Increased agricultural and industrial activities in recent years have tremendously enhanced a variety of chemicals in the environment. Besides, emissions from automobile exhaust and use of chemicals in the public health programme have also significantly contributed to add new entities. As most of these chemicals are neurotoxic, brain is a soft target to their deleterious effects. Among the metals, arsenic is widely distributed due to its natural existence and anthropogenic activities. Since arsenic is present as a contaminant in ground water in certain regions, humans are readily encountered with it and thus risk to develop neurological abnormalities is enhanced in exposed individuals. Monocrotophos, an organophosphate pesticide has extensive usage in agriculture and veterinary practices. Exposure to monocrotophos has been associated with neurobehavioral abnormalities including motor and cognitive deficits in humans. In view of increasing risk of human exposure to arsenic and monocrotophos and their associated health effects, there is serious concern to develop suitable neuroprotective and therapeutic measures. Pharmacological agents and herbal extracts have been used to protect toxic effects of environmental neurotoxicants. Curcumin, an active ingredient of turmeric is known to possess multiple pharmacological properties. Experimental studies and clinical trials have been carried out to assess its neuroprotective effects in neurological and psychiatric disorders. Another herb, *Bacopa monnieri* used in
the Ayurvedic system of Indian medicine for centuries as a brain tonic has been used to improve memory and cognitive functions. Besides, it has also been found to be effective in the treatment of epilepsy and other neurological disorders. In the present dissertation, attempts have been made by the candidate to review the mechanism of neurobehavioral toxicity of arsenic and monocrotophos and neuroprotective efficacy of curcumin and *Bacopa monnieri* briefly.

1.1 Arsenic

Arsenic, a heavy metal is widely distributed in the environment as a contaminant due to its natural existence and anthropogenic sources (Rodriguez et al., 2003; Banu et al., 2009; Das et al., 2010). Although arsenic exists in three states (elemental -0, trivalent - As$^\text{III}$ and pentavalent – As$^\text{V}$), it has been found to exist both in the inorganic and organic forms in the environment (Rodriguez et al., 2003). The important trivalent forms of inorganic arsenic are arsenic trioxide, sodium arsenite and arsenic trichloride while pentavalent forms are arsenic pentoxide and arsenates (lead and calcium arsenates). Common organic arsenic compounds are arsanilic acid, methylarsonic acid, dimethylarsinic acid (cacodylic acid) and arsenobetaine. The organic forms of arsenic are usually less harmful than the inorganic forms. Of the three forms of arsenic, the trivalent form of arsenic is considered to be more toxic due to its ability to bind with the sulphydryl group of proteins and disrupting the antioxidant defense (Aposhian and Aposhian, 1989; Shila et al., 2005a; Yu et al., 2006).

Arsenic compounds are used in the manufacture of insecticides, fungicides, weedicides and antifouling paints (Jacks and Bhattacharya, 1998; Bhattacharya et al., 2002). Other potential uses of arsenic compounds are in the production of alloys, pigments and certain arsenic compounds (Rodriguez et al., 2001; Alam et al., 2002). In the United States, around 74% of arsenic was found present in products used for wood preservation (ATSDR, 1991). It is also used in the pharmaceutical and glass industries and in the manufacture of sheep-dips, leather preservatives and poisonous baits (Chaineau et al., 1990; Yih et al., 1997; Chiou et al., 1997; Rodriguez et al., 2001; Alam et al., 2002). Gallium arsenide and indium arsenide are used in the production of certain
semiconductor devices such as field-effect transistors and microwave integrated circuits and in optoelectronics. In some countries, arsanilic acid and its derivatives are added in the feed of cattle and poultry at a concentration 25 - 45 mg/kg as growth-stimulating agent (USEPA, 1984). The physicochemical properties of arsenic are briefly presented in Table – 1.

Table – 1. Physicochemical properties of arsenic

<table>
<thead>
<tr>
<th>Name</th>
<th>Arsenic</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Common allotropic forms</strong></td>
<td>Yellow, black and grey</td>
</tr>
<tr>
<td><strong>Oxidation states</strong></td>
<td>0, +3, +5</td>
</tr>
<tr>
<td><strong>Element category</strong></td>
<td>Metalloid</td>
</tr>
<tr>
<td><strong>Phase</strong></td>
<td>Solid</td>
</tr>
<tr>
<td><strong>Symbol / Atomic number</strong></td>
<td>As / 33</td>
</tr>
<tr>
<td><strong>Atomic mass</strong></td>
<td>74.9216 g.mol⁻¹</td>
</tr>
<tr>
<td><strong>Electronegativity</strong></td>
<td>2.0</td>
</tr>
<tr>
<td><strong>Density</strong></td>
<td>5.7 g.cm⁻³ at 14°C</td>
</tr>
<tr>
<td><strong>Melting point</strong></td>
<td>814 °C (36 atm)</td>
</tr>
<tr>
<td><strong>Boiling point</strong></td>
<td>615 °C (sublimation)</td>
</tr>
<tr>
<td><strong>Sublimation point</strong></td>
<td>887 K, 1137 °F, 615 °C</td>
</tr>
<tr>
<td><strong>Vanderwaals radius</strong></td>
<td>0.139 nm</td>
</tr>
<tr>
<td><strong>Ionic radius</strong></td>
<td>0.222 nm (-2) 0.047 nm (+5) 0.058 (+3)</td>
</tr>
<tr>
<td><strong>Isotopes</strong></td>
<td>8</td>
</tr>
<tr>
<td><strong>Electronic shell</strong></td>
<td>[As] 3d¹⁰ 4s² 4p³</td>
</tr>
<tr>
<td><strong>Energy of first ionisation</strong></td>
<td>947 kJ.mol⁻¹</td>
</tr>
<tr>
<td><strong>Energy of second ionisation</strong></td>
<td>1798 kJ.mol⁻¹</td>
</tr>
<tr>
<td><strong>Energy of third ionisation</strong></td>
<td>2736 kJ.mol⁻¹</td>
</tr>
<tr>
<td><strong>Standard potential</strong></td>
<td>- 0.3 V (As⁵⁺/ As⁻)</td>
</tr>
</tbody>
</table>

(Source: IPCS, 1992)
The principal natural source of arsenic in the environment is volcanic activity with minor contributions by exudates from vegetation and wind blown dusts. Man made emissions to air arise from the smelting of metals, combustion of fuels especially low-grade brown coal and use of pesticides (Goyer and Amdur, 1991; Polissar et al., 1990; Klaassen, 2001). Inorganic arsenic occurs naturally in the soil and in certain kind of rocks especially in minerals and ores that contain copper or lead. On heating of ores during smelting, most of the arsenic goes up the stack and enters into the air as a fine dust. Arsenicosis, skin lesions, cardiovascular diseases, reproductive problems, psychological, neurological and mental abnormalities are quite common in humans following arsenic exposure (Ratnaike et al., 2003; Duker et al., 2005; Khan et al., 2006; Kapaj et al., 2006; Brinkel et al., 2009). In view of increasing health problems associated with arsenic through drinking water, the World Health Organization (WHO) lowered the limit of arsenic in ground water from 50 µg/l to 10 µg/l (WHO, 2001). Although adverse health effects of arsenic involving various body organs have been reported, brain is an easy target and quite vulnerable towards arsenic induced neurotoxicity. The present review is focused on the neurobehavioral toxicity of arsenic in humans and experimental animals.

1.1.1 Exposure, Absorption and Distribution
High levels of arsenic in ground water in certain regions of Asian countries (India, China, Bangladesh, Nepal, Taiwan, Myanmar and Thailand) and many other regions of the globe (South America, Mexico, Argentina and Chile), (Figure - 1) are cause of concern due to associated health problems in humans (Kapaj et al., 2006; Mukherjee et al., 2006; Brinkel et al., 2009). Less than 10 µg/l of arsenic has generally been found in drinking water sources (WHO, 2011). High levels of arsenic have been reported in carbonate spring waters in New Zealand, Romania and the United States (0.4–1.3 mg/l), artesian wells in Taiwan and China (up to 1.8 mg/l) and groundwater in Cordoba, Argentina (up to 3.4 mg/l) and India (up to 3.2 mg/l). Exposure of general population to arsenic has also been reported through folk medicines and by consuming contaminated sea food (Foa et al., 1984; Francesconi and Edmonds, 1987; WHO, 1992; ATSDR, 2005; Vahidnia et al., 2007).
Figure – 1. High levels of arsenic in ground water in certain regions of Asian countries and many other regions of the globe
Further, consumption of arsenic contaminated food is quite common in regions having high levels of arsenic in ground water and a potential source of arsenic exposure (Del Razo et al., 2002; Rahman et al., 2003; Chakraborti et al., 2004; Huq and Naidu, 2005). Human exposure to arsenic could occur through air, soil and in occupational settings due to its extensive uses (WHO, 2000; Pacyna and Pacyna 2001; Ramanathan et al., 2003). Exposure to arsenic could occur through inhalation, ingestion and absorption into the blood stream (Figure – 2). Arsenic exposure through air is usually a minor exposure route for the general population. Occupational exposure to arsenic occurs primarily among workers involved in copper smelting (Jarup et al., 1989), burning arsenic-rich coal in power plants (Ranft et al., 2003) and using pesticides containing arsenic (Ishinishi et al., 1986). The soil can be heavily contaminated (more than 90 mg/kg) with arsenic in close vicinity of copper smelting units (Wong et al., 1992). The median concentration of arsenic in soil and dust has been found 502 and 857 mg/kg near smelting unit in San Luis Potosi, Mexico. The median concentration of arsenic in the urine of children living nearby was 196 µg/g of creatinine (range 69–594 µg/g of creatinine). Exposure to arsenic through inhalation may also occur during production of gallium arsenide in the microelectronics industry, demolition of oil-fired boilers and metal ore mining (Taylor et al., 1989; Fairfax et al., 1993; Sheehy and Jones, 1993).

Arsenic is rapidly absorbed and distributed in the body and stored in the vital organs such as liver, kidney, heart and lungs (Klaassen, 1996; ATSDR 2000; Rodriguez et al., 2003). In muscles and neural tissues, it is present in low amount (Klaassen, 1996) while its quantity is very high in skin, hair and nails as compared to other body tissues except blood (Liebscher and Smith, 1968; Lindgren et al., 1982). Arsenic is quickly absorbed in the blood and transported into the body organs. Arsenic is cleared relatively rapidly from the blood. Low levels (normally less than 0.25 mg/kg) of arsenic in the form of organo-arsenicals (e.g. arsenobetaine) have been found to be present in the sea food. Organic arsenic compounds in seafood are also readily absorbed (75–85%). As the
Figure – 2. Arsenic - Human exposure and associated health risks
seafood have low biological reactivity and are rapidly excreted in urine, these are
known to be non toxic. Wine made from grapes contaminated with arsenic
pesticides may contain appreciable levels of arsenic (up to 0.5 mg/l) in the
trivalent inorganic form (Hughes et al., 1994). Human and animal data indicate
that over 60 - 90% of the ingested dose of dissolved inorganic trivalent or
pentavalent arsenic is absorbed from the gastrointestinal tract (Hall, 2002).
Airborne arsenic is usually in the form of arsenic trioxide. The amount of arsenic
excreted in urine was found to be 40–60% of the estimated inhaled dose in
workers exposed to arsenic trioxide dusts in smelting units (ATSDR, 1991).
Arsenic compounds are well absorbed within 24 hours and redistributed to the
liver, lungs, intestinal wall and spleen where they bind to the sulfhydryl groups of
tissue proteins. Factors affecting the extent of absorption of arsenic from the
lungs include the chemical form, particle size and solubility (Pinto et al., 1953;
Natusch et al., 1974). Arsenic has the capacity to replace phosphorus in the
bone and thus it may remain for years (Arena and Drew, 1986; Ellenhorn and
Barceloux, 1988). Effects of chronic arsenic poisoning therefore could be seen
even years after exposure.

1.1.2 Metabolism and Excretion
Methylation has been considered to be a major mechanism of arsenic
biotransformation and detoxification. Both arsenites and arsenates undergo
enzymatic methylation in cells of various organisms to produce
monomethylarsonic acid and dimethylarsenic acid (Figure – 3). These reactions
are catalyzed by methyltransferase that use S – adenosyl – methionine as
cofactor and are important because methylated arsenicals are more rapidly
excreted in urine than inorganic arsenicals (Zhao et al., 1997). During the
process of methylation, inorganic arsenic is reduced from AsV to AsIII in
mammals. The process of oxidation and reduction takes place in the plasma
while the process of methylation takes place in the liver (Nemeti and Gregus,
2002; Rossman, 2003). The metabolism of arsenic takes place in two important
processes by catalytic enzyme arsenic (3+) methyltransferase (As3MT) in which
arsenite is converted into monomethyl arsonate (MMA\textsuperscript{V}) followed by monomethyl arsonous acid (MMA\textsuperscript{III}), which is further converted into dimethyl arsenic acid (DMA\textsuperscript{V}) and finally to dimethyl arsinous acid (DMA\textsuperscript{III}) (Thomas et al., 2007). GSH conjugation with Asi\textsuperscript{III} through As3MT is a multi step methylation process involving formation of arsenite triglutathione followed by monomethylarsionic diglutathione [MMA(SG)\textsubscript{2}] and finally dimethylarsinic glutathione [DMA(SG)]. MMA(SG)\textsubscript{2} may undergo spontaneous degradation to form MMA\textsuperscript{III} followed by DMA\textsuperscript{III}. DMA(SG) may undergo degradation to form DMA\textsuperscript{III} and DMA\textsuperscript{V}, a major metabolite found in urine. Therefore, reduction may occur enzymatically with As3MT or glutathione S-transferase Omega and nonenzymatically, in the presence of endogenous reductants such as glutathione and thioredoxin under favorable physiological conditions (Kenyan et al., 2008).

**Figure – 3. Metabolism of arsenic**

Earlier, arsenic methylation was believed to be a simple arsenic-detoxification reaction. Increasing evidences however, suggest it to be a more complex metabolic process, important in modulating the arsenic toxicity. The toxicological profiles of arsenic metabolites vary *in vivo*. MMA\textsuperscript{III} and DMA\textsuperscript{III} have consistently been reported to be more toxic than any other metabolites of inorganic arsenic, possibly because of their higher cellular uptake (Dopp et al., 2010). Thus, GSH depletion due to utilization during arsenic metabolism may be crucial in arsenic
induced oxidative stress. It has been suggested that when uptake exceeds a certain value, the methylation mechanism becomes saturated and its efficiency declines as exposure increases. However, analysis of inorganic arsenic, MMA and DMA in the urine of different groups of individuals (nonexposed, occupationally exposed and volunteers) has not supported the methylation threshold hypothesis. After oral intake of radiolabelled pentavalent arsenic in humans, 66% was excreted with a half-time of 2.1 days, 30% with a half-time of 9.5 days and 3.7% with a half-time of 38 days (Pomory et al., 1980). Numerous studies have indicated inter-individual difference in arsenic toxicity which depends on the methylation and metabolic patterns (Steinmaus et al., 2006; Tseng et al., 2007; Tseng, 2009). Recent studies have shown that methylated arsenites are more toxic than inorganic arsenic (Dopp et al., 2010). Extensive studies with different cultured cells have been conducted during the past few years to elucidate the molecular basis for the manifestations of arsenic toxicity observed in humans (Li and Chou, 1992; Lee and Ho, 1995; Barchowsky et al., 1996; Jingbo et al., 2002; Kumagai and Sumi, 2007).

1.1.3 Toxic Effects
Arsenic exposure has been associated with health problems including hypertension (Chen et al., 1995), cardiovascular diseases, developmental abnormalities, diabetes, hearing loss, fibrosis of the liver and lung, hematological disorders, reproductive disturbances, blackfoot disease and cancer (Abernathy et al., 1999; Tchounwou et al., 1999; Sordo et al., 2001; Ratnaike et al., 2003; Duker et al., 2005, Khan et al., 2006). Exposure to inorganic arsenic may cause nausea, vomiting, diarrhea, shock, coma, convulsions, inflammation, ulceration of mucous membranes, skin diseases, sensory disturbances, visual disturbances and blindness (Ahsan et al., 2000; Smith et al., 2000; Kapaj et al., 2006; Spallholz et al., 2004; Khan et al., 2007). A number of studies have clearly demonstrated that arsenic may affect the functioning of the nervous system leading to neurobehavioral abnormalities in exposed individuals (Kapaj et al., 2006; Vahidnia et al., 2007; Brinkel et al., 2009).
1.1.3.1 Neurobehavioral toxicity in humans: Arsenic and its inorganic compounds have long been known to be neurotoxic (Vahidnia et al., 2007). Exposure to arsenic in humans has been reported to affect both the central and peripheral nervous system (Vahidnia et al., 2007). Peripheral neuropathy following arsenic exposure is well documented (Chhuttani and Chopra, 1979; Schoolmeester and White, 1980; Brouwer et al., 1992; Heaven et al., 1994; Kapaj et al., 2006; Vahidnia et al., 2007). A decrease in peripheral nerve conduction velocity has been observed following chronic exposure to arsenic dust (Blom et al., 1985; Kishi et al., 2001; Vahidnia et al., 2007; Sinczuk-Walczak et al., 2010). Alterations in motor behavior, impaired learning and concentration are the common CNS manifestations of arsenic exposure in humans (Bolla-Wilson et al., 1987). Epidemiological studies suggest neurological and cognitive deficits in humans due to arsenic exposure (Wasserman et al., 2004; Von Ehrenstein et al., 2007; Rosado et al., 2007). An association between arsenic ingestion and increased risk of microvascular diseases of brain has been reported (Chiou et al., 2005). Gharibzadeh and Hoseini (2008) suggested that arsenic exposure may be a risk factor for Alzheimer’s disease by inducing apoptosis in cortical neurons. Chronic exposure to arsenic leads to various neurological problems including cognition, mental retardation, intellectual function, verbal and speech impairments and long-term memory (Calderon et al., 2001; Tsai et al., 2003; Wasserman et al., 2004; Von Ehrenstein et al., 2007; Rosado et al., 2007). Urinary arsenic levels have been inversely associated with the IQ and cognitive performance in arsenic exposed children (Wasserman et al., 2004; Wang et al., 2007). Studies also revealed that exposure to arsenic may cause serious implications such as social instability, social discrimination and family related problems in humans (Ratnaike et al., 2003; Kapaj et al., 2006; Brinkel et al., 2009). Numerous studies have shown that long term exposure to arsenic may affect memory, attention, verbal, IQ and ability to understand speech (Kapaj et al., 2006; Brinkel et al., 2009). Rosado et al., (2007) found that arsenic affected the cognitive development in children living near the areas contaminated with arsenic and lead. It was found that arsenic exposure combined with lead
may have synergistic effect (Rosado et al., 2007). Long term exposure to arsenic was associated with neurobehavioral dysfunctions in adolescent (Tsai et al., 2003).

1.1.3.2 Neurobehavioral toxicity in experimental models: Arsenic easily crosses the blood brain barrier and accumulates in the brain regions and exerts its neurotoxic effects (Rodriguez et al., 2001; Jin et al., 2006; Rosado et al., 2007). A number of experimental studies have been carried to investigate the morphological, physiological, pharmacological and neurochemical effects following arsenic exposure. Morphological structure of cerebellum acquired from electron microscope showed delayed maturation of purkinje cells and their defective migration in rats exposed to sodium arsenite during rapid brain growth period from postnatal day 4 to 10 (Dhar et al., 2007). Involvement of multiple mechanisms in arsenic induced neurobehavioral toxicity has been suggested (Figure – 4). Alterations in dopaminergic, cholinergic, serotonergic and glutamatergic systems have been reported in rats and mice exposed to arsenic (Valkonen et al., 1983; Itoh et al, 1990; Nagaraja and Desiraju, 1993, 1994; Tripathi et al 1997, Kannan et al., 2001). Arsenic has been found to alter levels of biogenic amines in the brain and affect the behavioral and neurochemical functions in developing and adult rats (Itoh et al., 1990; Nagaraja and Desiraju, 1993; Tripathi et. al., 1997; Rodriguez et al., 1998; Kannan et al., 2001). Intragastric intubation of arsenic as sodium arsenite (10 or 20 mg/kg body weight) in rats for 2 or 3 weeks increased DA levels in mid brain and cortex and 5-hydroxyindoleacetic acid (5-HIAA) levels in the mid brain (Rodriguez et al., 2001). Increased plasma adrenocorticotropic hormone (ACTH) levels were found to be associated with increased NE levels in the medulla pons at a low dose but not in hypothalamus. It was suggested that alterations in brain monoamines are not dependent with alterations in plasma ACTH (Delgado et al., 2000). Mejia et al., (1997) found decreased norepinephrine levels in hippocampus and increased 5-HT levels in mid brain and frontal cortex following co-exposure to lead and arsenic in mice. Experimental studies on rodents have suggested that exposure
to arsenic affects the learning and memory (Nagaraja and Desiraju, 1994; Rodriguez et al., 1998, 2003; Wang et al., 2009; Luo et al., 2009). Alteration in motor behavior has also been reported in arsenic exposed rats (Rodriguez et al., 2001, 2002, 2003). In another study, dual response on motor activity following arsenic exposure was studied (Itoh et al., 1990). Xi et al., (2009) found that transplacental and early life exposure to arsenic could affect learning and memory function and neuromotor reflex in rat offspring. Luo et al., (2009) reported that arsenic affects the spatial learning associated with ultrastructural changes and decreased NR2A expression in rat hippocampus. Exposure to sodium arsenate or sodium arsenite through drinking water in rats decreased the activity of AChE in brain (Volkonen et al., 1983; Nagaraja and Desiraju, 1994; Tripathi et al., 1997; Kannan et al., 2001) and also inhibited the synthesis and liberation of acetylcholine in brain slices (Kobayashi et al., 1987). Patlolla and Tchounwou, (2005) found that arsenic trioxide significantly decreased the activity of serum AChE in a dose-dependent manner in rats. Decreased AChE activity in brain and plasma of rats following their exposure to sodium arsenite has also been observed (Yousef et al., 2008). Interestingly, decrease in the activity of AChE following exposure to arsenic and gallium arsenite was correlated with impairment in the learning and memory in rats (Flora et al., 2009). Consistent with this decreased activity of nitric oxide synthase and AChE following arsenic exposure was associated with impaired learning and memory functions (Kopf et al., 2001).

![CNS abnormalities](image)

**Figure – 4. Arsenic induced neurobehavioral toxicity**
Enhanced oxidative stress in brain has been suggested to be a potential mechanism in arsenic neurotoxicity (Flora and Gupta, 2007; Sinha et al., 2008; Das et al., 2010; Flora, 2011). Arsenic enhances generation of free radicals including hydroxyl radicals, superoxide anions, dimethyl arsenic peroxy radical, dimethyl arsenic radical, nitric oxide and impairs the antioxidant system in the brain and other biological tissues and therefore increases the oxidative stress (Barchowsky et al., 1996; Wang et al., 1996; Gurr et al., 1998; Lyn et al., 2000). Besides, arsenic has high affinity to GSH and thus enhances vulnerability towards oxidative stress by causing an imbalance between pro-oxidant and antioxidant homeostasis (Aposhian and Aposhian, 1989; Wang et al., 1996; Chen et al., 1998; Shila et al., 2005a, b, c). Chronic exposure to arsenic in rats was found to decrease the production of brain nitric oxide associated with an increase in the production of reactive oxygen species (Zarazua et al., 2006). Involvement of neuronal nitric oxide synthase (nNOS) and nitric oxide levels has also been shown in arsenic neurotoxicity (Chattopadhyay et al., 2002; Zarazua et al., 2006; Flora et al., 2009; Rios et al., 2009).

In view of increasing risk of human exposure to arsenic, there is a lot of concern among the health scientists if its neurotoxicity could be prevented and thus to develop strategies for the management of arsenic neurotoxicity. Although experimental studies have been carried out to investigate the neuroprotective efficacy of pharmacological agents and herbal extracts in arsenic induced neurotoxicity (Shila et al., 2005a, b, c; Flora and Gupta, 2007; Sinha et al., 2008; Banu et al., 2009; Flora, 2011), consistent findings with promising results have not been observed. Therefore, there is a search of agent which could protect arsenic induced neurotoxicity.

Pesticides in general are a group of chemicals used to kill pests and have extensive applications in agriculture, public health programmes, homes and gardens. The consumption of pesticides has increased many folds after the Green Revolution in India and many other parts of the globe. Synthetic pesticides are popular among farmers because of their easy availability, simple application,
increased efficacy and above all high economic returns. Pesticides depending on their uses are broadly classified as insecticides, rodenticides, herbicides, fungicides and fumigants. Further, based on the chemical type and mechanism of action, insecticides are classified as organophosphates, organochlorines, carbamates and pyrethroids. Use pattern of pesticides however, differs from region to region and country to country. Among different classes of insecticides, organophosphates are distributed in the environment due to their extensive usage in agriculture, homes, public health programme to control pests and vectors and veterinary practices to control ectoparasites. Incidences of human exposure to organophosphates are therefore frequent both under occupational and non-occupational conditions due to their indiscriminate and excessive use (Simcox et al., 1999; McCauley et al., 2001; Curl et al., 2002; Fenske et al., 2002). Exposure to organophosphates has also been linked to be a risk factor in the etiology of various neurodegenerative diseases including Parkinson’s and Alzheimer’s disease (Firestone et al., 2005; Wang et al., 2011; Das et al., 2011; Wingo et al., 2012). High levels of residues of organophosphates detected in the dietary products and biological tissues of exposed individuals are again a matter of concern due to associated health effects (CSE, 2005; Bjorling-Poulsen et al., 2008; Moreno-Banda et al., 2009; WHO, 2009; Srivastava et al., 2011).

1.2 Monocrotophos

Among the organophosphates, production and consumption of monocrotophos has been very high in India and estimated to be 5118 metric tones during 2007 – 2008 (Ministry of agriculture, 2007). Like other organophosphates, monocrotophos (Dimethyl (E)-1-methyl-2-(methylcarbamoyl) vinyl phosphate) consists of the vinyl phosphate group (Figure – 5). Monocrotophos is in high demand in India and many developing countries and is extensively used to control pests specially chewing, sucking and boring insects on a variety of crops including cotton, rice and sugarcane (IPCS, 1993). As monocrotophos is highly hazardous and has adverse effects on human health, the World Health Organization has classified the monocrotophos in the category 1b of chemicals. The primary target of monocrotophos like other organophosphates is
acetylcholinesterase, involved in the metabolism of acetylcholine, an important neurotransmitter in the central and peripheral nervous system. Inhibition of acetylcholinesterase causes accumulation of acetylcholine leading to over stimulation of both muscarinic and nicotinic cholinergic receptors in brain. The phenomenon, known as cholinergic syndrome or Intermediate syndrome is associated with excessive sweating, salivation, bronchosecretion and bronchoconstriction, miosis, diarrhoea, tremors, muscular twitching and other CNS effects.

![Figure 5. Structure of monocrotophos](image)

Monocrotophos in pure state is crystalline, colourless and hygroscopic. In commercial form, monocrotophos is reddish-brown to dark brown clear viscous liquid and has mild ester odour. It is unstable in low molecular weight alcohols and glycols but stable in ketones and higher molecular weight alcohols and glycols (Gallo and Lawryk, 1991). Monocrotophos is relatively stable at acidic and neutral pH values and hydrolysed in alkaline solutions (Roberts and Hutson, 1999). It remains stable while stored in glass and polyethylene containers (Gallo and Lawryk, 1991). The physicochemical properties of monocrotophos are summarized in Table – 2.

1.2.1 Exposure, Absorption and Distribution

Exposure to monocrotophos in humans has been reported both under occupational and non-occupational conditions (WHO, 2009). Monocrotophos has low persistence in the environment as it is biodegradable in field conditions (USEPA, 1985). However, studies have shown that monocrotophos may persist for much longer time in soil as its degradation decreases with depth and at low temperatures (Tariq et al., 2006). Although organophosphates do not persist for long in the environment, monocrotophos and its metabolites have been detected
in milk, muscles and cattle meat (WHO, 2009). Although use of monocrotophos on dietary products has been restricted, high levels of monocrotophos residues detected in food products including vegetables, cereals and fruits exhibit its persistence in the environment and thus enhance the risk of human exposure and associated health effects (WHO, 2009).

**Table – 2. Physicochemical properties of monocrotophos**

<table>
<thead>
<tr>
<th>Name</th>
<th>Monocrotophos</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Synonyms</strong></td>
<td>Phosphoric acid, dimethyl [1-methyl-3-(methylamino)-3-oxo-1-propenyl] ester; (E)-phosphoric acid dimethyl ester, ester with 3-hydroxy-N-methylcrotonamide; 3-(dimethoxyphosphinyloxy) -N-methyl-cis-crotonamide; dimethyl 2-methylcarbamoyl- 1-methylvinyl phosphate</td>
</tr>
<tr>
<td><strong>Trade name</strong></td>
<td>Azodrin, Bilobran, Crisodrin, Monocil, Monocron, Monopaz</td>
</tr>
<tr>
<td><strong>Molecular formula / molecular weight</strong></td>
<td>C$<em>7$H$</em>{14}$NO$_5$P / 223.2</td>
</tr>
<tr>
<td><strong>Common names</strong></td>
<td>Monocrotophos, (Approved by BSI, E-ISO, F-ISO, JMAF)</td>
</tr>
<tr>
<td><strong>IUPAC name</strong></td>
<td>Dimethyl(E)1-methyl-2-(methylcarbamoyl) vinyl phosphate</td>
</tr>
<tr>
<td><strong>CAS chemical name</strong></td>
<td>(E)-dimethyl-1-methyl-3-(methylamino)-3-oxo-1-propenyl phosphate (9Cl)</td>
</tr>
<tr>
<td><strong>CAS registry number</strong></td>
<td>6923-22-4</td>
</tr>
<tr>
<td><strong>Physical state</strong></td>
<td>Reddish-brown mixture of solid and liquid (at 25-30°C)</td>
</tr>
<tr>
<td><strong>Odour</strong></td>
<td>Mild ester</td>
</tr>
<tr>
<td><strong>Melting point</strong></td>
<td>25-30°C (technical), 54-55°C (pure)</td>
</tr>
<tr>
<td><strong>Boiling point</strong></td>
<td>125°C (at 0.0005 mmHg)</td>
</tr>
<tr>
<td><strong>Vapour pressure</strong></td>
<td>0.29 mPa (20°C)</td>
</tr>
<tr>
<td><strong>Solubility</strong></td>
<td>Soluble in water, acetone and alcohol, very slightly soluble in mineral oils</td>
</tr>
<tr>
<td><strong>Half life in aqueous</strong></td>
<td>at pH 5.0 - 96 days; at pH 7.0 - 66 days; at pH 9.0 - 17 solution days</td>
</tr>
</tbody>
</table>

(Source: IPCS, 1993)
It has been found that monocrotophos is readily absorbed through the skin. However, monocrotophos can be absorbed by ingestion and inhalation also and is toxic by all routes (Venkatesh et al., 2006). Absorption of monocrotophos into the body has been studied by measuring the concentration of its principle metabolite dimethyl phosphate (DMP) in the urine of human sprayers largely exposed through skin (Schulze-Rosario and Loosli, 1994). Urinary levels of monocrotophos ranged from 0.02 to 1.9 mg monocrotophos / 24 hours (Kummer and van Sittert, 1986) and from 0.6 to 19 mg during 3 days exposure (Van Sittert and Dumas, 1990). The average half life of DMP elimination from the body was found to be 18 hours (Kummer and van Sittert, 1986). After entering into the body system, monocrotophos could differentially be distributed in all tissues through the blood (at a concentration of 12 μg/g). The concentration of monocrotophos however was found to differ in body organs - 13 μg/g in the lungs and brain, 11 μg/g in the kidneys, 1.8 μg/g in the liver and a total of 52 mg in the stomach (Gelbke and Schlicht, 1978).

1.2.2 Metabolism and Excretion

Biotransformation of monocrotophos has been studied both in animals and humans (Beynon et al., 1973; Lee, 1987; Mucke, 1994; Skripsky and Loosli, 1994). Studies carried out on rats suggest that the metabolism of monocrotophos is primarily a detoxification process involving the ester cleavage of monocrotophos leading to produce N-methyl acetoacetamide through the hydrolysis of P-O vinyl linkage. It is further degraded to produce 3-hydroxy-Nmethyl butyramide. Various metabolites of monocrotophos formed during the biotransformation are mentioned in Figure – 6. Lee, (1987) studied the metabolism of monocrotophos in Wistar rats. Common signs of organophosphate poisoning (trembling, twitching, salivation, chromoda-chyorrhoea and piloerection) were evident thirty minutes after single dose exposure of $^{14}$C-monocrotophos (2mg/kg b.w). These effects could be observed up to 3 h after monocrotophos exposure (Lee, 1987). About 82% of the administered
radioactive dose of $^{14}C$-monocrotophos was detected in the urine after 96 h. Interestingly, 76% of the administered radioactive dose was detected in the urine after 12 h in these studies. In the tissues, very low level of radioactivity was detected after 96 h. After 7 days following monocrotophos exposure, the levels of radioactivity were determined in urine, faeces, expired $CO_2$ and also in specific tissues. The distribution of radioactivity was independent of sex (Lee, 1987). The principal urinary metabolites - N-methyl acetoacetamide and 3-hydroxy-N-methyl butyramide accounted for approximately 13-17% and 8% of the radioactive dose respectively (Menzer and Casida, 1965; Lee, 1987).

![Figure 6. Biotransformation of monocrotophos](image_url)

Figure – 6. Biotransformation of monocrotophos

Another metabolic route of monocrotophos has also been suggested in which $N$-demethylation of monocrotophos is converted to the $N$-hydroxymethyl derivative followed by $O$-dealkylation to form des-$O$-methylmonocrotophos (Menzer and Casida, 1965, Roberts et al., 1999). As $N$-demethylated derivatives are potential inhibitors of acetylcholinesterase, they have been suggested to play important role in toxicological studies (Roberts et al., 1999). Bull and Lindquist, (1966) in another study assessed excretion of urinary metabolites after administration of $^{32}P$-labelled monocrotophos (5 mg/kg bw, i.p) in Wistar rats. Urine and faecal sample were collected every 2 h for the first 12 h and then at intervals until 48 h after treatment. It was found that after the first 6 h of treatment, about 45% of the administered dose of monocrotophos rapidly excreted in the urine while 61% of
radioactivity was excreted after 48 h in the urine and 6% excreted in the faeces. Over a period of 48 h, DMP - 40%, monocrotophos - 28%, hydroxymethyl monocrotophos - 19%, O-demethyl monocrotophos - 10% and phosphoric acid - 3% were the metabolites excreted in the urine (Bull and Lindquist, 1966).

1.2.3 Toxic Effects
1.2.3.1 Toxic effects in humans: Human exposure to monocrotophos is quite imminent due to its extensive uses and presence as a contaminant in the dietary products (WHO, 2009). Because of increased incidences of human toxicity, use of monocrotophos is banned or restricted in many countries. Cases of acute monocrotophos poisoning and deaths have been reported from Sri Lanka, Indonesia, Egypt, Brazil and many other places (WHO, 2009). These incidences have been found either accidental or not taking proper protective measures. A high rate of mortality due to monocrotophos exposure in humans has also been reported from different regions and States of India (WHO, 2009). Like other organophosphates, dermal exposure to monocrotophos may cause sweating and involuntary muscle contractions. It may cause pain, tears, pupil constriction and blurred vision if comes in contact with eyes. The systemic effects following exposure to monocrotophos include nausea, vomiting, diarrhoea, headache, dizziness, salivation etc. (Health council of the Netherland, 2003; WHO, 2009). Such effects may be seen immediately after exposure or delayed for 12 hours. Chronic exposure to monocrotophos may affect the central nervous system and cause weakness, fatigue, involuntary muscle contractions, slurred speech, twitching (Chambers and Oppenheimer, 2004; Costa et al., 2006; Venkatesh et al., 2009). Severe poisoning may cause paralysis of the body extremities, unconsciousness, convulsions and coma (Chambers and Oppenheimer, 2004; Costa et al., 2008; Venkatesh et al., 2009). Besides, psychosis irregular heart beat, respiratory failure and cardiac arrest are other symptoms of monocrotophos exposure like organophosphate poisoning (Costa et al., 2008). In a study on farmers in Egypt using organophosphates including monocrotophos, 50% of the workers exhibited neurological symptoms including loss of reflexes (Amr, 1995;
Pesticide News, 1996). High levels of pesticides including monocrotophos in blood were detected in a study on randomly selected individuals residing in four different villages of Punjab (India) surrounded by the agricultural fields (Mathur et al., 2005).

1.2.3.2 Toxic effects in experimental models: Numerous experimental studies have been carried out to investigate toxic effects of monocrotophos in vertebrate and non-vertebrate models. Generally, monocrotophos produces systemic and toxic actions in insects and is highly toxic to aquatic invertebrates, birds, bees and mammals, moderately toxic to fish and earthworms and non-toxic to microorganisms and algae (Khanobdee et al., 1999; Wang et al., 2009; Rao et al., 2001, 2005). Acute effect of monocrotophos and its two thiol analogs, 2-butenoic acid-3-(diethoxyphosphinoothionyl) methyl ester (RPR-II) and 2-butenoic acid-3-(diethoxyphosphinoothionyl) ethyl ester (RPR-V) was also studied in fish Oreochromis mossambicus (Rao, 2004). Both the analogs RPR II and RPR-V were found to be 65 fold toxic than monocrotophos, assessed by inhibitory and recovery pattern of acetylcholinesterase activity in brain, gill and muscles 96 hours after exposures (Rao, 2004). Effect of monocrotophos and chlorpyrifos on locomotor behaviour and acetylcholinesterase activity of subterranean termites, Odontotermes obesus was studied by paper contact method (Rao et al., 2005). Chlorpyrifos was found to be 3.22 fold more toxic than monocrotophos as assesses by the LC 50 values (Rao, 2004). Both monocrotophos and chlorpyrifos significantly decreased the locomotor behaviour activity after 24 hours and inhibited the acetylcholinesterase activity in subterranean termites (Rao et al., 2005). Monocrotophos was found to be toxic to Daphnia magna both in acute and chronic studies (Wang et al., 2009). Interestingly, concurrent exposure to bifenthrin and sodium dodecyl benzene sulphate had antagonistic effect on the monocrotophos induced toxicity in these studies (Wang et al., 2009). Studies have also been carried out to investigate the neurotoxic effect of monocrotophos in different organisms (Qadri et al., 1994; Rao, 2001, 2004). Recently, in vitro studies on PC-12 cells revealed that monocrotophos enhanced apoptosis
involving caspase cascade and xenobiotic metabolizing cytochrome P-450s. It was suggested that apoptosis is the principle mechanism in the neurotoxicity of monocrotophos (Kashyap et al., 2010, 2011).

Monocrotophos has been reported to be embryotoxic both in rats and rabbits (Janardhan et al., 1983). Exposure to monocrotophos caused a decrease in the weight of fetuses and new born and also affected the growth of developing animals at the time of weaning. Involvement of multiple mechanisms in the neurotoxicity of monocrotophos has been suggested. Acute exposure to monocrotophos has been reported to cause adverse neurobehavioral effects in mammals (Mandhane and Chopde, 1995). Hypoactivity and impaired motor coordination in rats and mice as assessed by their performance on rotating rod and decreased motor activity have been observed following acute monocrotophos exposure (Mandhane and Chopde, 1995). Potentiation of pentobarbitol induced sedation and haloperidol induced catalepsy has also been reported following exposure to monocrotophos in rats and mice (Mandhane and Chopde, 1995). Based on these findings, it was suggested that monocrotophos is a potent CNS depressant.

Neuromuscular weakness associated with significant changes in myofibril membrane lipid compositions and increased ratio of cholesterol to phospholipids has been reported as a result of monocrotophos poisoning in rats (Venkatesh et al., 2006). Inhibition in the activity of acetylcholinesterase in brain and red blood cells in monocrotophos treated rats was also observed. Activity of acetylcholinesterase in red blood cells exhibited a significant recovery while remained decreased in brain of monocrotophos treated rats one week after exposure (Mikkelsen et al., 2004; Venkatesh et al., 2006). Venkatesh et al., (2009) found that muscle mitochondrial ATP synthase activity associated with decreased reactive cystein group of ATP synthase subunit is included in rats following acute exposure to monocrotophos. No effect on respiration was observed in monocrotophos treated rats in these studies. The decreased mitochondrial ATP synthase activity in monocrotophos treated rats was
prevented by treatment with nitric oxide synthase inhibitors suggesting the role of nitric oxide in the process of muscle weakness (Venkatesh et al., 2009).

Acute exposure to monocrotophos has been reported to cause hyperglycemia and stressogenic effects in rats 2 h after treatment (Joshi and Rajini, 2012). These changes were associated with decreased activity of acetylcholinesterase in brain, adrenal and liver and found to be prevented by acetylcholine antagonists (Joshi and Rajini, 2012). The vulnerability of rat pups as compared to young rats following exposure to mevinphos, monocrotophos, dicrotophos and phosphamidon has recently been reported (Moser, 2011). The difference in age-related neurotoxicity was associated with the metabolic capacity and type of chemical used in this study (Moser, 2011). In a recent study, Masoud et al., (2011) observed that acute exposure to monocrotophos caused organophosphate induced delayed neuropathy (OPIDN) in rats associated with impaired motor activity and learning involving dopaminergic system. Effect of combined exposure to dichlorovos and monocrotophos on biochemical alterations in rat brain and blood has also been studied (Dwivedi et al., 2010). Exposure to monocrotophos and dichlorvos alone decreased the levels of norepinephrine, dopamine and serotonin, activity of monoamine oxidase and acetylcholinesterase and depleted GSH:GSSG ratio in rat brain (Dwivedi et al., 2010). Repeated exposure to monocrotophos developed tolerance in rats (Khanobdee et al., 1999). Effect of monocrotophos on tremors and convulsions was studied by Khanobdee et al., (1999) in detail. Exposure to monocrotophos (3 mg/kg, i.p for 14 days) caused severe tremors and convulsions during the first five days in rats. These changes were decreased to 50%, one week after monocrotophos treatment. No such changes were observed 10 days after monocrotophos treatment in this study (Khanobdee et al., 1999). More recently, post-lactational exposure to monocrotophos was found to impair brain cholinergic and non-cholinergic functions of rats associated with enhanced oxidative stress in the brain (Sankhwar et al., 2011; 2012). These changes were found to persist even after withdrawal of monocrotophos exposure (Sankhwar et al., 2011; 2012).
Although use of monocrotophos is banned in the U.S. and many other countries, injudicious use of monocrotophos and associated neurobehavioral toxicity in humans has aroused a great concern among the health scientists to use protective and preventive approaches to minimize its neurotoxicity. In view of this, pharmacological agents and herbal extract have been used to explore their neuroprotective efficacy against organophosphate induced neurotoxicity. However, not many studies have been carried out on these aspects.

1.3 Curcumin
The potential of curcumin, an ingredient of turmeric *Curcuma longa* (Zingiberaceae) rhizomes, commonly referred as ‘Haldi’ in India is known for its wound healing property and treatment of a variety of inflammatory conditions for centuries (Gujral et al., 1953; Maheshwari et al., 2006; Strimpakos and Sharma, 2008; Aggarwal and Sung, 2009). Turmeric has a long history of its use as a household remedy for the prevention and treatment of skin diseases, stomachic, tonic and blood purifier. More interestingly, use of turmeric as a folk medicine continues even today and is well documented in the traditional system of Indian medicine in the management of other diseases like anorexia, coryza, cough, biliary disorders, diabetic wound, hepatic disorder, rheumatism and sinusitis (Ammon et al., 1992; Eigner and Scholz, 1999; The Wealth of India, 2001). The rhizomes of *Curcuma longa* are oblong, ovate, pyriform, short branched and widely used as powder in curry in many Asian countries. Later, it was introduced as a spice to the western world in the 14\textsuperscript{th} century by the European explorers (Aggarwal et al., 2007). Of various constituents present in turmeric, curcumin is pharmacologically more active while the yield of curcumin ranges from 2 - 5% and depends on the variety. As curcumin has wide range of beneficial medicinal properties, much attention has been given in recent years to use it as a natural and complementary medicine. The chemistry and therapeutic potential of curcumin in the management of neuropsychiatric disorders are briefly reviewed.
1.3.1 Chemistry

The chemistry of turmeric and its constituent has been a subject of intense interest. Turmeric contains both volatile (essential) and nonvolatile oils, protein, fatty oil minerals, carbohydrates, moisture and ash content (Chattopadhyay et al., 2004). The major constituents of turmeric and their approximate percentage are described in Figure – 7. The aromatic properties of turmeric are due to volatile oils and its yellow color is due to the curcuminoids especially curcumin, a polyphenol (Jayaprakasha et al., 2005). Other curcuminoids present in turmeric are demethoxycurcumin and bis-demethoxycurcumin (Figure – 8).

![Turmeric Plant with Rhizome](Image)

(Modified from - Strimpakos and Sharma, 2008)

**Figure – 7. Major constituents of turmeric**

![Curcuminoids and other constituents](Image)

**Figure – 8. Curcuminoids present in turmeric**

Historically curcumin was first isolated by Vogel in 1842 and its structure was characterized by Milobedzka et al., (1910). Later, it was synthesized and confirmed in 1913 (Lampe and Milobedzka, 1913). The chemical structure of
Curcumin is unique in its own consisting of two methoxy and two phenolic groups and three conjugated double bonds (Figure – 9). The phenolic groups present in the curcumin have been found to impart its strong antioxidant and antiinflammatory activity while the ketonic group and double bonds present have metal chelating property (Dinkova-Kostova and Talalay, 1999; Suzuki et al., 2005). The tautomeric forms of curcumin including enolate (alkaline) and bis-keto (acidic and neutral) exist in equilibrium (Figure – 10) under normal physiological conditions (Jovanovic et al., 1999). Curcumin (Molecular weight - 368.38) is less soluble in water and highly soluble in ethanol, dimethylsulfoxide and acetone. The maximum absorbance of curcumin in methanol is 430 nm while it absorbs light at 415 - 420 nm in acetone. It is degraded in the presence of light therefore care is taken while handling the biological samples containing curcumin.

**Figure – 9. Curcumin - Structure and pharmacological spectrum**

**1.3.2 Pharmacology and Molecular Targets**

Eversince the first report of curcumin in the treatment of biliary disorders appeared in The Lancet in 1937, much interest aroused among the scientists to
explore its therapeutic uses and utilize appropriately. Further, treatment with curcumin has been found to correct cystic fibrosis in homozygous DeltaF508 cystic fibrosis transmembrane conductance regulator knock out mice (Egan et al., 2004). A number of preclinical and clinical studies have been carried out since then and found that curcumin has wide pharmacological spectrum and has promising properties including wound healing anti-inflammatory, anti-carcinogenic, anti-mutagenic, anti-ischemic and many others (Shukla et al., 2003, 2008; Duvoix et al., 2005; Maheshwari et al., 2006; Strimpakos and Sharma, 2008; Yousef et al., 2008; Aggarwal and Sung, 2009; Xu et al., 2011; Singla and Dhawan, 2012). The pharmacological effects associated with turmeric and curcuminoids are briefly presented in Figure 9.

Figure 10. Tautomeric forms of curcumin

In view of multiple pharmacological properties, it has been suggested that curcumin may influence a number of biological and molecular targets including transcription factors, growth factors and their receptors, cytokines, enzymes and genes regulating cell proliferation and apoptosis. Upregulation and downregulation of various transcription factors has been reported following treatment with curcumin (Aggarwal et al., 2006; Maheshwari et al., 2006; Strimpakos and Sharma, 2008; Aggarwal and Sung, 2009). These transcription factors are known to modulate cellular functions involving apoptosis, cell mediated response, signaling cascade, inflammatory response and several others. Curcumin can suppress the survival and proliferation of tumor cells by suppressing NF-kB-regulated gene products. It has been known to inhibit the expression of various transcription factors including NF-kB, β-catenin, signal transducers and activators of transcription protein (STAT)-1, CBP, STAT3, STAT5, EpRE, Egr and apoptosis protein (AP)-1 associated with the
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tumorigenesis, inflammation and angiogenesis. A number of genes such as cyclin D1, inducible nitric oxide synthase (iNOS), 5-lipoxygenase (LOX), matrix metalloproteinase-9 (MMP9), cyclooxygenase (COX)-2, IL-5, tumor necrosis factor (TNF), interleukin (IL)-8 and IL-12 associated with the apoptosis, cell invasion and adhesion have been found to be downregulated following exposure to curcumin. Curcumin suppresses the expression of different kinases including epidermal growth factor receptor (EGFR), IKK, HER2, Src, c-Jun N-terminal kinase (JNK), JAK2, TYK2, TNF and protein kinase A (PKA) and exerts its anti-carcinogenic and anti-inflammatory effects (Aggarwal et al., 2006; Maheshwari et al., 2006; Strimpakos and Sharma, 2008; Aggarwal and Sung, 2009). The mechanism of anti-tumor, anticancer and anti-proliferative action of curcumin has been suggested due to the induction of apoptosis, antioxidant property, affinity towards cytochromes and induction of the expression of HSP70 gene through initial depletion of intracellular Ca^{2+} followed by suppression of p53 gene function in the target cells. Numerous evidences have been provided to show that curcumin suppresses the proto-oncogenes, the transcriptional factor e-jun/AP-15l and protein kinase activity. Besides, it has also been reported to upregulate various enzymes like GST, GSH-px, Hemeoxygenase and Xanthine oxidase involved in the antioxidant defense system suggesting that it has strong antioxidant potential.

Curcumin directly interacts with the COX-2, LOX, glycogen synthase kinase (GSK)-3b, phosphorylase-3 kinase, xanthine oxidase, N-aminopeptidase, amyloid protein, human a1-acid glycoprotein, autophosphorylation activated protein kinase, DNA polymerase, focal adhesion kinase (FAK), glutathione, albumin, P glycoprotein, pp60 src tyrosine kinase, thioredoxin reductase (TrxR), tubulin, topoisomerase II, ubiquitin isopeptidase and toll-like receptor (TLR)4. The binding constant (IC50) of curcumin to these targets starts at nanomolar levels, as in GSK3b, 5-LOX, β-amyloid and TLR4 and progresses to micromolar levels in glutathione S-transferase (GST), TrxR, DNA polymerase-1 and tubulin. Curcumin has also been found to bind to certain divalent metal ions such as Fe,
Cu, Mn and Zn suggesting its metal chelating property (Maheshwari et al., 2006; Strimpakos and Sharma, 2008; Aggarwal and Sung 2009). Curcumin has been shown to inhibit neutrophil response and induce NOS in activated macrophages (Brouet and Ohshima, 1995).

1.3.2.1 **Anti-ageing agent:** Ageing is manifested by the accumulation of changes in an organism or object over the time (Kregel and Zhang, 2007). In humans, it is a multidimensional process of physical, psychological and social change. It is also a major risk factor for the neurodegenerative diseases including Alzheimer’s and Parkinson’s disease. Viani et al., (1991) reported that synaptic plasma membrane Na⁺, K⁺-ATPase is sensitive to age related parameters. Phospolemman, an accessory protein is associated with Na⁺, K⁺-ATPase (Crambert et al., 2002). It has been proposed that this protein may activate Na⁺, K⁺-ATPase in the CNS (Feschenko et al., 2003). Mattson, (1998) found that Na⁺, K⁺-ATPase activity is sensitive to lipid peroxidation. Bala et al., (2006) observed increased activity of Na⁺, K⁺-ATPase in specific brain regions in 6 and 24 month old rats treated with curcumin and suggested that it could be due to anti-lipid peroxidative property of curcumin. Due to this, levels of lipofuscin, a by product of lipid peroxidation were found to be decreased and associated with anti-lipid peroxidative action and anti-ageing effects of curcumin (Bala et al., 2006). Curcumin is an electrophilic compound that triggers the Nrf2/ARE signaling pathway associated with activation of antioxidant enzyme, phase-2 enzymes (heme oxygenase, Hsp70, thioredoxin reductase and sirtuins) and prevents from the oxidative stress induced diseases (Calabrese et al., 2008). Hence, it has been found to work as a vitagen and known to almost universal remedy.

1.3.2.2 **Effect in Alzheimer’s disease:** Role of inflammatory mediators in the production and accumulation of β-amyloid peptide (IL-1B, p JNK) ROS and lipid peroxidation products (8-iso-PGF₂α) in Alzheimer’s disease conditions has been well accepted (Akiyama et al., 2000). There is a lot of concern to introduce anti-inflammatory drugs to reduce the plaque burden in Alzheimer’s disease. Curcumin has been found to decrease the expression of NfKB, iNOS and JNK...
(Pendurthi et al., 1997; Weber et al., 2006) with no side effects. Administration of curcumin has been reported to attenuate cognitive deficits, neuroinflammation and plaque pathology in experimental models of Alzheimer’s disease (Frautschy et al., 2001; Yang et al., 2005; Garcia-Alloza et al., 2007). Treatment with amyloid β has been found to be associated with increased activity of AChE. Curcumin prevented from learning and memory deficits by inhibiting the upregulation of acetylcholinesterase (AChE) and maintaining the levels of acetylcholine at synaptic site as in Alzheimer’s disease (Yang et al., 2005). Hafner-Bratkovic, (2007) showed that curcumin labels prion plaques in vCJD brain which makes curcumin derivatives potentially useful for diagnosis and for the development of new probes for positron emission tomography (PET). Low dose of curcumin (160 ppm) was found to reduce the astrocyte marker glial fibrillary acidic protein and decrease the insoluble beta amyloid (Aβ), soluble Aβ and plaque burden in animal model of Alzheimer’s disease (Lim et al., 2001). Further, it has been suggested that strong anti-inflammatory property and dienone system in the structure of curcumin could reduce the production of β-amyloid and its burden from neuronal cells through binding capacity (Lim et al., 2001).

Plaque deposition is tightly associated with neurotoxicity, as exhibited by dystrophies and distorted neuritis (Knowles et al., 1999; Le et al., 2001; Garcia-Alloza et al., 2006). Curcumin was found to inhibit the formation of Aβ oligomers and fibrils in vitro (Ono et al., 2004; Yang et al., 2005). Garcia-Alloza et al., (2007) reported that curcumin may cross the blood brain barrier, label senile plaques and cerebrovascular amyloid angiopathy and prevent/reduce amyloid deposition in vivo. Yang et al., (2005) suggested that curcumin directly binds with small β-amyloid species to block aggregation and fibril formation in vitro and in vivo. Iron accumulation within specific brain regions displaying selective vulnerability to neurodegeneration has been reported in the brain of Alzheimer’s disease patients (Lovell et al., 1998; Pinero et al., 2000, 2001). Interestingly, use of curcumin in the diet by Indian population has been associated with reduced
risk to develop Alzheimer’s disease (4.4 fold) as compared to Americans (Ganguli et al., 2000; Lim et al., 2001).

1.3.2.3 Effect in Parkinson’s disease: Numerous studies on experimental models of Parkinson’s disease have been carried out to investigate the neuroprotective efficacy of curcumin. Protective efficacy of curcumin against 1-methyl-4-phenyl-1,2,3,6-tetrahydro pyridine (MPTP) induced oxidative stress in the brain of mice has been demonstrated (Rajeswari, 2006). Simultaneous treatment with curcumin (80 mg/kg body weight, i.p, 7 days) and MPTP (40 mg/kg, i.p, total dose) in mice decreased the lipid peroxidation and increased reduced glutathione levels (GSH) associated with increased activity of superoxide dismutase and catalase in striatum and mid brain on 3rd and 7th day of treatment as compared to those treated with MPTP alone (Rajeswari, 2006). In another study, curcumin (80 mg/kg body weight, i.p, 7 days) or its metabolite tetrahydrocurcumin (60 mg/kg body weight, i.p, 7 days) were found to decrease the activity of monoamine oxidase, an enzyme involved in the metabolism of catecholamines and increased the levels of dopamine and 3,4-dihydroxyphenylacetic acid (DOPAC) in the striatum of mice treated with MPTP (40 mg/kg, i.p, total dose) (Rajeswari and Sabesan, 2008a, b). The inhibitory effect of tetrahydrocurcumin on monoamine oxidase activity was found to be more than curcumin in these studies. Cytoprotection of curcumin against 1-methyl-4-phenylpyridinium ions (MPP⁺) was also studied in vitro on PC12 cells (Chen et al., 2006). Interestingly, curcumin protected PC12 cells against MPP⁺ induced cytotoxicity and apoptosis by enhancing expression of Bcl-2, attenuating intracellular ROS levels, over expressing iNOS and decreasing the loss of mitochondrial membrane potential (Chen et al., 2006). Based on these findings, neuroprotective potential of curcumin in the treatment of Parkinson’s disease was suggested (Chen et al., 2006).

Pre-treatment with curcumin (50 mg/kg body weight, p.o., 4 days prior to lesioning) in 6-OHDA lesioned rats (12 µg, unilateral lesioning) showed significant protection in the number of TH-positive cells in the substantia nigra
and dopamine levels in the striatum. The neuroprotection in the 6-OHDA model of Parkinson’s disease was associated with the antioxidant capacity of curcumin and its capability to reach the brain (Zbarsky et al., 2005). High levels of Fe$^{2+}$ in the substantia nigra have been reported in the patients suffering from Parkinson’s disease (Griffiths et al., 1999). It was suggested that curcumin may chelate Fe$^{2+}$ that is needed for Fenton’s reaction to generate hydroxyl radicals and inactivate its toxic effects in experimental studies (Reddy and Lokesh, 1994; Daniel et al., 2004).

Effect of curcumin on the expression of leucine rich repeat kinase 2 (LRRK2) gene associated with familial and sporadic Parkinson’s disease was studied in rat mesencephalic cells. Expression of LRKK2 mRNA and protein in N27 cells was found to be upregulated while no effect on other genes like α-synuclein and parkin was observed on exposure to curcumin (Ortiz-Ortiz et al., 2010). Bioconjugates of curcumin involving diesters of demethylenated piperic acid, valine and glutamic acid were prepared to compare their neuroprotective efficacy with curcumin in N27 dopaminergic cultured cells (Harish et al., 2010). Glutamic acid derivative of curcumin was found to exhibit improved neuroprotection as compared to curcumin and its two other derivatives as evident by its capability to enhance cellular GSH levels associated with increased accumulation of curcumin and improved concentration of glutamic acid in the non-toxic range (Harish et al., 2010).

**1.3.2.4 Effect in stroke / ischemia:** A number of studies have been carried out to investigate protective and preventive efficacy of curcumin using different doses, duration and routes in animal models of stroke. Treatment with curcumin (100, 300 mg/kg, i.p., 30 minutes after middle cerebral artery occlusion, MCAO) in rats reduced ischemia induced lipid peroxidation both in the ipsilateral and contralateral hemispheres of brain (Thiyagarajan and Sharma, 2004). Decreased activity of superoxide dismutase and glutathione peroxidase in the ipsilateral hemisphere of brain in MCAO rats was also found increased following curcumin treatment. Increased edema and peroxynitrite in MCAO rats was decreased
following curcumin treatment. In another study, neuroprotective effect of curcumin (50, 100 or 200 mg/kg, i.p) against ischemia reperfusion insult in rat fore brain was observed by Ghoneim et al., (2002). Pretreatment with curcumin (100 mg/kg, p.o) for 5 days prior to MCAO for 2 hours and for another 3 days after MCAO in rats resulted in significant improvement in grid walking and rotarod performance as compared to ischemic rats (Shukla et al., 2008). Treatment with curcumin in MCAO rats also inhibited lipid peroxidation and intracellular calcium levels and increased superoxide dismutase activity in corpus striatum and cerebral cortex and reduced the infarct area as compared to MCAO rats (Shukla et al., 2008).

Treatment with curcumin (30 mg/kg, i.p. or by supplementation of the AIN-76 diet 2 g/kg for 2 months) decreased lipid peroxidation, mitochondrial dysfunction, apoptotic indices and glial activation in gerbils subjected to transient global ischemia for 5 minutes as compared to sham (Wang et al., 2005). Hyperactivity due to transient global cerebral ischemia was also modulated following curcumin treatment (Wang et al., 2005). The protective effect of curcumin in these studies was attributed to its bioavailability as curcumin injected intraperitoneally reached the brain within 60 minutes after crossing the blood brain barrier. Rathore et al., (2007) found that treatment with curcuma oil in cerebral ischemia in rats could protect neuronal death due to its anti-oxidant effects and anti-apoptotic property via caspase-dependent pathway. Curcumin treatment was found to scavenge oxygen free radicals, reduce nitric oxide overproduction by inhibiting iNOS expression in astrocyte and prevent ONOO− induced cerebral capillaries endothelial cell damage (Jiang et al., 2007).

1.3.2.5 Effect in epilepsy: Pretreatment with curcumin was found to decrease severity of epilepticus in rats (Taniura et al., 2006; Sng et al., 2006). Such an effect was suggested due to histone acetyltransferase inhibitory activity, CBP/p300 attenuated histone modifications and IEG expression of curcumin. Curcumin pre-treatment was found to increase expression of immediate early response genes - c-fos and c-jun in kainic acid lesioned rats (Taniura et al.,
Role of astrocytes in kainic acid induced hippocampal cell death was studied in mice (Shin et al., 2007). Treatment with curcumin prevented upregulation of caspase-3, GFAP, eNOS and heme oxygenase (HO)-1 expression in hippocampal astrocytes in kainic acid induced epileptic mice. Based on these findings, it was further suggested that curcumin may prevent apoptosis by blocking the signaling pathway and protect the blood brain barrier and associated neuronal death (Shin et al., 2007). Sumanont et al., (2007) showed that treatment with manganese complexes of curcumin and diacetyl curcumin may inhibit kainic acid induced oxidative stress suggesting protective effect of curcumin in neuronal damage.

1.3.2.6 Effect in tardive dyskinesia: Although molecular mechanisms in the pathophysiology of tardive dyskinesia, a motor disorder of the orofacial region are not well understood, role of oxidative stress has largely been suggested in its etiology. Increased levels of lipid peroxidation in blood and cerebrospinal fluid of clinical cases and animal models of tardive dyskinesia have been observed (Burger et al., 2003; Lohr et al., 2003). Bishnoi et al., (2008) found that pretreatment with curcumin was able to reverse haloperidol induced behavioural and neurochemical changes associated with orofacial dyskinesia possibly due to its antioxidant properties (Kulkarni and Dhir, 2010). It was also suggested that curcumin could possibly inhibit calcium entry and COX/LOX enzymes associated with its protective effect in preventing orofacial dyskinesia (Bishnoi et al., 2008). Naidu and Kulkarni, (2001a, b) found that cyclooxygenase enzyme inhibitors may attenuate the development of tardive dyskinesia in rats.

1.3.2.7 Effect in stress and depression: While the mechanism of depression is multifactorial, stress has been suggested to be one of the contributing factors to precipitate the affective state. Abnormalities of the monoaminergic system associated with sympathetic nervous system, endocrine and immune systems in depressive state are well accepted (Szelenyi and Selmeczy, 2002). Several studies have been carried out to investigate the antidepressant effect of curcumin on animal models of depression. Treatment with curcumin (5, 10 mg/kg, p.o) was
found to decrease the duration of immobility both in the tail suspension and forced swim test (Xu et al., 2005b). The behavioral effects indicating antidepressant activity of curcumin was correlated with increased levels of serotonin, dopamine and norepinephrine in frontal cortex and striatum in these studies (Xu et al., 2005b). Further, increased levels of biogenic amines in depressed mice were associated with decreased monoamine oxidase activity following curcumin treatment (Xu et al., 2005b). Kulkarni et al., (2008) also observed that curcumin inhibited the immobility time in mice subjected to forced swim test and suggested that the antidepressant activity of curcumin is modulated by central dopaminergic and serotonergic systems. They also showed that combination of piperine may enhance the bioavailability of curcumin and has potential antidepressant effect as compared to curcumin alone (Kulkarni et al., 2008; Bhutani et al., 2009). In another study, Xu et al., (2005a) found that hyperactivity and learning deficits by step down passive avoidance in olfactory bulbectomised rats are protected by curcumin treatment. The behavioral changes due to olfactory bulbectomy were correlated with alterations in the levels of biogenic amines and their metabolites and were reversed following curcumin treatment (Xu et al., 2005a). To further confirm the antidepressant activity, rat model of forced swim test was employed. A decrease in immobility time on forced swim test was observed in curcumin treated rats (Xu et al., 2005a). Curcumin has been shown to have antidepressant effect in mouse model of behavioral despair tests (Yu et al., 2002). Curcumin was found to modulate the concentration of serotonin and catecholamines in rat brain which are important mediators involved in the age related neurological disorders and depression (Mazzio et al., 1998). Anti-depressant effect of curcumin in mouse model of forced swim test has been suggested to be mediated by interaction with 5-HT(1A/1B) and 5-HT2C receptors (Wang et al., 2008). Involvement of central 5-HT(1A/1B/7) receptors in the antidepressant effect of curcumin has been suggested (Li et al., 2009). Curcumin was found to modulate adenylate cyclase activity, cAMP levels and CREB via suppressing central 5-HT(1A/1B/7) receptors in chronic unpredictable mild stress rat model of depression (Li et al., 2009).
Rats subjected to chronic stress had impaired escape performance associated with HPA axis dysfunction and downregulation in brain derived neurotropic factor (BDNF) and cyclic AMP response element binding protein (CREB) proteins in hippocampus and frontal cortex (Xu et al., 2006). Interestingly, treatment with curcumin (5 or 10 mg/kg, p.o.) could check downregulation in BDNF and CREB proteins in hippocampus and frontal cortex associated with behavioral deficits (Xu et al., 2006). In another study, Xu et al., (2007) found that curcumin protected hippocampal neurons in response to chronic stress by upregulating 5-HT1A receptors and BDNF. Sub-effective doses of curcumin and fluoxetine, a selective serotonin reuptake inhibitors were found to have synergistic antidepressant effect (Wang et al., 2008). Neuroprotective efficacy of curcumin in depression has been suggested to be modulated via BDNF/TrkB signaling (Wang et al., 2008). In an interesting study on rat cortical neurons, Wang et al., (2010) further demonstrated that MAPK and PI-3K pathways associated with BDNF signaling are activated by curcumin and may stimulate the phosphorylated CREB leading to neuroprotection. It was found that curcumin induced heat shock protein associated with the protection of cells from various types of stress and also reduced oxidative damage and amyloid pathology as in the Alzheimer’s disease (Calabrese et al., 2004)

1.3.2.8 Effect in diabetic encephalopathy: A number of studies have indicated that diabetes causes neuronal damage by intracellular glucose leading to diabetic encephalopathy, characterized by impaired cognitive functions and neurochemical and structural abnormalities. Diabetes has also been suggested to be a predisposing factor for depression, anxiety, stroke and other cerebrovascular disorders. Enhanced generation of free radicals in chronic hyperglycemia has been found in the development of diabetic encephalopathy. Chronic treatment with curcumin (60 mg/kg body weight/ day, p.o) for 10 weeks in diabetic rats (streptozotocin 65 mg/kg, i.p., once only) ameliorated cognitive deficits and cholinergic dysfunctions (Kuhad and Chopra, 2007). Treatment with curcumin could check increased nitrite production and oxidative stress in cerebral
cortex and hippocampus of diabetic rats (Kuhad and Chopra, 2007). Antinociceptive activity of curcumin has been demonstrated in attenuating diabetic neuropathic pain (Sharma et al., 2006, 2007). Treatment with insulin (10 IU/kg/day, s.c.) and its combination with curcumin (60 mg/kg, p.o.) attenuated thermal hyperalgesia and hot plate latencies associated with decreased brain nitrite and plasma TNF-α levels in diabetic rats (streptozotocin 200 mg/kg, i.p. once only). Curcumin treatment in diabetic rats was found to inhibit diabetes induced increase in the gene expression of AChE, cholinergic and insulin receptors in cerebellum (Peeyush et al., 2009). The study also showed that curcumin could modulate glucose transportation by inhibiting the expression of Glut3 and check diabetes mediated cerebellar disorders (Peeyush et al., 2009).

Rajakrishnan et al., (1999) reported that curcumin is neuroprotective and shows protective efficacy in diabetic neuropathy (Sharma et al., 2007). In diabetes, glucose levels in blood could be reduced by curcumin (Arun and Nalini, 2002) by stimulating pancreatic β-cells (Srivivasan et al., 2003) and enhancing the activation of PPAR-γ (Murugan and Pari, 2006). Curcumin also reduced the production of pro-inflammatory cytokines such as TNF-α, interleukin-1beta (IL-β) and interleukin-8 (IL-8) (Chan, 1995; Cho et al., 2007) and prevented from diabetic encephalopathy. Rastogi et al., (2008) found that curcuminoids, polyphenols of Curcuma longa have protective effect by enhancing antioxidant defense mechanism and controlling the mitochondrial dysfunction in the brain of diabetic rats. Curcumin has been reported to produce a strong inhibitory effect on superoxide anion and hydrogen peroxide production (Iqbal et al., 2003). Sharma et al., (2006) found that the antioxidant properties of curcumin counteracted the oxidative stress produced in hyperglycemia and streptozotocin induced renal dysfunction in diabetic rats. The studies have suggested that curcumin inhibited xanthine oxidase and NADPH oxidase which are important source of free radical generation (Ghoneim et al., 2002). Pari and Murugan, (2007) found that teterahydrocurcumin was more effective as compared to curcumin in reducing oxidative stress in the brain of diabetic rats.
1.3.2.9 Effect in chemical induced neurotoxicity: Increased industrial and agricultural activities have distributed metals, pesticides and other chemicals in the environment. Most of these chemicals easily cross the blood brain barrier and affect functioning of the brain leading to neurobehavioral abnormalities. A number of studies have been carried out involving curcumin to investigate its neuroprotective efficacy. Lead induced oxidative stress in brain was found to be decreased in rats co-treated with curcumin (100 mg/kg body weight, p.o.) and lead (50 mg/kg body weight, p.o) for 45 days (Shukla et al., 2003). A significant decrease in lipid peroxidation and increase in GSH levels, superoxide dismutase and catalase activity was observed in cerebellum, corpus striatum, hippocampus and frontal cortex of rats simultaneously treated with lead and curcumin. The protective changes were found to be associated with decrease in lead levels in all the brain regions (Shukla et al., 2003). Treatment with curcumin was found to retain spatial reference memory in lead exposed rats (Dairam et al., 2007). In another study, Daniel et al., (2004) suggested that curcumin has metal binding property by forming a complex and may protect lead and cadmium induced lipid peroxidation in rat brain. Antioxidant and iron binding property of curcumin and certain other spice ingredients were shown in vitro and associated with its neuroprotective efficacy (Dairam et al., 2008). The chelating property of curcumin was suggested to decrease the load of metals in the brain regions (Mitchell, 2000). El-Demersdash et al., (2009) found that enhanced oxidative stress in brain following arsenic treatment could be reduced in rats simultaneously treated with arsenic and curcumin. In another study, decreased activity of brain AChE in arsenic treated rats was found to be recovered in rats co-treated with arsenic and curcumin (Yousef et al., 2008). Behavioral, neurochemical and ultrastructural alterations by aluminum in rat brain were found to be protected by curcumin treatment (Sethi et al., 2009). Soudamini et al., (1992) reported that curcumin could significantly scavenge the free radical species generated in rat brain by paraquat, cyclophosphamide and carbon tetrachloride as revealed by decrease in lipid peroxidation.
Antioxidant and hypolipidaemic activity of curcumin was suggested to be involved in preventing ethanol induced brain damage (Rajakrishnan et al., 1999). Treatment with curcumin in alcohol exposed rats decreased brain cholesterol, phospholipids, free fatty acid levels and lipid peroxidation associated with increased levels of GSH as compared to those exposed to alcohol alone (Rajakrishnan et al., 1999). Administration of curcumin has been found to decrease the lipid peroxidation in rat brain treated with ethanol, due to its antioxidant property. Administration of curcumin was also found to decrease the levels of phospholipids and free fatty acids and thereby protecting the membrane from disruption and preventing ethanol-induced neurotoxicity (Rajakrishnan et al., 1999). Curcumin has been found to attenuate ethanol induced cell death associated with inhibition in the activation of p38 mitogen activated protein kinase (MAPK). The studies also revealed activation of MAPK phosphatase – 1 (MKP-1), a negative regulator of p38 MAPK in HT22 cells by curcumin in modulating ethanol induced cytotoxicity (Pae et al., 2009). Sohda et al., (1993) reported that brain cytochrome P4502E1 is responsible for the metabolism of ethanol into acetaldehyde but curcumin has inhibitory action against Cyt-P450 due to its antioxidant property (Oetari et al., 1996). Jagota and Reddy, (2007) showed that ethanol induced changes in 5-HT, a major neurotransmitter regulating the circadian rhythm and its metabolite, 5-HIAA in suprachiasmatic nucleus (SCN) and pineal were partially restored with phase shifts in SCN and pineal following administration of curcumin in rats. Cytoprotective effect of curcumin in HT22 hippocampal cells has also been suggested (Pae et al., 2009).

Curcumin has potent antioxidant activity for nitric oxide related radical generation unlike tocopherol (Chan et al., 1998). Curcumin was found to block iNOS expression by suppressing JAK – Stat inflammatory signaling (Kim et al., 2003a, b). Jung et al., (2006) observed that curcumin prevented microglia from neurotoxic alterations caused by excessive nitric oxide production by suppressing nitric oxide generation both from primary and microglial cells.
mediated by lipopolysaccharide and other pathological stimuli such as IFN-γ and Aβ (25-35) in a dose-dependent manner. Curcumin was found to have potential to inhibit expression of iNOS (Camacho-Barquero et al., 2007) and also had nitric oxide scavenging effect (Nanji et al., 2003).

Although curcumin has multiple pharmacological spectrum with enormous potential to modulate biochemical and molecular targets in different neurological and psychiatric disorders, concerted efforts are required to assess its neuroprotective potential in chemical induced neurotoxicity.

1.4 Bacopa monnieri

Bacopa monnieri (B. monnieri) is also called Brahmi, a name derived from Brahma, the creator God of the Hindu pantheon of deities. It is a perennial creeping plant belonging to the family Scrophulariaceae found in wet, damp and marshy areas throughout Indian subcontinent (Sandhya et al., 2012). B. monnieri has been classified as a Medhya Rasayana and documented in the Charaka Samhita and used in the traditional Ayurvedic system of Indian medicine for centuries as a brain tonic to enhance memory, learning and concentration (Das et al., 2002; Russo and Borrelli, 2005; Mathew et al., 2011) (Figure - 11). It has also been used to provide relief to the patients suffering from anxiety and epilepsy (Udupa and Singh, 1993). Studies have clearly shown that B. monnieri enhances memory and cognition in experimental models of learning deficits (Singh and Dhawan, 1982, 1992, 1997; Vohora et al., 2000; Joshi and Parle, 2006; Sandhya et al., 2012). It has been reported to reduce oxidative stress and protect from free radical damage in neurological disorders due to its antioxidant potential (Jyoti and Sharma, 2006; Jyoti et al., 2007; Anbarasi et al., 2006). Due to these pharmacological effects, the main focus of the research has been to explore its cognitive-enhancing effects, specially memory, learning, concentration, insomnia and nervous tension in neurological disorders and in chemical induced neurotoxicity (Sharma et al., 1987; Roodenrys et al., 2002; Jyoti and Sharma, 2006; Jyoti et al., 2007; Singh et al., 2010, 2012).
1.4.1 Chemistry

The chemistry of *B. monnieri* has been well studied due to its potential neuropharmacological effects (Sastri et al., 1959; Chatterji et al., 1965; Chandel et al., 1977; Russo and Borrelli, 2005). *B. monnieri* is a composite mixture of brahmine, herpestatine and a mixture of three bases. The herb also contains saponins, monnierin, hersaponin, d-mannitol, bacoside A and bacoside B (Chopra et al., 1956, Sastri et al., 1959). Bacoside A usually co-occurs with bacoside B and the latter differs only in optical rotation. On acid hydrolysis, bacosides yield a mixture of aglycones, bacogenin A₁, A₂, A₃ and two genuine saponins. Bose and Bose in 1931 for the first time isolated the alkaloid brahmine from *B. monnieri*. Chatterji et al., (1965) found that the bacoside A and B the active constituents of *B. monnieri* are responsible for the memory enhancing action (Singh and Dhawan, 1992). Interestingly, bacoside A has been found to be involved in antioxidant defense (Anbarasi et al., 2006) and bacoside B plays crucial role in memory enhancing activity (Rastogi, 1990) (Figure – 12). Besides, improving the learning and memory in rats, these active ingredients have also been found to inhibit the amnesic effects of scopolamine, electroshock and immobilization stress (Dhawan and Singh, 1996).

The neuropharmacological properties of *B. monnieri* are due to the presence of significant amount of betulic acid, stigmastanol, bet-sitosterol, bacopaside I, bacopaside II, bacopaside X, bacopasaponin C, bacopaside N2 and the minor components were bacopasaponin F, bacopasaponin E, bacopaside N1,
bacopaside III, bacopaside IV and bacopaside V (Murthy et al., 2006; Chakravarty et al., 2001, 2003; Hou et al., 2002; Russo and Borrelli, 2003b).

![Bacoside A](image)

![Bacoside B](image)

**Figure – 12. Structure of bacoside A and B**

### 1.4.2 Pharmacology and Molecular Targets

The nootropic action of *B. monnieri* is well recognized. A number of neuropharmacological properties have been reported by many investigators (Singh and Dhawan, 1997; Bhattacharya et al., 1999, 2000a, b; Russo and Borrelli, 2005). The bacosides are known to inhibit prostaglandin synthesis and lysosomal membrane stabilization and hence possesses anti-inflammatory activity (Jain et al., 1994). The triterpenoid saponins and their bacosides are known to enhance neurotransmission process by nerve impulse transmission (Singh and Dhawan, 1997). Besides, the bacosides modulate kinase activity, neuronal synthesis and restores synaptic activity and synaptic transmission attributed to repair injured neurons (Singh and Dhawan, 1997). It has also been reported that it involve in the modulation of the expression of certain enzymes.
involved in generation and scavenging of reactive oxygen species in the brain (Chowdhuri et al., 2002). The protective effect of *B. monnieri* has been demonstrated in *in vitro* system against DNA damage in astrocytes and human fibroblasts (Russo et al., 2003 a, b), stabilize mast cells, lysosomal membrane and inhibit prostaglandin synthesis associated with anti-inflammatory activity (Samiulla et al., 2001). Role of *B. monnieri* to inhibit DNA replication in cancer cell lines attributed to anticancer effect has also been shown (Elangovan et al., 1995). *B. monnieri* has relaxant effect on pulmonary arteries, aorta, trachea and bronchial tissues as it inhibits the calcium-ion influx into cell membranes (Channa et al., 2003). *B. monnieri* has ability to stimulate and synthesize GABA, a neurotransmitter in the central nervous system and leads to improved acquisition, memory and adaptation to new conditions. It inhibits the breakdown of cholinesterase, an enzyme involved in the metabolism of acetylcholine, a key neurotransmitter in the CNS and has positive effects in patients with Alzheimer’s and dementia. Bacosides present in *B. monnieri* have the potential to increase protein and RNA turnover in specific brain areas through membrane dephosphorylation (Singh et al., 1990). It has also been reported that *B. monnieri* enhances protein kinase activity in the hippocampus to impart its nootropic action (Singh and Dhawan, 1997). Administration of *B. monnieri* for two weeks was found to reverse the colchicine induced decrease of acetylcholine levels, cholinesterase activity and the muscarinic-cholinergic receptors in the frontal cortex and hippocampus of rats (Bhattacharya et al., 1999). Besides effects on the cholinergic system (Bhattacharya et al., 1999, 2000), serotonergic system has also been found to be modulated in the cognitive enhancement by *B. monnieri* (Meneses, 1999; Parker and Medora, 2005).

### 1.4.2.1 Nootropic effects:
Nootropics also known as smart drugs are used to enhance the cognitive abilities in humans. Nootropics primarily increase the levels of neurochemicals including neurotransmitters, enzymes and hormones and improve the supply of oxygen in the brain and also stimulate nerve growth. Preclinical and clinical studies have been carried out to validate the
neuroprotective potential of *B. monnieri* in healthy subjects and patients suffering from different neurological disorders. The bacosides present in *B. monnieri* have been broadly investigated for their neuropharmacological properties associated with nootropic action. Numerous studies have found that active saponins - bacosides A and B, present in the extract of *B. monnieri* enhanced learning and cognitive ability in rats (Malhotra and Das, 1959; Singh and Dhawan, 1982, 1992; Russo and Borrelli, 2005; Uabundit et al., 2010). Rats subjected to learning performance tests following treatment of *B. monnieri* exhibited better acquisition, improved retention and shortened reaction time than controls (Singh and Dhawan, 1982). BacoMind, a standardized phytochemical composition derived from the plant of *B. monnieri* showed significant decrease in the latency to reach shock free zone (SFZ), decrease number of mistakes and increase in the inflexion ratio and discrimination index against scopolamine induced impairment in learning and memory in rodents (Kasture et al., 2007). Chronic treatment of *B. monnieri* extract in patients with anxiety neurosis and in children have reported memory and learning enhancing effects in clinical studies (Singh and Singh, 1980; Sharma et al., 1987). The commercial preparation has shown remarkable nootropic activity in young subjects (Dave et al., 1993). Improvement in short-term memory and delayed recall, increase in working memory and reduction in error-making have been reported following 4, 6 and 12 weeks supplementation of *B. monnieri* in clinical trials indicating significant changes in cognitive tasks (Roodenrys et al., 2002; Raghav et al., 2006; Calabrese et al., 2008; Stough et al., 2008). Interestingly, improvement in cognitive performance has been found to be associated with improved nerve impulse transmission leading to faster learning and better mental power in the individuals. Further, Stough et al., (2001) reported that treatment with *B. monnieri* in humans caused significant improvement in speed of visual information processing, learning rate, memory consolidation and decreased forgetting rate. Significant improvement in verbal learning, memory acquisition and retention has also been reported (Morgan and Stevens, 2010).
1.4.2.2 **Effect in learning and memory**: Involvement of central cholinergic system in the regulation of cognitive functions is well accepted. Loss of cholinergic neurons in hippocampal region is the major feature of Alzheimer’s disease while clinical relief could be brought about by administration anti-cholinesterases. Neuroprotective effects of *Bacopa monnieri* associated with cognitive enhancement in Alzheimer’s disease model have been suggested (Limpeanchob et al., 2008; Uabundit et al., 2010). Administration of *B. monnieri* for 2 weeks in rats reversed cognitive deficits induced by colchicines and ibotenic acid (Bhattacharya et al., 1999). It has been suggested that the behavioral effects of cholinergic degeneration can be alleviated by the reduction in noradrenergic function (Sara, 1989). *B. monnieri* is known to decrease norepinephrine and increase 5-HT levels in the hippocampus, hypothalamus and cerebral cortex (Singh and Dhawan, 1997). It has been found to modify the concentrations of acetylcholine by influencing the other neurotransmitter systems.

Administration of *B. monnieri* extract for 12 weeks improved verbal learning, memory consolidation and speed of early information processing in healthy human subjects (Stough et al., 2001). Sharma et al., (1987) also observed positive effects on learning and memory following exposure to *B. monnieri* extract for 12 weeks. Clinical trials on humans have shown that *B. monnieri* significantly enhances the retention of new information but rate of learning remains unaffected suggesting that *B. monnieri* may decrease forgetting of newly acquired information (Roodenrya et al., 2002). *B. monnieri* extract also known to improve the speed of visual processing, learning rate and memory consolidation in different population (Negi et al., 2000; Stough et al., 2001). Interestingly, low doses or acute exposure of combined extract of *B. monnieri* with *Ginkgo biloba* did not exhibits significant effect on learning, attention, short term learning and memory, memory consolidation and problem solving capacity (Nathan et al., 2001, 2004; Maher et al., 2002). Although many clinical trials on *B. monnieri* have been carried out to investigate its cognitive enhancing activity, consistent results have not been observed. The differential effects of *B. monnieri* could be
due to differences in dose, duration and timing of exposure in healthy individuals (Nathan et al., 2001, 2004; Maher et al., 2002).

1.4.2.3 Effect in Parkinson’s disease: Neuroprotective efficacy of B. monnieri in animal models of Parkinson's disease has been investigated (Hosamani and Muralidhara, 2009, 2010; Jadiya et al., 2011; Singh et al., 2012). B. monnieri has been found to reduce alpha synuclein aggregation, prevent dopaminergic neurodegeneration and restore the lipid content in Caenorhabditis elegans (C. elegans) and exhibit its anti-parkinsonian property (Jadiya et al., 2011). In a study on drosophila, increased oxidative stress, decreased motor performance and lower levels of dopamine induced by rotenone were found to recover following exposure to B. monnieri. Protective effect of B. monnieri was suggested by modulating the antioxidant defenses system (Hosamani and Muralidhara, 2009). Singh et al., (2012) found that B. monnieri is effective against paraquat and 1-methyl-4-phenyl-pyridinium iodide (MPP+) induced neurotoxicity in rats and suggested that B. monnieri extract could be used as therapeutic agent in age-related neurodegenerative diseases like Parkinson’s disease.

1.4.2.4 Effect in epilepsy: The historical claims of B. monnieri have been suggested to have curative effects in epilepsy (Ganguly et al., 1967). As epilepsy involves multiple neurotransmitter systems, treatment with B. monnieri could protect cholinergic, serotonergic and GABAergic modifications in the epileptic rat models (Paulose et al., 2007; Mathew et al., 2011). Alterations in the 5-HT levels, 5-HT<sub>2C</sub> receptors and gene expression in the cerebellum of epileptic rats were found to be reversed at control levels following treatment with B. monnieri (Krishnakumar et al., 2009). A number of studies on different pre-clinical models such as pentobarbitone hypnosis, electroshock and chemoconvulsions in mice and rats have been carried out to assess anti-epileptic effects (Shukia et al., 1987; Dhawan and Singh, 1996; Singh and Dhawan, 1997; Vohora et al., 2000). Hersaponin, an active constituent of B. monnieri has been known to prevent seizures in mice (Ganguly et al., 1967). Anticonvulsant effect of B. monnieri has been demonstrated by Martis, (1992). Treatment of B. monnieri extract showed
significant improvement in acquisition and retention of memory against phenytoin-induced cognitive deficit in mice (Vohara et al., 2000). Anti-epileptic potential of aqueous extract *B. monnieri* has been demonstrated in experimental studies (Shanmugasundaram et al., 1991).

### 1.4.2.5 Anti-amnesic effect:

Amnesia may be associated with various neurological problems including Alzheimer’s disease, ageing and chronic drug abuse or head injury. Besides, it may also be induced by a range of competitive and non-competitive NMDA receptor antagonists such as AP5, NPC, phencyclidine, ketamine and MK801 which block long term potentiation induction (Bliss and Collingridge, 1993; Gruart et al., 2006; Harney et al., 2006). Singh et al., (1990) found that bacopaside induced membrane dephosphorylation, together with increase in protein and RNA turnover in specific brain areas. Further enhanced protein kinase activity in hippocampus may be attributed to its memory enhancing activity. The dose-dependent anti-amnesic effect of *B. monnieri* on diazepam-induced anterograde amnesia has also been shown (Prabhakar et al., 2008). Although anti-amnesic effects of *B. monnieri* have been associated with its antioxidant and anticholinesterase activities, exact mechanism of its action remains elusive (Tripathi et al., 1996; Bhattacharya et al., 2000a, b; Das et al., 2002). Saraf et al., (2010) in separate study, found that anti-amnesic effect of *B. monnieri* in scopolamine induced amnesia has been associated with kinase-CREB pathway. L-NNA (a nitric oxide synthase inhibitor) induced anterograde amnesia was significantly reversed by *B. monnieri* by modulating calmodulin and pCREB/CREB levels (Anand et al., 2010). Amnesic effects of hyoscine, electroshock and immobilization stress in rats were also found to be reversed *B. monnieri* (Dhawan and Singh, 1996).

### 1.4.2.6 Anti-depressant activity:

Anti-depressant activity of *B. monnieri* in rodent models of depression has been investigated extensively (Sairam et al., 2002; Chatterjee et al., 2010; Ramanathan et al., 2011). *B. monnieri* exhibited its anti-depressant activity in forced swim and learned helplessness rat model of imipramine (15 mg/kg i.p) suggesting its anti-depressant activity (Sairam et al.,
2002). Although the mechanism by which *B. monnieri* exhibits its anti-depressant activity is not known, it has been suggested that besides affecting the cholinergic system, bacosides may modulate monoaminergic neurotransmission process and exerts its nootropic effect. Enhanced levels of dopamine and serotonin have been reported following treatment with bacosides that could be associated with its anti-depressant activity among the aged rats (Rastogi et al., 2011). Zhou et al., (2007) isolated three new triterpene glycosides, bacopasides VI-VIII (1-3) along with three known analogues, bacopaside I (4), bacopaside II (5) and bacopasaponsin C (6) from the whole plant of *B. monnieri*. The isolated analogues (4, 5 and 6) of *B. monnieri* were found to have anti-depressant activity while evaluated on forced swimming and tail suspension tests in mice.

**1.4.2.7 Anti-anxiety agent:** Experimental studies on rats revealed greater anxiolytic effects of *B. monnieri* at a higher dose as compared to LZP, a standard benzodiazepine. Interestingly, amnesic effect of benzodiazepines the major side effect was ameliorated by *B. monnieri* (Bhattacharya and Ghosal, 1998). The anxiolytic effects of *B. monnieri* have been found to be associated with the suppression of fear in mice (Shanker and Singh, 2000). The supplementation with *B. monnieri* extract has also led to significant decreases in anxiety symptoms, levels of disability and mental fatigue. Limited clinical trial of one-month in 35 anxiety patients demonstrated that administration of Brahmi syrup (30 ml daily in two divided doses) resulted to decrease the anxiety symptoms, level of anxiety, level of disability and mental fatigue and an increase in immediate memory span (Singh and Singh, 1980). Nootropic activity of *B. monnieri* has been found to be effective in the treatment of anti-anxiety effects (Russo and Borrelli, 2005).

**1.4.2.8 Anti-stress effect:** Stress has been known to be associated with most of the diseases including neurological and neuropsychiatric disorders. Monoamines including noradrenaline, dopamine and 5-HT, the major neurotransmitters present in the brain have functional role in stressful conditions (Tsigos and Chrousos, 2002; Gonzalo et al., 2003). Pretreatment with *B. monnieri*
significantly protected against acute and chronic stress induced changes in adrenal gland weight, spleen weight, plasma glucose, alanine and aspartate aminotransferase, ulcer index and creatine kinase suggesting its adaptogenic activity (Rai et al., 2003a). Further, it was also suggested that it imparts anti-stress activity by attenuating the systemic HPA axis response (Rai et al., 2003a, b). Studies also exhibited that *B. monnieri* significantly protected against stress induced ulcers and oxidative stress attributed to its anti-stress and antioxidant potential (Sairam et al., 2001). *B. monnieri* has been found to alleviate stress induced alterations in plasma corticosterone and levels of monoamines like norepinephrine, 5-HT and dopamine in brain regions, which are more vulnerable to stressful conditions (Sheikh et al., 2007). Anti-stress effects of *B. monnieri* by modulating Hsp70 expression, superoxide dismutase and cytochrome P450 has also been observed in rats (Chowdhuri et al., 2002). Clinical studies performed in India confirm that the bacosides can reduce anxiety in stressed individuals and contribute to improve brain functions (Chowdhuri et al., 2002). Perment, a polyherbal Ayurvedic formulation containing equal amount of *Clitoria ternatea, Withania somnifera, Asparagus racemosus, B. monnieri* was found to exhibit anti-depressant and anxiolytic by modulating adrenergic and serotonergic system (Ramanathan et al., 2011).

**1.4.2.9 Effect in chemical induced neurotoxicity:** A number of studies have been carried out to explore the protective effect of *B. monnieri* in chemical induced neurotoxicity in experimental models (Jyoti and Sharma, 2006; Jyoti et al., 2007; Singh et al., 2010, 2012; Shinomol et al., 2011, 2012a, b; Sumathi et al., 2012). Implication of *B. monnieri* extract for its various neurological uses may be attributed due to its antioxidant potential and presence of bacosides. Bacoside A3 from *B. monnieri* extract was found to inhibit superoxide released from polymorphonuclear cells in nitroblue tetrazolium assay (Pawar et al., 2001). *B. monnieri* extract has the ability to modulate endogenous markers of oxidative stress associated with antioxidant defenses system and cholinergic function in brain regions of mice (Shinomol and Muralidhara, 2011). S-nitroso-N-acetyl-
penicillamine (SNAP), a nitric oxide donor increases the intracellular oxidants and initiates the fragmentation of DNA in cultured rat astrocytes cells. Co-treatment with *B. monnieri* reduced the intracellular oxidants and prevented DNA damage in these cells suggesting its antioxidant potential (Russo et al., 2003b).

*B. monnieri* has been found to be well tolerated without any known side effects (Russo and Borrelli, 2005) and the LD50 in rats was determined to be as high as 2.7 g/kg p.o (Hota et al., 2009). However, doses of *B. monnieri* at therapeutic levels have not been associated with any known side effects. Single (20-30 mg) or multiple (100-200 mg) daily doses of *B. monnieri* were well tolerated without adverse effects (Singh and Dhawan, 1992).