*Curcuma longa* (Turmeric) is a rhizomatous herbaceous perennial plant of the ginger family, Zingiberaceae which is native in tropical South Asia. Interest in this herb has grown in recent years based on its reported putative beneficial pharmacological effects, which include antioxidant, anti-inflammatory and cancer-preventive properties (Sharma *et al*., 2005). Curcuminoids, a major constituent of *Curcuma longa* is a mixture of three, different chemical moieties namely Curcumin, Demethoxycurcumin, and Bisdemethoxycurcumin (Tonnesen, 1989). Although earlier literature reveals that in various studies Curcumin and curcuminoids are treated as the same entity possibly because CUR constitutes the major part (almost 77%) in the mixture (Inano *et al*., 2000). However, the present study was carried out with an aim to evaluate the role of individual isolated curcuminoids, that is, CUR, DMC, BDMC and CUR MIX in different pharmacological activities. Since isolated curcuminoids are not readily available in commercial market so we had isolated these curcuminoids in “Indian system of medicine” laboratory of DIPSAR, University of Delhi.

The percentage yield of ethanolic extract was found to be 9.67%. The percentage yield of isolated Curcumin, DMC and BDMC were found to be 0.393%, 0.087% and 0.016%, respectively. Different compositions of the mobile phase for HPTLC analysis were tried in order to obtain high resolution and reproducible peaks and was best achieved using chloroform: methanol (48:2, v/v) at 425 nm. The Rf value of Curcumin, DMC and BDMC were found to be 0.67, 0.48 and 0.30 respectively in the mobile phase chloroform: methanol (48:2 v/v). The percentage purity of Curcumin, DMC and BDMC was found to be 98.31, 98.23 and 97.72% respectively. The best separation in HPLC was achieved at retention time 9.26, 11.15 and 13.38 min; BDMC, DMC and Curcumin respectively. The percentage purity of Curcumin, DMC and BDMC was found to be 99.19, 98.82 and 99.25% respectively.

The structures of isolated curcuminoids were identified by spectral data comparison of their published values (Masuda *et al*., 1998). These three compounds were identified on the basis of the spectral evidence. The IR spectral data of Curcumin, DMC and BDMC shows O-H stretching on 3450, 3447, 3207 cm\(^{-1}\) frequency; OCH\(_3\) stretching on 2830, 2840 cm\(^{-1}\), absent; C=O stretching on 1650, 1630, 1626 cm\(^{-1}\) ; C-C=C asymmetric stretching on 1510, 1504, 1509 cm\(^{-1}\) ; C=C olefi nic stretching on 1500, 1500, 1500 cm\(^{-1}\) ; phenol or tertiary
alcohol on 1420, 1410, 1430 cm$^{-1}$ respectively. Only one peak, OCH$_3$ stretching, was not found in BDMC due to the absence of OCH$_3$ group. Apart from this other bands were observed: 810, 720 and 700 cm$^{-1}$, being assigned to the C=C-H aromatic stretching (Alexzendru et al., 2005). The results confirmed our isolated compound.

1 H and 13 C NMR spectrum of isolated curcuminoids were determined in DMSO at operating frequencies 300 and 75 MHz, respectively. The chemical shifts and coupling constant (J) of curcuminoids were interpreted and confirmed (Park et al., 2005). The results are shown in table no 5 & 6.

MASS spectral data of Curcumin, DMC and BDMC showed prominent molecular ion peak on m/z: 368 (M+); 338 (M+); 308 (M+), respectively. The yield of ethanolic extract was found to be 9.67 % which was in compliance with the previous studies (Ayurvedic Pharmacopoeia., 1999). The yield of individual curcuminoids was found to be 0.393, 0.087 & 0.016 % respectively. Simultaneous analysis of Curcumin, DMC and BDMC from C. longa by HPTLC fingerprinting reveals the R value 0.67, 0.48 & 0.30 respectively, which is in accordance with earlier studies (Parmasivum et al., 2009). The percentage purity was calculated by estimating AUC of each standard and isolated curcuminoids which was in accordance with previous literatures (Kamble et al., 2011), further supported by HPLC analysis. The spectral data of IR, NMR & MASS spectroscopy confirms the structure of isolated curcuminoids viz. Curcumin, DMC, BDMC reported previously (Masuda et al., 1998). IR spectroscopy of these isolated curcuminoids reveals broad band at the frequency of 3207 cm$^{-1}$ in BDMC, which were in conformity with the earlier reported structures (Alexzendru et al., 2005). The absence of absorption bands in the aliphatic C–H stretching regions (3000-2800 cm$^{-1}$), corresponding to the methoxyl group can be used to distinguish BDMC from Curcumin and DMC. A singlet, corresponding to the two methoxyl groups in curcumin was observed at d 3.95 in the 1 H NMR spectrum. For DMC, the corresponding signal was observed at d 3.85 (Table 5). The 13 C NMR signals for the methoxyl groups of Curcumin and DMC occurred at d 55.8 (Table 6) (Park et al., 2005). The curcuminoids were identified by comparing with reference standards literatures of Mass spectra showed, m/z: 368 (M+); 338 (M+); 308 (M+), for Curcumin, DMC and BDMC, respectively. The above
characteristic fragment ions were in accordance with previous reports (Masuda et al., 1998 and Alexzendru et al., 2005). Further experiments (in vivo & in vitro studies) were carried out using the purified curcuminoids.

Plant kingdom is a rich source of active components that have been shown to promote the specific cellular and humoral immune response in different ways. This property has increased the interest of the scientific community to discover and develop various therapeutic interventions to be used as medicine against several ailments. This study was conducted to investigate the individual efficacy of curcuminoids (CUR, DMC & BDMC) and their mixture on Freund’s complete adjuvant induced arthritis and the possible mechanisms underlying this effect.

Rheumatoid Arthritis (RA) is an autoimmune inflammatory disease of unknown etiology, which is characterized by a chronic inflammation of synovial joints that leads to a progressive destruction of articular and periarticular structures, leading to severe morbidity and disability (Müller-Ladner U et al., 2005). This disease also affects the tissues surrounding the joints such as skin, blood vessels, and muscles (Asolkar LV et al., 1981). NSAIDs along with major steroids are used to suppress the symptoms, while DMARDs (disease-modifying anti-rheumatic drugs) along with newer therapies like anti-tumor necrosis factor (anti-TNFα) therapy & anti-CD20 therapy are often required to inhibit or cease the underlying immune process (Ling-dong Quan et al., 2008).

The currently available remedies for RA are only temporarily effective and often result in undesired gastrointestinal side effects (Morgan RW., 1999). In addition to this RA patients also seemed to prefer alternative medicine compared to the conventional ones (Alan R. Gaby., 1999). This highlights the need for clinically safe and efficacious natural anti-inflammatory agents. This search attracted significant attention to the plant based moieties that are used in traditional medicine because these drugs elicit fewer side effects, are long acting anti-inflammatory and are inexpensive (Dharamsiri MG et al., 2003). Natural compounds, such as Curcuminoids, may circumvent the side effects of non-steroidal anti-inflammatory drugs and offer new opportunities for RA therapy.
The presently employed Freund’s complete adjuvant (FCA) induced rat adjuvant arthritis (AA) model shares many features of human RA (Shivaprasad H. et al., 2011). The high sensitivity of this screening model to anti-arthritic moieties further supports the selection of presently best available model for RA (Ward JR and Cloud RS., 1996). The study has determined the anti-inflammatory property of Curcuminoids using cellular and histopathological analysis in AA rat model. Furthermore, in order to clarify the immunological activity of curcuminoids, we also investigated its effect on TNF-α, IL-1β, IL-6 and IL-10 release from synoviocytes and mRNA expression of these cytokines in AA rats. We expect that all of these experiments could provide a scientific basis and technological support for alternative RA therapy.

Several mechanisms have been proposed to rationalize the anti-inflammatory effect of curcuminoids, which includes inhibition of arachidonic acid metabolism, matrix metalloproteinase (MMP) & COX-2, LOX, ILs, TNF & NF-κB (Purusotam Basnet and Natasa Skalko-Basnet., 2011). In RA, synoviocytes and synovial macrophages produce a wide array of inflammatory mediators including prostaglandins, reactive oxygen species and proinflammatory cytokines such as IL-1β, IL-6 & TNF-α. Several studies have reported that IL-1β and TNF-α are the key pro-inflammatory cytokines mediating cartilage degradation in patients with RA (Andrew D. Rowan et al. 2003).

CFA administration to rodents produces an arthritic state that is believed to have closely resemble the human rheumatoid arthritis condition, which is characterized by inflammation of joint and surrounding tissue, hyperalgesia and elevation of the concentration of pro-inflammatory cytokines including IL-1β, IL-6 and tumor necrosis factorα (TNF-α), which are closely related with the pathological changes (Yue et al., 2004; Elenkov et al., 2005). Freund’s adjuvant injection into the hind paw induces “primary” inflammatory signs and hyperalgesia at the site of inoculation within hours after injection. Subsequently, “secondary” inflammation and pro-nociceptive signs appear between the 10th and 15th day post-inoculation and are particularly evident in the contralateral paw (Eiseman JL., 1982 and Zimmermann M., 1983). This secondary inflammation is latently associated with the development of the systemic phase of adjuvant arthritis. In the present study this latent
systemic arthritic response, which is characterized by swelling of the non-injected contralateral hind paw and tail, was first evident at 12 days post-adjuvant injection into the ipsilateral hind paw.

Changes in body weight have also been used to assess the course of the disease and the response to therapy of anti-inflammatory drugs. As the incidence and severity of arthritis increases, the changes in the body weights of the experimental rats also occurred during the course of the experimental period. The loss of the body weight during arthritic condition was also supported by earlier observations on alterations in the metabolic activities of diseased rats. Earlier finding suggests that absorption of C-glucose and C-leucine in rat’s intestine was reduced in the case of inflamed rats. But on the treatment with anti-inflammatory drugs, the decrease in absorption was nullified and it shows that the anti-inflammatory drugs correct the decreased/ deranged absorption capacity of intestine during inflammation. The increased body weight during treatment of standard drug and curcuminoids may be due to restoration of absorption capacity of the intestine.

Curcuminoids effectively improved the arthritic symptoms throughout the experiment, leaving the arthritic score and index closer to that of the healthy control group. Paw swelling (expressed as arthritic index) is an important factor in determining the degree of inflammation and therapeutic efficacy of the plant extract in question. A significant reduction in paw swelling (evident by table 7) was observed in CUR, DMC, BDMC, CM and STD treated groups, which indicates the anti-inflammatory potential of Curcuminoids in the order CUR=CM> DMC> BDMC. Surprisingly, the CIA inhibition by CUR was found to be more pronounced than DMC & BDMC suggesting that presence of curcumin in Curcuminoids might be whole and sole responsible for the observed anti-inflammatory effects.

Paw swelling parameter is employed here as an index of measuring the anti-arthritic activity of Curcuminoids at the dose level of 60 mg/kg/b.w./po. Curcuminoids administration showed significant reduction in paw volume and maintained the body weight when compared with the arthritic control group. This depicts a close link between the extent of inflammation and loss in body weight.
The diminished Hb and RBC levels represents anemic condition of arthritic rats which could be attributed to abnormal storage of iron in reticuloendothelial system & synovial tissue and failure of bone marrow to respond to anemic condition. Immune system stimulation against invading antigens might be the possible cause of increase in WBC count of arthritic rats and its significant restoration in Curcuminoids treated group supports its immunomodulatory effect. Restoration of ESR count by Curcuminoids shows its potency in counteracting arthritis in the order of CUR > DMC > BDMC.

Arthritic rats showed alterations in plasma protein concentrations that are manifested as in increase in the globulin fraction and decrease in albumin fraction. Earlier studies revealed that inflammatory mediators increase the permeability of vascular tissues to albumin attributing to decrease in its serum levels (Sanmugapriya Ekambaram et al., 2010; Dolly Mehta and Asrar B. Malik, 2010). Thus Curcuminoids might have a suppressive action on inflammatory mediators as observed by increase in albumin and decrease the globulin level in immuned rats. The presence of inflammation can also be determined by measurement of plasma acute phase proteins. Tissue injury leads to formation of ceruloplasmin in liver following its release into circulation (Denko CW, 1979). Therefore, the elevated level of ceruloplasmin in the serum is an indication of prolong tissue injury during the chronic arthritis (Fisher CL and Gill CW., 1975). Administration of Curcuminoids normalized the deranged levels of fibrinogen and ceruloplasmin which reveals its potential effect in tissue repair.

Pro-inflammatory cytokines directly contribute to cartilage and bone destruction via enhancement of proliferation of fibroblast, increased production of PGE2, synthesis of collagen by synovial cells and to induce biosynthesis and secretion of MMPs and osteoclasts (T. Thalhamer et al. 2008).

It was earlier reported that increased expression of pro-inflammatoty cytokines including
TNF-α, IL-1β and IL-6, plays a significant role in pathophysiology of arthritis development and were detected in both bone regions and serum sample from arthritic patients (Marije I et al.). IL-6 acts as a stimulator of both B and T cell functions; it also promotes proliferation of plasmablastic precursors in the bone marrow (Tonino Alonzi et al. 1998).

In RA as well as FCA, the primary site of inflammation is the synovial tissue, which can release cytokines into the systemic circulation causing measurable plasma levels of TNFa, IL-1b and IL-6 to shoot up. Compelling evidence suggests that the secretion of cytokine IL1 is increased on exposure of mononuclear cells to type II collagen. These cytokines including TNF-α are also released by superoxide radicals (via mechanisms not yet fully defined), macrophages/monocytes and synovial lining cells (synoviocytes) (Sipe et al., 1994). Interestingly, the administration of curcuminoids drastically reduced the levels of these three pro-inflammatory cytokines to almost basal levels. It is, therefore, proposed that Curcuminoids, being an important negative regulator of these pro-inflammatory cytokines related to RA might deactivate the inflammatory response of infiltrating and proliferating synovial cells. Hence, it is plausible to suggest that part of the beneficial anti-inflammatory and cartilage/bone protective effects of Curcuminoids may be mediated through the inhibition of TNFa and IL-b. This, in turn, would lead to reduced production of free radicals and subsequent damage.

Anti-inflammatory cytokine IL-10 is of great concern as it inhibits the production of pro-inflammatory cytokines like IL-1β, TNF-α and IL-6 (A M van Furth et al. 1995). Earlier reports revels clinical and preclinical importance of IL-10 administration against RA (Kathrin Schwager et al. 2009). Increase in IL-10 levels after Curcuminoids administration depicts its anti-arthritis action by shifting the balance of cytokine parameters in the serum towards the production of anti-inflammatory cytokines and away from pro inflammatory cytokines. The mRNA expression of TNF-α, IL-1β and IL-6 were ironically suppressed in the synovium of rats suggesting the anti-inflammatory effects of Curcuminoids on AA rats. Anti-inflammatory cytokines (IL-10) inhibits the production of pro-inflammatory cytokines (IL-1β, TNF-α & IL-6).
Bone destruction is a predominant feature in RA (E M Gravallese, 2002). Radiographic changes in RA conditions are useful diagnostic measures which indicate the severity of the disease. Soft tissue swelling is the earlier radiographic sign, whereas prominent radiographic changes like bony erosions and narrowing of joint spaces can be observed only in the developed stages (final stages) of arthritis (A M van Furth et al. 1995). The radiographic features of the rat joints in adjuvant induced arthritic model are shown in Figure 19. In adjuvant induced arthritic rat (group II), soft tissue swelling along with narrowing of the joint spaces were observed which implies the bony destruction in arthritic condition. The standard drug Diclofenac sodium treated groups have prevented this bony destruction and also there is no swelling of the joint. Similar to histopathological studies, CUR & CM treatment for 28 days have shown significant prevention against bony destruction by showing less soft tissue swelling and narrowing of joint spaces when compared with DMC and BDMC treated groups.

Erosion of trabecular and cortical bone is common, leading to characteristic erosions seen in radiography (Satyanarayana Medicherla et al., 2006). Notably CUR and CM treatment demonstrated no radiographic damage supporting the protective effect of Curcuminoids treatment in the reduction of bone destruction.

Neurodegenerative disorders is a class of neurological diseases marked by extensive neuronal loss in the brain (R. C. Herdman and B. B. Potter., 1990). Progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta region leads to the progression of Parkinson’s disease (PD). This is followed by depletion of striatum dopamine content (H. Yuan et al., 2006). When up to 80% of the dopamine-producing cells are damaged, and are not able to produce enough dopamine, then the motor symptoms (bradykinesia, resting tremor, rigidity, and postural disturbances) of PD appear. 6-OHDA, a potent neurotoxin, can severely damage dopaminergic neurons in substantia nigra, leading to a significant decrease in dopamine levels, followed by precise behavioral, biochemical, and pathological changes distinctive in PD. These toxic effects can be attributed to the formation of various reactive oxygen species, lipid peroxidation, depletion of reduced glutathione, and mitochondrial complex I deficits (A. Schober., 2004). 6-OHDA-lesioned rat model has a
measurable motor deficit, which can be seen by apomorphine-induced contralateral rotations (S. M. Papa and T. N. Chase., 1996). Although progress has been made in the symptomatic treatment of PD since the discovery of L-dopa in the 1960s, no effective neuroprotective therapy is yet available (M.-F. Chesselet and F. Richter., 2011). This provoked us to evaluate the efficacy of a widely used dietary spice; turmeric-(Curcuma longa), which is considered useful in neurodegenerative disorders, both in vivo (G. M. Cole, B. Teter, and S. A. Frautschy 2007) and in vitro [H. Hatcher., 2008; B. B. Aggarwal and B. Sung., 2009].

Unilaterally 6-OHDA-lesioned rats are suitable for evaluating in vivo behavioral recovery in studies of neuroprotective drugs, gene therapy, and the regeneration of transplants from neural or embryonic stem cells (Deumens R et al., 2002; Inden M et al., 2003; Kitamura Y & Nomura Y., 2003). Since 6-OHDA is strongly neurotoxic in the in vivo rat nigrostriatal DA pathway, the injection of 6-OHDA alone has been shown to reduce the number of TH-immunopositive neurons in the level of TH protein in striatum by more than 90% (Hefti F., 1980). In addition, marked DA depletion causes the supersensitivity of DA receptors in the striatum, and the administration of apomorphine then induces contralateral rotation (Ungerstedt U., 1971; Schwarting RKW & Huston JP., 1996; Deumens R et al., 2002). Although 6-OHDA is one of the most common neurotoxins used to experimentally model nigral degeneration in vivo as well as in vitro, it has also been reported that 6-OHDA is present in both rat and human brains as well as in the urine of L-DOPA-treated PD patients (Blum D et al., 2001). Under physiological conditions, 6-OHDA is rapidly and non-enzymatically oxidized by molecular oxygen to form hydrogen peroxide and the corresponding p-quinone (Gee P & Davison AJ., 1989; Graham DG et al., 1978). Various downstream mechanisms for its toxicity have been proposed, including the production of reactive oxygen species (ROS), the interaction of one or more of its oxidation products with nucleophilic groups of several intraneuronal proteins, or with peptides like glutathione (Graham DG et al., 1978; Gee P & Davison AJ., 1989), and the inhibition of mitochondrial complexes I and IV (Blum D et al., 2001, Glinka YY & Youdim MB., 1995). After the injection of 6-OHDA into the SNpc, dopaminergic neurons undergo degeneration within 24 h.
6-OHDA was found to produce the deleterious effect on motor coordination of rats as evident by significant shortening of the time of fall from the rotating rod. All the three curcuminoids, that is, CUR (260.33), DMC (243.83), and BDMC (193.40), significantly improved motor coordination compared to lesioned, 57.16, group. However, BDMC was found to be inferior followed by DMC in comparison to other curcuminoid, that is, CUR & CM which showed similar activity (Figure 21).

Actophotometer study was carried out to evaluate the potential depression caused by 6-OHDA induced neurodegeneration, BDMC (133.16) showed no improvement in locomotor activity whereas CUR (191.66) and DMC (161.00) showed significant improvement in 6-OHDA induced depression (69.00) caused by striatal neurodegeneration (Figure 21).

6-OHDA was injected in right coordinates of brain, thereby damaging nigrostriatal dopaminergic neurons. Injection of D2 agonist, that is, apomorphine, increases the D2 stimulation in the left hemisphere of brain resulting in peculiar circling frequency in the lesioned animals. Effect of 6-OHDA in producing the lesion was prevented significantly by CUR and CM and not by other two curcuminoids, that is, DMC and BDMC (Figure 21). It signifies that CUR is superior in preventing the damage caused by 6-OHDA compared to its other congeners.

Although the etiology of Parkinson disease has not been fully elucidated, the generation of reactive oxygen species (ROS), leading to oxidative stress, together with a relative paucity of antioxidant defenses in the substantia nigra and nigrostriatal dopaminergic pathway, is widely considered as the final biochemical cause of neuronal death (Gille et al., 2004). The brain is thought to be vulnerable to oxidative damage due to its high oxygen consumption, presence of high levels of polyunsaturated fatty acids, and the non-regenerative nature of neurons (Floyd and Carney, 1992). The free radical induced damage is major mechanism through which setting of Parkinson’s symptoms are evident in the model. Glutathione and GPx protect cells from oxidative stress. Depletion of GSH is the earliest biochemical change in the brains of PD patients (Perry et al., 1982). The enzyme GPx is involved in reducing hydrogen peroxide to water, in the presence of reduced glutathione. Central nervous system
cells are vulnerable to the free radicals toxicity because they have a high rate of
catecholamine oxidative metabolic activity (Fendri et al., 2006). We observed reduced levels
of GSH and GPx in the PD mouse corpus striatum. Improvement of GSH and GPx activities
after curcuminoids treatment observed in the present study suggests that curcuminoids
induces antioxidants in the PD mouse brain. It is substantiated by significant decrease in
GSH levels (0.95) in the lesioned group compared to sham control (1.91). These levels were
restored with CUR (1.65), DMC (1.36), and BDMC (1.31) treatment. However, CUR and
CM was found to be more effective than the other two curcuminoids.

GPx and GR levels were also reduced with 6-OHDA and were subsequently found to
increase in a manner in which GSH levels were restored (Table 10). In none of the treated
groups GSH levels were found to be enhanced compared to sham group indicating that
curcuminoids are not responsible for the upregulation of the enzyme rather they only prevent
the degradation of the antioxidant enzymes. 6-OHDA is known to undergo nonenzymatic
degradation to produce superoxide radicals; furthermore, it enhances the release of iron from
ferritin which catalyzes the conversion of hydrogen peroxide into free hydroxyl radicals.
These superoxide and hydroxyl radicals are neutralized by SOD and catalase. SOD and
H2O2-removing enzymes protect the cell against reduced intermediates of oxygen produced
during normal aerobic metabolism, but they seem unable to cope when production of O2 and
H2O2 is excessive. SOD converts O2 to H2O2 (Freeman and Crapo, 1982; Fridovich, 1975).
Catalase, which is found at very low levels in the brain, also removes H2O2. Restoration of
the activities of SOD and CAT due to pretreatment with curcuminoids further demonstrates
the protective role of curcuminoids in 6-OHDA toxicity and is supported by various studies
(Perumal et al., 1992; Zafar et al., 2003a,b; 2 Ahmad et al., 2005a,b), in which parkinsonism
was partially protected by the application of antioxidants. The level of these SOD and
catalase enzymes was found to be reduced to 0.76 and 3.58, respectively, upon
administration of 6-OHDA when compared to sham group. The enzyme levels were restored
with the administration of CUR, DMC, and BDMC; 1.73, 1.43, and 1.28 for SOD and 6.98,
5.21, and 5.18 for catalase, respectively.

The elevated levels of TBARS in the brain of PD mouse observed in the present study
suggest that lipid peroxidation is increased in the PD mouse striatum. The high amount of TBARS was also reported in the brain of MPTP-treated monkeys (Marzatico et al., 1993) further supporting our study. Treatment of PD mouse with Curcuminoids reducing TBARS level observed in the present study suggests that Curcuminoids prevents lipid peroxidation. Restoration of enzymes are corroborated with fall in thiobarbituric acid-reactive substances (TBARS) upon administration of CUR, DMC, and BDMC (19.67, 25.83, and 27.00) which was found to be enhanced upon administration of 6-OHDA (33.50) when compared to sham group (12.17).

The neurotransmitter, DA plays a key role in body movement and motor control. Reduced levels of catecholamines and oxidative stress are the contributing factors of neurodegeneration in PD and this leads to the loss of motor function seen in the patients with PD (Mishra et al., 2000; Halliwell, 2001; Koutsilieri et al., 2002; Jenner, 2003; Olanow, 2007). Our present study suggests that DA, DOPAC and HVA levels are reduced in the corpus striatum of the PD mouse. These catecholamine levels also found to be decreased in PD patients (Hinterberger, 1971; Piggott et al., 1999). Increase of these catecholamine levels after curcuminoids treatment suggests that curcuminoids induces catecholamines in the PD mouse corpus striatum. The mean levels of DA, DOPAC, and HVA in the rat striatum are shown in Table 11. Rats in lesion group showed significant reduction of striatal dopamine, DOPAC, and HVA levels when compared with those of vehicle-treated rats. Curcuminoid pretreatment afforded a significant restoration in their content in the order: CUR=CM > DMC > BDMC.

These findings were further supported by immunohistochemical staining of dopaminergic neurons in contralateral striatal region of brain by tyrosine hydroxylase (TH) antibodies. These primary TH antibodies recognize tyrosine hydroxylase enzyme as antigens, and Figures 22 showed depletion in percentage staining of TH immunopositive neurons with marked damage to specific subnuclei of the substantia nigra pars compacta, with severe obliteration of their neuromelanin-laden projection neurons in lesioned as compared to sham group. Curcuminoids-pretreated groups did not show any marked reduction in percentage area of TH-immunopositive neuron in L+C, 95%; L+DMC, 75% and L+BDMC, 68%
compared to sham group signifies the shielding of dopamine receptors/neurons by curcuminoids. Curcumin was found to be superior compared to its other derivatives in this shielding.

Ameliorative effect of CUR in apomorphine induced increase in contralateral rotations may be either due to blockage of D2 receptor by CUR or by preventing the neuronal loss of dopaminergic neurons. Ability of CUR and its other counterparts in blocking D2 receptors needs to be studied either by radiolabelling these compounds or docking these compounds in silico with D2 receptor structure.

Although oral bioavailability of CUR is poor (>2%) but it crosses BBB due to its high lipophilic nature and low molecular weight. Due to its penetration in the brain, it has been widely explored in various neurological disorders. In a previous study (L. Zecca et al., 2003), it was found that CUR binds and destabilizes amyloid beta plaques and has pronounced beneficial effect in the treatment of AD. Furthermore the structural similarity with BBB permeable dyes like congo red and thioflavin T (K. A. Malkus et al., 2009), confirms its high penetration in brain to make it a suitable chemical compound for treatment of neurological disorders. It is due to the presence of two aromatic rings in the structure of CUR that attaches to amyloid beta plaques, and these two aromatic rings are essential for its biological activity. CUR contains two phenyl methoxy groups: DMC contains one and BDMC contains none. Since CUR is showing maximum, DMC intermediate and BDMC least activity, this suggest that phenyl methoxy group in chemical structure plays a key role in ameliorating PD. Contrary to these findings, their antimetastatic activity of CUR, DMC, and BDMC was found to be in the order of BDMC > DMC > CUR, (B. J. Kelley and D. S. Knopman., 2008) increased penetration of these compounds into brain in order of CUR > DMC > BDMC, explains higher potency of CUR in shielding brain against 6-OHDA-induced PD. Furthermore, these curcuminoid induced restoration of dopamine levels in the brain was indirectly observed by D2 binding studies, a direct evidence of measuring the dopamine level in brain homogenates with chromatographic/immunological methods will gives authenticity to the radioligand binding study. HPLC estimation of dopamine levels in homogenates of substantia nigra region shows that CUR is more potent in restoring the
dopamine levels and other metabolites compared to its other counterparts. Formation of lewy bodies is a hallmark of Parkinson induced brain lesion as seen in the histopathological examination of brains substantia nigra region, absence of lewy bodies in 6-OHDA-induced parkinson’s model exhibits a lacunae in the models being used therefore study of these compounds in MPTP-induced model is also solicited which is clinically more relevant (F. Yang et al., 2005). Ability of individual curcuminoids in crossing BBB will further support our findings that CUR is more potent than DMC and BDMC. Since curcuminoids have shown promising effect improving 6-OHDA-induced brain lesions, a regenerative potential of these compounds can be studied to make them more suitable for therapeutic use rather than of mere prognostic importance. Apart from induced neuronal death through ROS, 6-OHDA is also known to induce apoptosis by activation of apoptotic genes and caspase enzymes. Ability of these curcuminoids in inhibiting apoptotic genes and caspase enzymes need to be explored out individually (S. Yodkeeree et al., 2009). Moreover, ability of CUR and its analogs to bind to dopamine receptors as agonist is another mechanism for which a study is solicited to corroborate this hypothesis. However, CUR, DMC, and BDMC have shown a prognostic beneficial effect in preventing the neurodegeneration.

In vitro screening has been performed to assess the effect of CUR, DMC, BDMC & CM on cellular proliferation of MCF-7 cells at different time points, using the MTT assay. Reduction of MTT in isolated cells is regarded as an indicator of cell redox activity and the reaction is attributed mainly to mitochondrial enzymes and electron carriers (Bernas and Dobrucki, 2000).

Many studies have demonstrated inhibitory effects of curcuminoids on tumorigenesis and tumor growth in vitro and in vivo. These observations suggest a need for further research on the chemopreventive and anticancer activities of curcumin and its derivatives. In the present work we have shown that curcuminoids are potent inhibitors of MCF-7 cell proliferation in the order DMC ≥ CM > CUR > BDMC. The growth of MCF-7 cells was suppressed by CUR, DMC, BDMC & CM in a time and dose dependent manner.

Our results show that DMC and CM is a better inhibitor than CUR and BDMC suggesting
that there is no synergistic action of CUR & BDMC in inhibiting cell proliferation. A similar study was conducted in 1998 by Simon et al., 1998. As the literature survey for this study was very less and therefore to develop a consensus this study was planned to investigate the role of individual curcuminoids and its mixture in inhibiting MCF-7 cells proliferation. Although our study corroborates the earlier findings, it is further needed to investigate to molecular mechanism of each individual curcuminoids.

In other pharmacological screenings systems, it has been reported that CUR effectively possesses a more potent activity than DMC and BDMC. Our results indicates that methoxyl groups are not essential for the activity; demethoxycurcumin, the best inhibitor, possesses one methoxyl group, while curcumin and bisdemethoxycurcumin possess two and no methoxyl groups, respectively, and are less inhibitory.

The in vivo & in vitro studies conducted for evaluation of pharmacological activities of individual isolated curcuminoids i.e curcumin, demethoxycurcumin & bisdemethoxycurcumin and their combination clearly demonstrated that individual curcuminoids have different pharmacological actions. The present in vivo study revealed a promising anti-arthritic & neuro-protective activity of isolated curcuminoids with curcumin showing the maximum efficacy indicating that the other two curcuminoids might not have any pharmacological synergistic action, while on the other hand in vitro studies on breast cancer cell line raveled the importance of DMC among the other curcuminoids. Thus, further pharmacological screening has to been done to explore the pharmacological action of individual curcuminoids at molecular level for further development of these plant derived molecules into highly beneficial therapeutic option in terms of safety, bioavailability, efficacy, availability as well affordability.

Further studies to explore the mechanism of action, efficacy and safety in clinical trials studies of individual curcuminoids are extremely important as it can be used in daily routine dietary therapy.