. In 1993, five independent groups almost simultaneously reported for the first time interconnection between the RAS–RAF–MEK–ERK and the cAMP–PKA pathways. They all established that cAMP blocked growth signal-induced activation of the MEK/ERK signalling cascade in fibroblasts, fat cells, and smooth muscle cells. They also pinned down the location of this inhibition somewhere between RAS and c-RAF, but the exact mechanism remained unsolved at that time (Burgering et al., 1993; Cook et al., 1993; Graveset et al., 1993).

Various ways of cross regulation between PACAP and NGF have been reported. In particular, PACAP increased the basal and NGF-stimulated phosphorylation of the tyrosine kinase A receptor (Lazarovici and Fink 1999), while NGF stimulates the expression of the PAC1-R (Jamen et al., 2002). The two neurotrophic agents can also
synergize to promote, through different transduction pathways, prolonged phosphorylation of ERK1 and -2 through Rap1 (Kao et al., 2001; Sakai et al., 2004), which is involved in the induction of neurite outgrowth. Downstream of the activation of the ERK pathway, NGF induces a sustained and strong expression of the neuron-specific activator p35 and of the cyclin-dependent kinase 5 (Harada et al. 2001), which are required for NGF-induced neurite outgrowth. PACAP and NGF also activate Rac1 through both a PI3-kinase-independent and -dependent pathway. ERK, a participating protein in the MAPK signaling pathway, can be activated or inhibited by cAMP. cAMP can inhibit ERKs in a variety of ways, most of which involve the cAMP-dependent protein kinase (PKA) and the inhibition of Ras-dependent signals to Raf-1. However, cAMP can also stimulate cell proliferation by stimulating ERKs. This occurs through the induction of specific genes via phosphorylation of the transcription factor CREB by PKA. Though ERKs do not appear to be a requirement for this phosphorylation of CREB, the MAPK pathway does play into crosstalk again, as ERKs are required to phosphorylate proteins downstream of CREB (Sakai et al., 2004).

**Mechanisms of cAMP/PKA and p38 MAPK cross-talk:** As for the MEK/ERK pathway, cAMP/PKA seems to have opposite effects on p38 MAPK activity depending on the cell-type examined. But in contrast to ERK2 which could influence PKA activity through activation or inhibition of PDE, no effect of the p38 MAPK module on PKA has been reported, so far. Several studies have reported PKA-dependent activation of p38 MAPK in a variety of cells, including NIH3T3, macrophages, SK-N-MC, PC12, MC3T3-E1, CHO, colon cancer cells, adipocytes, rat primary granulosa cells, and adult mouse cardiomyocytes. However, forskolin has also been shown to inhibit p38 MAP kinase
activation in e.g. HUVEC cells and in thymocytes (Delghandi., 2005; Hunzicker-Dunn et al., 2006; Rahman et al., 2004). Currently, several different mechanisms by which cAMP/PKA can interfere with the p38 MAPK module have been described. PKA can influence the activity of the p38 MAPK cascade through: (i) Rap1-mediated regulation, (ii) PTPs acting on p38 MAPK, (iii) protein phosphatases targeting upstream activators of p38 MAPK, (iv) glia maturation factor, (v) the p160ROCK kinase, or (vi) the small GTPase Rit.

**Neuronal Calcium Signaling:** Calcium (Ca2+) is a ubiquitous intracellular signal responsible for controlling numerous cellular processes. Under resting conditions, free cytosolic calcium levels in neurons are maintained around 200 nM. Upon electrical or receptor-mediated stimulation, calcium levels rise to low micromolar concentrations by a mechanism of extracellular calcium influx or calcium release from intracellular stores (Marambaud, et al., 2009) Extracellular calcium concentrations are several magnitudes higher compared to cytosolic calcium levels. Calcium signaling is involved in many different intracellular and extracellular processes ranging from synaptic activity to cell-cell communication and adhesion. In the brain, calcium is essential in the control of synaptic activity and memory formation, a process that leads to the activation of specific calcium dependent signal transduction pathways and implicates key protein effectors, such as CaMKs, adenylate cyclases, cyclic-AMP, MAPKs and CREB. (Greer PL, et al., 2008) Properly controlled homeostasis of calcium signaling not only supports normal brain function but also maintains neuronal integrity and long-term cell survival. Emerging knowledge indicates that calcium homeostasis is not only critical for cell
physiology and health, but also, when deregulated, can lead to neurodegeneration via complex and diverse mechanisms involved in selective neuronal impairments and death.

![Diagram of depolarization-mediated ERK signaling](image)

**Figure 1.** Model of depolarization-mediated ERK signaling via PKA and Rap1/B-Raf following the exposure of KCl. Membrane depolarization of PC12 cells induces opening of L-type calcium channels leading to calcium influx. Calcium can then activate a membrane Rap1/B-Raf signaling pathway to cytoplasmic ERKs via PKA. This requirement for PKA may reflect the regulation of cAMP signaling via stimulation of calmodulin-activated adenylate cyclases at the membrane. CaM, calmodulin; ACase, adenylate cyclase.

**Forskolin:** Forskolin (also called Coleonol) is a labdane diterpene that is produced by the Indian Coleus plant (*Coleus forskohlii*). Forskolin is commonly used to raise levels of cyclic AMP (cAMP) in the study and research of cell physiology. Forskolin resensitizes cell receptors by activating the enzyme adenylyl cyclase and increasing the intracellular levels of cAMP. Cyclic AMP acts by activating cAMP-sensitive pathways such as protein kinase A and Epac. The forskolin-induced cAMP also induced sustained
increase in ERK1/2 phosphorylation within 0.25–6 h and then led to neuronal differentiation (Park, et al., 2012).

**RV and its pharmacological properties:** The polyphenolic compound RV (3,4',5-trihydroxystilbene) is a naturally occurring phytochemical which has been found in more than 70 plant species, including herbs and human food products such as grapes, berries, and peanuts. RV was first isolated in 1940; however, little attention was paid to it until its benefits in coronary heart disease were studied in 1992. Since then, increasing evidence has indicated that RV may be useful in treating cardiovascular diseases, cancers, pain, inflammation, tissue injury, and in reducing the risk of neurodegenerative disorders, especially Alzheimer's disease (AD) (Albani, et al., 2010). The natural phytocompound RV has been considered for many years as a potential anticancer drug, but recently it has come to the attention of neuroscientist too, as it displays neuroprotective actions. RV has neuroprotective features both in vitro and in vivo in models of AD, but it has proved to be beneficial also in ischemic stroke, Parkinson's disease, Huntington's disease, and epilepsy (Richard et al., 2011). A limited number of therapeutic options are available to treat neurodegenerative diseases; however, these are primarily treating symptoms and not interfering with the disease process. RV derivatives are highly promising molecules that could be used to both prevent and/or treat neurodegenerative diseases such as AD. Indeed, RV derivatives may effectively modulate multiple mechanisms of the neurodegenerative disease pathology (Li, et al., 2012).

RV exists as two isomeric forms: the biologically inactive cisform, and the active trans-form. UV exposure converts the trans-form to cis-form. Trans-form of RV is synthesized for in vivo, in vitro and ex vivo experiments. The purified form of RV
extracted from Knotweed is also available as dietary supplements. RV is a stilbene and its estrogenic effects mimic diethylstilbestrol, a synthetic estrogen. RV is thought to have a cholesterol lowering effect which hypothetically is responsible for the reduced risk of heart disease in people having a Mediterranean diet. The fact that there is an epidemiologic link between moderate red wine consumption (in spite of high fat diet), and decreased incidence of cardiovascular disease in the French population (the French Paradox) suggests that some red wine ingredients like RV do have beneficial effects. (Anekonda TS, 2006). Recent reviews (Baur, *et al.*, 2006; de la Lastra, *et al.*, 2007) have reviewed the effects of RV. Since 1992 a number of studies suggested that RV can prevent or delay multiple diseases including cancer, cardiovascular diseases and ischemic injuries (neuroprotective effects in response to brain ischemic injuries) (Gao, *et al.*, 2006). Other studies have shown that RV increases resistance to stress and extends the lifespan of yeast and vertebrates. The mechanism by which RV confers these beneficial effects is not clear. A series of studies have demonstrated that RV mimics the effect of calorie restriction in lower organisms by interacting with specific genetic pathways (Calabrese, *et al.*, 2001; Ghosh, 2008). Calorie restriction, defined as consumption of around 60% of normal diet, has anti-ageing effects in organisms ranging from yeasts to mammals. Calorie restriction has some obvious limitations in humans but the new category of drugs called CR mimetics, e.g., RV, are promising in prevention and treatment of human diseases (Roth, *et al.*, 2005). Like other oral medications and dietary supplements, the efficacy of oral (PO) RV administration depends on its absorption, metabolism distribution, and clearance. Our knowledge about RV’s pharmacokinetics and bioavailability in human is limited. Rat studies show that after intravenous (IV)
administration of purified RV, plasma concentration of aglycone drop rapidly with a half life of about 0.13 h, and then increased 4-8h after drug administration. In this study, the bioavailability of oral RV was estimated to be about 38% (de la Lastra, et al., 2005).

Wallace et al in 2005 showed that after IV and PO administration of RV in human volunteers, a very small amount of RV found in the plasma and most of the oral dose was found in urine.

The close relationship between RV and mitochondrial function became evident in several ischemic brain injury studies performed by Zini et al. (Zini, et al., 2002). they suggested that the mechanisms by which RV might conserve mitochondrial function in response to ischemic insult is by reducing ROS generation (antioxidant properties) and by stabilizing the mitochondrial membrane. RV has long been considered to be an antioxidant; however the mechanisms by which RV responds to oxidative stress are not clear. Recent studies suggested that RV suppresses peroxidation of lipids and other macromolecules. Floreani et al. (Floreani, et al., 2002) showed that RV induces catalase and quinone reductase 1(QR1) activities in cardiac tissue and decreases the amount of ROS generated by Menadione. Thus, RV might act directly as a ROS scavenger and/ or might induce cellular natural antioxidants. by increasing expression of paraoxonase 1 gene ,RV inhibits oxidation of low-density lipoprotein (LDL) particles (a known risk factor for coronary heart disease and myocardial infarction), (Holvoet, et al., 2004). It also changes the qualities of pro-oxidants in alcohol. (Turrens, et al., 1997)

**RV induced activation of MAP kinases activation during neuronal differentiation:**

RV, a natural polyphenolic compound has shown a wide pharmacological window with powerful antioxidant activities both in vitro (Dasgupta et al., 2007) and in vivo (Della-
morte *et al.*, 2009) experimental models of neuronal injuries and neuronal disorders. However, it is hypothesized that the pharmacological properties of RV might have routed through some different mechanisms other than the conventional one (Dong *et al.*, 2008).

In one of such studies in SHYSY5Y (human neuroblastoma cell line), it has been observed that RV causes upregulation of ERK1/2 during retinoic acid induced differentiation in cells (Miloso *et al.*, 1999). Dasgupta *et al.* (2007) have demonstrated the activation of mitochondrial biogenesis and neurite out growth in Neuro2a cells via activation of AMP kinase pathway. These studies have provided some initial clues regarding the neuronal differentiating potential of RV. However, the link of the activation of MAP kinases with neuronal differentiation are still missing and are to be explored because they are the key regulators for switching on/off the neural differentiation, induced by different growth factors (Kriegshein *et al.*, 2009; Monaghan *et al.*, 2008).

These studies have provided some initial clues regarding the neural differentiating potential of RV. However they do not link the MAP kinases activation with neural differentiation, which are the key regulators for switching on/off the neural differentiation, induced by different growth factors.
Figure 2. The RAS/RAF/MEK/ERK pathway is activated by NGF binding to receptor tyrosine kinase (RTK), which leads to the activation of the small G-protein RAS. Subsequently, RAF, MEK and ERK are activated in a cascade of phosphorylation events. Through the phosphorylation of many targets, MAPKs regulate cell fate. The cAMP pathway is activated when hormones/stimulus bind to receptors (GPCR) coupled to heterotrimeric G proteins and lead to the activation of adenylyl cyclase, which converts ATP into cAMP. The second messenger cAMP acts through many effectors and has many cellular effects. These two pathways interconnect or crosstalk at MEK1/2. This allows cells to regulate the distribution, duration, intensity and specificity of the response.

Brain and its susceptibility towards toxicants: Brain is the most complex and vital organ of the body and possesses the ability to control all physical as well as physiological activities of the body including behavior, learning, talking, memorizing, organizing, listening, performance of routine skills and interaction with one’s social environment. The brain cells/neurons are in their post mitotic phase and have little/negligible power of regeneration except the cells of some zone in the brain. Although the brain has only two
percent of total body weight yet it consumes more than twenty percent of the total oxygen used by body. Due to high blood supply, the brain cells are highly vulnerable towards chemical entities, and a small damage could lead to lifelong disability (Roberts, et al., 2012). Even at a low dose, neurotoxic hazards can cause injury during the early nervous developmental period that has no effect in adult brain. The early developing human nervous system exhibits susceptibility to many toxicants and there is evidence that chemical exposure during development can cause long lasting neurological deficits (Bellinger DC, 2012). Among the documented hazards are certain industrial chemicals, pesticides, tobacco smoke, alcohol and certain drugs, as well as maternal stress (Miodovnik A, 2011). Because of a growing recognition of an apparent increase in the incidence of developmental disabilities, considerable attention has been focused on the effects of exposures to environmental pollutants, including organophosphate and chlorinated pesticides (Freeman, et al., 2005). Current methods for DNT, which can be found in guidelines issued by the USEPA in 1998 during the process of harmonization between the USEPA and OECD (OPPTS 870-6300), include measures of gross morphology in the brain, a range of behavioral assays, neurochemistry, biomarkers of gliosis and cytotoxicity, and measurement of surrogate biomarkers specific to certain classes of neurotoxic chemicals, such as the use of plasma cholinesterase inhibition as a biomarker for organophosphate pesticide developmental neurotoxicity (Kamanyire, et al., 2004). Many of these tests are complex and expensive in terms of scientific resources, time and animal use and it is not clear how the data from such a complex battery of tests will be integrated. Moreover, there is increasing evidence in the experimental literature that chemical exposures can disrupt neurodevelopment in the absence of gross
morphological perturbations, that neurochemical changes can be transient, and thus may be missed in a routine screen, and that behavioral alterations may only be revealed under challenging test conditions. Occupational medicines have traditionally been studied for their neurotoxic and other adverse consequences in the workers themselves (dick FD, 2006). Literature is full of neurotoxicity of metals (such as lead and mercury), polychlorinated biphenyls (PCBs), and many pesticides (Kashyap, et al., 2011), solvents and other industrial chemical to adult brain and nervous system.

However, neuro-developmental studies on the children born by pregnant workers exposed to the same industrial chemicals have not been pursued much intensely. Millions of women have been joined as work force during recent decades in developed as well as in developing countries. Female employees often continue working throughout their pregnancy and large proportions are of a reproductive age (Ostrea, et al., 2006). Attention has been paid to the possible reproductive toxicity risks, such as birth defects, etc. but the existing studies have shown ambiguous results due to methodological and design problems (Ostrea, et al., 2006). Assessment of neurodevelopment may require many years of follow-up and is therefore even more difficult to document. Evidence on the consequences of industrial chemical exposures among pregnant workers is therefore rather scant, despite the magnitude of this public health concern. Improved insight into developmental neurotoxicity is crucial, because these effects may occur at exposure levels that are lower than existing occupational exposure limits, which aim at protecting the nervous system of the adult workers themselves.

**Organochlorine Pesticides:** Pesticides are a group of chemicals used to kill the “pest” species. The word pesticide may also be referred into subcategories like insecticides,
herbicides, fungicides, or other pest control formulations. Pesticides may be inherently toxic and often harm to non-target organisms and can lead to adverse health effects after exposure. Organochlorine pesticides are a large class of chlorinated hydrocarbon compounds. Organochlorine pesticides have the phenomenon of bioamplification and accumulate in the food web. Thus, they stay in the environment and fatty tissues of animals long after the being applied. DDT, now banned in many countries due to biomagnifications problem with is pesticide. Endosulfan (50-200 µM) generated both superoxide anion and hydrogen peroxide in a dose-and time-dependent manner, enhanced the production of these reactive oxygen species, decreased superoxide dismutase, glutathione peroxidase, and catalase activities in SH-SY5Y cells. Additionally, this pesticide induced lipid peroxide and induced caspase-3 (100 µM) and nuclear NFκappa B activity in SH-SY5Y cells (Jia et al., 2007). Wang et and colleagues reported a comprehensive study of pesticide levels and bio-accumulation characteristics in human adipose tissues among residents of Southeast China and showed that POPs pesticides were frequently detected, including 2,4'-DDD, 2,4'-DDE, 2,4'-DDT, 4,4'-DDD, 4,4'-DDE, 4,4'-DDT, α-HCH, β-HCH, γ-HCH, δ-HCH, hexachlorobenzene (HCB), and mirex (Wang et al., 2011). Other detected pesticide species were dicofol, methamidophos and chlordimeform, which have rarely been reported. DDT persists in environment for a very long time and used against mosquitoes to check transmission of malaria. Some scientists also studied the association between neurodevelopmental and concentration of DDT and its metabolite DDE in blood during pregnancy. The results revealed no visible changes in the neonatal behavior, but mental and motor developments were negatively associated with DDT exposure during infant ages (Eskenazi et al., 2006). In a case control study by
Ren et al. (2011) reported a dose-response relationship the exposure of o,p'-DDT and metabolites, α-HCH, γ-HCH, α-endosulfan in placenta and anencephaly as well as spina bifida (Ren et al., 2011).

**Organophosphate pesticides:** The organophosphates are commonly known as insecticides used throughout the world for agriculture (Eskenazi et al., 2007). These substances work preliminary by inhibiting the enzyme acetylcholinesterase, which break down the neurotransmitter acetylcholine in both the peripheral and the central nervous system. Acute pesticide neurotoxicity is well known from occupational exposure studies, poisoning events, and suicide data in adults (Grandjean and Landrigan, 2006). Developmental neurotoxicity may be caused by similar mechanisms, which may lead to more permanent effects, as acetylcholine has crucial functions during brain development (Eskenazi et al., 2007). Two cohort studies (from the same research group) were based on an agricultural worker population in California (Young et al., 2005; Eskenazi et al., 2007). The effects of prenatal exposure to organophosphates were assessed by maternal urinary excretion of pesticide metabolites showing negative associations between the prenatal exposure and abnormal reflexes in the infant, as determined by the Brazelton Neonatal Behavioral Assessment Scale (Young et al., 2005). At ages 6–24 months, the results showed lower mental development on the Bailey Scales and higher pervasive disorder scores on the Child Behavior Check List questionnaire, as compared to unexposed controls (Eskenazi et al., 2007). These results were independent of other potential neurotoxicants measured, such as lead, dichlorodiphenyltrichloroethane (DDT), and PCB, in blood samples. Additionally, four cross-sectional studies (Handal et al., 2007; Handal et al., 2008) were carried out in Ecuador and Mexico, where mothers
working in agriculture or flower production were exposed to pesticides during pregnancy, while comparison groups were similar but not exposed to pesticides. The findings showed lower motor skills, communication and problem solving abilities, creativity, and lower visual acuity at age 6–61 months in infants with prenatal exposure. In older children at age 8 yr, Grandjean et al. (2006) reported lower visuospatial performance in prenatally exposed children and an additive effect of stunting. In all of these studies, the exact identity of the pesticides is unclear, as exposures involved mixtures of substances. From the evidence available, the organophosphates are likely the causative substance, as also supported by experimental studies. Although many different compounds were involved, they may share toxic mechanisms about developmental neurotoxicity (Bjorling-Poulsen et al., 2008). Duramed et al. reported that although chlorpyriphos (CPF), and its metabolites chlorpyrifos-oxon (CPO) and 3,5,6-trichloro-2-pyridinol (TCP), were not able to induce the expression of different cytokines alone, but these compounds in combination with endotoxin lipopolysaccharide (LPS) or house dust mite Dermatophagoides pteronyssinus (Der p1) allergen were able to induce the expression of IFN-gamma (Duramed et al., 2006). Many workers have been reported the negative health effects including developmental neurotoxicity of chlorinated pesticide in human systems and other species (Hein et al., 2010; Slotkin et al., 2009; Cole et al., 2011; Sledge et al., 2011). Rauh et al. (2011) studied the relationship between prenatal CPF exposure and neurodevelopment among cohort children at age 7 years. They measured prenatal CPF exposure using umbilical cord blood plasma (picograms/gram plasma), and 7-year neurodevelopment using the Wechsler Intelligence Scales for Children (WISC-IV)
and reported that increase in CPF exposure (4.61 pg/g), Full-Scale IQ declined by 1.4%, and Working Memory declined by 2.8%.

**Monocrotophos:** Monocrotophos (MCP) is a systemic insecticide and acaricide belonging to the vinyl phosphate group. It controls pests on a variety of crops, such as cotton, rice, and sugarcane. It is used to control a wide spectrum of chewing, sucking and boring insects (aphids, caterpillars, *Helicoverpa* spp, ites, moths, jassids, budworm, scale and stem borer, as well as locusts). MCP is not patentable and has therefore become an easily affordable pesticide. Its low cost and many possible applications have kept up its demand in India despite growing evidence of its negative impact on human health.

MCP is an organophosphorus compound that inhibits cholinesterase. It is highly toxic by all routes of exposure. MCP can be absorbed following ingestion, inhalation and skin contact. The acute oral lethal dose (LD50) for rats is 14 mg/kg. According to WHO, the ingestion of 120 mg MCP can be fatal to humans (International Programme on Chemical Safety, 1993). In the WHO 2004 edition of the Recommended Classification of Pesticides by Hazard and the guidelines to Classification (Forget, *et al.*, 1992), MCP is classified in the WHO Class Ib. i.e. as a highly hazardous pesticide. MCP can be absorbed following ingestion, inhalation and skin contact. When inhaled, it affects the respiratory system and may trigger bloody or runny nose, coughing, chest discomfort, difficulty breathing or shortness of breath and wheezing due to constriction or excess fluid in the bronchial tubes. Skin contact with organophosphates may cause localized sweating and involuntary muscle contractions. Eye contact will cause pain, tears, pupil constriction and blurred vision.
Following exposure by any route, other systemic effects may begin within few minutes or be delayed for up to 12 hours. These may include pallor, nausea, vomiting, diarrhoea, abdominal cramps, headache, dizziness, eye pain, blurred vision, constriction or dilation of the pupils, tears, salivation, sweating and confusion (Eddleston, et al., 2000). Severe poisoning will affect the central nervous system, producing lack of coordination, slurred speech, loss of reflexes, weakness, fatigue, involuntary muscle contractions, twitching, tremors of the tongue or eyelids, and eventually paralysis of the body extremities and the respiratory muscles. In severe cases there may also be involuntary defecation or urination, psychosis, irregular heartbeat, unconsciousness, convulsions and coma. Respiratory failure or cardiac arrest may cause death.

MCP is also highly neurotoxic as it cause inactivation of the cholinesterase in the blood and in a wide range of nerve, neuromuscular (skeletal, smooth and cardiac) and glandular tissues where these enzymes have a role in cell-to-cell communication and the hydrolysis of xenobiotics. These enzymes have possibly as yet unidentified roles such as cell development and growth. The inhibition of AChE leads to the accumulation of acetylcholine, the neurotransmitter at all ganglia in the autonomic nervous system and at many synapses in the brain, skeletal neuromuscular junctions, at some postganglionic nerve endings of the sympathetic nervous system and adrenal medulla (Julvez, et al., 2009).

**Free radical generation and oxidative stress:** Free radicals (O$_2^-$, NO$_2^-$, H$_2$O$_2$ and OH$^-$) generated predominantly during cellular respiration and normal metabolism are highly reactive molecules. Their overproduction can cause oxidative damage to biomolecules, (lipids, proteins, DNA), that can further lead to many chronic diseases such as
atherosclerosis, cancer, diabetics, rheumatoid arthritis, post-ischemic perfusion injury, myocardial infarction, and other degenerative diseases in humans (Freidovich et al., 1999; Yun-Zhong et al., 2002) ROS are mainly active in the brain and neuronal tissue as the excitatory amino acids and neurotransmitters, whose metabolism is factory of ROS, which are unique to the brain and serve as sources of oxidative stress. As ROS attack glial cells and neurons, which are post-mitotic cells and therefore, they are particularly sensitive to free radicals, leading to neuronal damage (Gilgun-Sherki et al., 2001). It has been reported that harmful effects of ROS on human cells may end in oxidative damage leading to programmed cell death i.e. apoptosis (Saxena, A.K et al., 1993)

Programmed cell death: It is also known as apoptosis and involves activation of specific intracellular pathways that leads to the controlled destruction of the cell (Nicotera and Lipton, 1999; Doyle et al., 2008). It results from DNA cleavage and by other autotypic processes causing nuclear shrinkage, chromatin clumping, and ultimately cell death (Portera-Cailliau et al., 1997). That results into aging, cerebral ischemia, and other neurodegenerative diseases is neuronal cells death either by necrosis or apoptosis (Graham and Chen, 2001; Doyle et al., 2008; Nakka et al., 2008). It is now established that apoptotic/necrotic cell death in the brain following neurodegenerative diseases is dependent on the type of cells involved and the nature of the stimulus. In the nervous system, genes have been identified which either (i) block apoptosis: Bcl-2 and Bcl-xL or(ii) promote apoptosis: Bax. In case of neuronal injuries leading to apoptosis, the majority of pro-apoptotic factors results in to the release of protein from the intermembrane space into the cytoplasm of which cytochrome-C has already been characterized. In the cytoplasm, cytochrome-C interacts with a protein caspase-9 that
further activate caspase-3. This leads to the chain of events including the activation of the caspase dependant DNAase leading to fragmentation of DNA and followed by cell death (Sims and Anderson, 2002; Fiskum, 2000).

**Need of Antioxidants:** Various epidemiological studies reported that many of antioxidant compounds shows antiinflammatory, antibacterial, anticarcinogenic, antitumor, antimutagenic, and antiviral activities to certain extent (Mitscher et al., 1996; Owen et al. 2000; Sala et al., 2002.). In many cases other than neurogenesis, increased oxidative stress is a widely associated in the development and progression of disease like diabetes (Baynes, 1991; Baynes, 1999; Ceriello, et al., 2000; Chang, et al., 1993; Halliwell et al., 1990; McLennan et al., 1991 and Young, et al, 1995). Though the intake of natural antioxidants has been reported to reduce risk of cancer, cardiovascular diseases, diabetes and other diseases associated with aging, there is considerable controversy in this area (Hertog 1995; Sun et al., 2002; Yang et al., 2001) Leukocytes and other phagocyte destroy bacteria, parasites and virus-infected cells with NO, O2 and H2O2, those are powerful oxidants and protect humans from infection. However, they can cause oxidative damage and leads to mutation of DNA and participate in the carcinogenic process if unchecked. Moreover, experiments and studies infer that antioxidants are needed to scavenge and prevent the formation of ROS and reactive nitrogen species (RNS); out of them, some are free radicals while some are not (Barry, 1996). There is growing evidence that oxidative damage to sperm DNA is increased when there is ascorbate insufficiency in diet. This strongly suggests the protective role of antioxidant in our daily diet. Animal cytochrome P450 enzymes are one of the primary defense systems that provides protection against natural toxic chemicals from plants, the
major source of dietary toxins. Even these enzymes are protective against acute toxic effects from foreign chemicals, yet they may generate some oxidative by products that damages DNA (Bruce, et al., 1993).

Both vegetarian as well as non vegetarian diet supplies various antioxidants such as vitamins C and E, β-carotene and coenzyme Q to human body. They are the most famous antioxidants, out of which, vitamin E is present in vegetable oils and found abundantly in wheat germ. Out if 8 natural state isomeric forms of vitamin E, α-tocopherol is the most common and potent isomeric form. Vitamin E can effectively prevent lipid peroxidation of plasma membrane as it is lipid soluble (Burton et al., 1989, 1990). Plants (fruits, vegetables, medicinal herbs) may contain a wide variety of free radical scavenging molecules such as phenolic compounds (Phenolic acids, flavonoids, quinons, coumarins, lignans, stilbenes, tannins etc.), nitrogen compounds (alkaloids, amines, betalains etc.), vitamins, terpenoids (including carotenoids) and some other endogenous metabolites which are rich in antioxidant activity (Zheng et al., 2001; Velioglu et al., 1998; Cotelle et al., 1996 and Cai et al., 2003).

**Neurodegenerative diseases and oxidative stress:** Neurodegenerative diseases collectively define as a state in which nerve cells from brain and spinal cord are lost leading to either sensory dysfunction (dementia) or functional loss (ataxia). Mitochondrial (Mt) dysfunctions and excitotoxicity and finally apoptosis have been reported as pathological cause for aging and major neurodegenerative diseases such as Parkinson’s disease (PD), Alzheimer’s disease (AD), Multiple Sclerosis (MS) and amyotrophic lateral sclerosis (ALS). Neurodegeneration have been hypothesized to be interplay of a number of factors including environmental and genetic predisposition but
Review of literature

Redox metal abuse occupies central role as most of symptoms stems out from abnormal metal metabolism (Mark 2004). Oxidative stress and free radical generation catalyzed by redox metals have been shown to play pivotal role in regulating redox reactions in vivo contributing RNS and ROS, main culprits in neurodegeneration.

**Monocrotophos and RV:** Surplus literature has supported vulnerability of neural cell to the environmentally existing toxicant. Among such toxicants, pesticides especially organophosphorus pesticides (OPs) have become leading area of research as a consequence of their reported effects on the brain both in vivo and in vivo (Julvez *et al*., 2009). Ops such as monocrotophos (MCP) have also shown to cause severe nervous system injury. As environmental exposure of MCP to human beings is more, it has become the big health concern worldwide and has also been banned in some countries like US. Our previous research on MCP induced neuronal toxicity on PC12 cells have shown mitochondria mediated apoptotic cell death via induction of oxidative stress, lipid peroxidation and changes in glutathione disulphide ratio (Kashyap *et al*., 2011). We have also recently demonstrated that in PC12 cells, MCP induced apoptosis and oxidative stress are regulated by specific xenobiotic metabolizing enzymes, CYPs (Singh *et al*., 2012) .Thus we identified possible cellular and molecular mechanisms for MCP induced apoptosis in neuronal cells. Other in vitro as well as in vivo studies have also suggested the apoptotic neural cell death caused by MCP treatment regulated via different signaling pathways (Kashyap *et al*., 2013; Singh *et al*., 2012 and Kazi *et al*., 2012).

As MCP has been shown to induce extensive oxidative stress in neural cells, we suggested that antioxidants can provide protection in the case of MCP toxicity. Thus in the present study we have shown the protective effects of RV in PC12 cells treated by
MCP. Several studies have shown the protection ability of RV from toxic compounds. Recently Huang et al (Huang, et al., 2011) showed neurological protection effects of RV in rats from Aβ peptide-induced neurotoxicity. In this study it was shown that RV suppresses the nitric oxide synthase (iNOS) expression that further reduces lipid peroxidation and apoptotic cell death. Pretreatment of RV have shown to save PC12 cells from 4-hydroxynonenal induce cell damage (Siddiqui et al 2010). Thus RV has immense potential to work as neuro-protectant against various xenobiotics and environmental stress.