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COMPARATIVE EFFECT OF PHOSPHODIESTERASE INHIBITORS ON INTRACEREBROVENTRICULAR COLCHICINE MODEL OF MEMORY IMPAIRMENTS IN RATS

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Abstract
Alzheimer's disease (AD) is a progressive, age related neurodegenerative disease, characterized by a progressive decline in memory, cognitive performance and loss of acquired skills leading to apraxia, agnosia and aphasia. Central colchicine induced cognitive dysfunction and oxidative stress in rats closely resembles with the characteristic features of Alzheimer's type of dementia. The present study was designed to investigate the comparative effect of vinpocetine (PDE1 inhibitor) and Ro 20-1724 (PDE4inhibitor) on intracerebroventricular (ICV) colchcine induced experimental sporadic dementia of Alzheimer's type. Enhancing cyclic nucleotides signaling by inhibition of phosphodiesterases (PDEs) is known to be beneficial in disorders associated with cognitive decline. Infusion of colchicine impaired learning and memory, increased oxidative– nitritive stress and induced cholinergic hypofunction in rats. Treatment with vinpocetine (5, 10 and 20 mg/kg i.p.) and Ro 20-1724 (0.5, 1 and 2 /kg i.p.) for 21 days following first ICV colchicines administration significantly improved learning and memory in Morris water maze and passive avoidance paradigms. Furthermore, both PDE 1 and 4 inhibitors significantly reduced the oxidative–nitritive stress, as evidenced by decrease in malondialdehyde (MDA) and nitrite levels, and restored the reduced glutathione (GSH) levels. Significant increase in acetylcholinesterase activity and lactate dehydrogenase levels was observed in the present model indicating cholinergic hypofunction and increase in neuronal cell damage. The possible mechanism of said effects of the compounds investigated may be due to their PDE inhibitory activity resulting in enhanced levels of second messengers which may be consisting to the anti-oxidant and anti-inflammatory action, and modulation of cholinergic activity and prevention of cell damage collectively leading to cognitive enhancement.

Keywords: - Alzherimer's diseases; Intracerebroventricular; Rat; PDE

Introduction
Neurological disorders are a heterogeneous group of diseases of the nervous system having different etiologies. A variety of CNS disorders including Alzheimer's disease (AD), Parkinson's disease, Huntington's disease, Amyotrophic lateral sclerosis (ALS), brain abscess, multiple sclerosis, spinal cord injury, cerebral stroke and traumatic brain injury are characterized primarily by neurodegeneration and neuroinflammation [1]. Though stroke and AD have somewhat different etiologies yet they share common features such as oxidative stress, excitotoxicity, neurotransmitter deficits, energy failure and neuronal cell death that leads to cognitive dysfunction [2,3]. Stroke is an acute cerebrovascular disease which occurs as a result of sudden interruption of blood supply [4] to a part of brain typically by a thrombus or embolus occlusion or hemorrhage due to rupture of blood vessels [5]. On the other hand, Alzheimer's disease is an age related [6], progressive, irreversible [7], chronic neurodegenerative disorder [8] characterized by the presence of senile plaques and neurofibrillary tangles, composed of extracellular deposits of Amyloid beta (Aβ) & hyperphosphorylated microtubule-

associated protein such as Tau [9]. Alzheimer's disease and Stroke have a common final finding i.e. cognitive impairment and this may occur as a result of multi-neurotransmitter deficits [10], decreased availability of intracellular molecules and axonal transporters through which the different neurons communicate with each other to maintain neuronal excitation and cognitive functioning [11,12]. Intracellular molecules also known as secondary messengers such as cyclic nucleotides i.e. cAMP and cGMP play a critical role in neuronal signaling and synaptic plasticity by activation of several pathways like cAMP /PKA /CREB, cGMP/PKG/CREB and factors like brain-derived neurotrophic factor (BDNF) [13], semaphorins, netrin-1&16 and nerve growth factor (NGF) [14], Neurotrophins 3,4,5-inhibitory factors associated with myelin and myelin associated glycoprotein (MAG) [15]. These pathways and factors are well known to help in neuronal survival, neurogenesis and protect neurons from injury [16]. Elevation of cAMP causes both short- and long-term increase in synaptic strength [17] and stimulates cholinergic amacrine cells to release acetylcholine [18]. But, the levels of cAMP and cGMP are reported to be decreased in neuropathological conditions including cerebral stroke and AD [19,20]. Thus, agents that enhance cAMP/PKA/CREB & cGMP/PKG/CREB pathways have potential for the treatment of stroke, AD and other neurological diseases [21]. Moreover, cAMP & cGMP mediate signaling of several neuro transmitters including serotonin, acetylcholine, glutamate & dopamine [22] which play important role in cognitive functioning [23]. Furthermore, elevation of cAMP & cGMP levels is known to restore the energy levels [24], reduce excitotoxic damage [25], prevent Aβ mediated neurotoxicity [20], enhance biosynthesis & release of neurotransmitters [23], inhibit apoptotic and necrotic cell death [26] leading to improvement in cognitive functioning [27,28]. Central administration of cAMP and cGMP has been reported to enhance neuronal survival and memory performance [29]. In view of the above, the enhancement and prolongation of cAMP and cGMP signaling can thus be helpful in dealing with neurodegenerative disorders including Stroke and AD. This can be accomplished by inhibiting the phosphodiesterase enzymes which metabolize these cyclic nucleotides. The family of PDEs has been classified into eleven isoforms based on their substrate selectivity for either cAMP or cGMP or both [16,30]. Among different isoforms of PDEs, PDE 1 & 4 is found to localize in hippocampus and cortex which are involved in cognitive functioning [31]. Therefore, as already mentioned above, one of the alternatives to enhance the levels of cAMP & cGMP secondary messengers or to enhance CREB phosphorylation can be achieved through inhibition of phosphodiesterases which are meant to decrease these cyclic nucleotides. Based on important and versatile role of cAMP and cGMP signaling in regulation of neuronal functions, the focus of the present study was designed to investigate the differential role of PDE 1 (cAMP& cGMP selective) and PDE 4 (cAMP selective) inhibition in experimental models of Stroke and Alzheimer type of dementia.

Materials And Methods

Animals

Male Wistar rats (weighing 220-250 gm) obtained from Central Animal House facility of the Institute were employed in the studies. The animals were kept in polyacrylic cages with wire mesh top and soft bedding. They were kept under standard husbandry conditions of 12 hr light and darkness cycle with food and water maintained at 22 ±2 ^{0}C and at humidity of ≤40 % and ≥ 50 %. The experimental protocols were approved by Institutional Animal Ethics Committee (IAEC) as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animal (CPCSEA), Government of India. Animals were acclimatized to laboratory conditions prior to experimentation.

Drugs and Chemicals

Phosphodiesterase inhibitor Ro 20-1724 was purchased from Tocris, Biosciences, UK (Nu Life, India). Its solution was prepared in normal saline. It was administered at a dose of 0.5, 1 & 2 mg/kg intraperitoneally.

Vinpocetine was obtained as an ex gratia sample from Covex Pharma, Germany. It was dissolved in acidic saline (pH 5.5) and administered in three different doses i.e. 5, 10 and 20 mg/kg. The total volume injected intraperitoneally ranged between 0.1–1 ml.

The doses of Ro 20-1724 [32] and vinpocetine [33] were selected on the basis of earlier reports, which had demonstrated their efficacy in other models and ranged from 1-5 and 2-20 mg/kg, respectively.

Colchicine (Sigma Chemicals Co., St. Louis, MO, USA) was dissolved in artificial cerebrospinal fluid (aCSF) and administered intracerebroventricularly (ICV) at a dose of 15 µg/5 µl/rat [34]. All other chemicals used were of analytical grade (AR). All the drugs and chemical & solutions were freshly prepared.

Experimental Models

ICV Colchicine induced Alzheimer type dementia

In animal models of central nervous system damage, colchicine, a microtubule-disrupting agent, is used as a neurotoxin [35,36]. Following its introduction directly into the brain, colchicine binds irreversibly to tubulin, the principal structural protein of the microtubule, and induces microtubular depolymerization and destabilization, with subsequent block of axonal transport and mitosis, resulting in neuronal cell death. The neurotoxicity is mediated through free radical production and the resultant oxidative stress [37]. In addition, colchicine causes loss of cholinergic neurons, destruction of cholinergic pathways, and decrease in cholinergic turnover [38]. The distribution of colchicine in the brain is unequal; its concentration in the hippocampus, the area most affected in Alzheimer's disease – is almost three times higher than in other brain regions [39]. The drug selectively blocks acetylcholine transferase in the hippocampus and basal forebrain - the brain areas responsible for memory consolidation [36,38]. Clinically, the result of central colchicine administration is progressive deterioration of learning and memory, i.e., cognitive impairment. Because microtubules have a major role in neuronal plasticity, microtubule disruption could be also directly responsible for cognitive defects [40]. The central manifestations of colchicine neurotoxicity in the animal model closely simulate Alzheimer's disease in humans [41]. Both the colchicine induced neurotoxicity as well as Alzheimer's disease is characterized by oxidative stress, microtubule disruption, decrease in cholinergic activity and progressive deterioration of cognitive functions [42]. Systemic administration of colchicine in rats also induced cognitive defects similar to those of Alzheimer and characterized by amnesia of recent learning and loss of formerly established memories [39]. Colchicine also increases the expression of inducible nitric oxide synthase [43] and cyclooxygenase-2, thereby increasing nitric oxide (NO) and prostaglandin's synthesis, respectively [44]. Nitric oxide, a precursor for free radicals reacts with the superoxide anions produced as a result of excitotoxicity, peroxidase activity of cyclooxygenase and increased oxidative stress after central administration of colchicine that give rise to toxic intermediates (peroxynitrite, nitric dioxide) [45,46]. Overproduction of nitric oxide is neurotoxic to cholinergic neurons [47].

Surgical procedure for ICV colchicines induced Alzheimer type dementia

Surgery was performed according to a protocol previously described by Kumar et al. [34,35]. Animals were anaesthetized with sodium pentobarbital (45 mg/kg, i.p.) and placed in a stereotaxic apparatus. The head was positioned in a frame and a midline saggital incision was made in the scalp. Two holes were drilled through the skull for placement of injection cannula into the lateral cerebral ventricles. The scalp was then closed with sutures. After surgery, animals received gentamycin (5 mg/kg, i.p.) injection to prevent sepsis. Animals were infused intracerebroventricularly (ICV) with either artificial cerebrospinal fluid (aCSF; 147mM NaCl, 2.9mM KCl, 1.6mM $MgCl_2$, 1.7mM $CaCl_2$ and 2.2mM dextrose) or 15 µg colchicine, dissolved in aCSF solution (5 µl), using a Hamilton microsyringe positioned in the injection cannula. In sham operated rats, the surgery was identical except for drilling of holes and placement of the cannula. Coordinates for the injection were 0.8 mm posterior to bregma, 1.8 mm lateral to saggital suture and 3.6 mm beneath the cortical surface. To promote diffusion, the microsyringe was left in place for a period of 2 min following injection. Special care was taken during the postoperative period to provide food and water inside the cage of the rat. Drugs were administered to different animals according to the grouping of experimental animals given below from day 1 of colchicine ICV injection and the treatment was continued for 21 days.

Grouping of Experimental Animals

1. Sham control
2. Vehicle control (aCSF only)
3. ICV Colchicine control
4. ICV COL + VIN (5 mg/kg, i.p.)
5. ICV COL + VIN (10 mg/kg, i.p.)
6. ICV COL + VIN (20 mg/kg, i.p.)
7. VIN (Per se, 20 mg/kg, i.p.)
8. ICV COL + Ro (0.5 mg/kg, i.p.)
9. ICV COL + Ro (1 mg/kg, i.p.)
10. ICV COL + Ro (2 mg/kg, i.p.)
11. Ro (Per se, 2 mg/kg, i.p.)

ICV- Intracerebrovenricular, COL- Colchicine and aCSF- Artificial cerebrospinal fluid

Biochemical Parameters

In ICV colchicine model, the biochemical estimations were carried out on the 22nd day after ICV colchicine treatment.

Tissue preparation

Animals were sacrificed by decapitation and brains were removed and rinsed with ice cold isotonic saline. Brain tissue samples were then homogenized with ice cold 0.1 M phosphate buffer [pH 7.4, 10 times w/v of the weight of the tissue]. The homogenates were centrifuged at 10,000×g for 15 min and aliquot of supernatants were separated and used for biochemical estimations [35].

Protein estimation

Protein was measured in all brain samples for oxidative parameters and acetylcholine esterase activity by the method of Lowry [48]. Bovine serum albumin (BSA) (1 mg/ml) was used as standard.

Acetylcholine esterase assay

The quantitative measurement of acetyl cholinesterase levels in brain was performed according to the method described by Ellman [49]. The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01M sodium phosphate buffer (pH 8), 0.10 ml of acetylthiocholine iodide and 0.10 ml of DTNB (Ellman reagent). The change in absorbance was measured immediately at 412nm spectrophotometerically. The concentration of acetylcholinestrase activity in the supernatant was expressed as nmol per mg protein.

Measurement of MDA

The quantitative measurement of MDA product of lipid peroxidation in brain was performed according to the method of Wills [50]. The amount of MDA was measured by reaction with thiobarbituric acid at 532nm using spectrophotometer (Shimadzu, UV-1700). The concentration of malondialdehyde in the supernatant was determined from a standard curve and expressed as nmol per mg protein.

Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO), was determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) Ethylenediame dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green [51]. Equal volumes of supernatant and Greiss reagent were mixed; the mixture was incubated for 10 min at room temperature in the dark and the absorbance at 540nm and was determined spectrophotometerically. The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as μmol/ mg protein.

Measurement of GSH

Reduced glutathione in brain was estimated according to the method described by Ellman [52]. One ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4°C for 1h. The samples were centrifuged at 1200 ×g for 15 min. To 1 ml of this supernatant, 2.7 ml of phosphate buffer (0.1M, pH 8) and 0.2 ml of 5,5′dithio-bis (2-nitrobenzoic acid) (DTNB) were added. The yellow color developed was read immediately at 412nm using a spectrophotometer. The concentration of glutathione in the supernatant was determined from a standard curve and expressed as μmol/mg protein.

Measurement of Myeloperoxidase (MPO) activity

Myeloperoxidase activity is an indicator of polymorphonuclear leukocyte accumulation (that infiltrate oxidative damaged region of the brain) and measured according to the method described by Mullane [53]. Each sample of tissue was washed with sterile normal saline, weighed and homogenized in an ice-cold solution containing 0.5% hexadecyltrimethylammonium bromide, dissolved in 10mM potassium phosphate buffer, pH 7.0. The resulting homogenate was centrifuged for 30 min at 20,000×g at 4° C. To an aliquot of the supernatant was added 1.6mM tetramethyl benzidine and 0.1mM hydrogen peroxide. MPO activity was calculated as the change in absorbance (ΔA) at 650 nm over 1 min and expressed as $\Delta A\ min^{-1}$ mg $protein^{-1}$.

Measurement of Lactate Dehydrogenase (LDH) Activity

LDH is an oxidoreductase enzyme which catalyzes the interconversion of lactate and pyruvate. When disease or injury affects tissues, the cells release LDH, where it is identified in higher than normal levels. Therefore, LDH is most often measured to evaluate the presence of tissue or cell damage. The level of LDH represents the necrotic cell death and level of LDH is increased in neuroinflammation [54]. Measurement of LDH was accomplished by using a commercial kit (Reckon Diagnostics, Baroda).

Behavioral Study

Morris water maze (MWM)

The maze consisted of white fiber glass circular pool (2m diameter), filled with an opaque mixture of water and white non-toxic dye maintained at 26-28 ^{0}C. The pool was located in the center of a sound attenuated room. The pool was divided into four equal quadrants: northeast, southeast, northwest, and southwest [55]. A hidden platform (9cm dia) submerged 1 cm below the surface of water was placed in the middle of one of the quadrant. During the acquisition phase of the experiment, each rat was given four trials each day until they took approximately 15 seconds to find the platform. During this time, the hidden platform was submerged in the centre of one of the 4 quadrants and

remained in that location for the entire duration of experiment. For each trial, the rat was taken from the home cage and placed into the water maze at one of the four quasi-randomly determined locations with its head facing and almost touching the pool wall. Trial began when the rat was released by the experimenter and ended when it climbed to the top of hidden platform. The mean escape latency was recorded. The maximum trial length was 60 seconds. If by that time, the rat was not able to locate the top of the platform, the trial was terminated. It was guided to the platform where it was allowed to stay for 20 seconds and then again the process of recording escape latency of another 60 seconds was started. The rat was removed from the platform and placed in the pool again to begin next trial. Normally, the escape latency declines during acquisition trial from 60s to around 15s when the animal learns the location of the hidden platform. In colchicine ICV model, initial acquisition latency (IAL), the 1st retention transfer latency (1 RTL) and 2nd retention transfer latency (2 RTL) were recorded on 13th day, 14th and 21st day, respectively after the colchicine ICV injection. The cut off time was 120 sec.

Assessment of Gross Behavioral Activity (Closed Field Activity)

Gross behavioral activity was assessed by digital Actophotometer. Each animal was observed over a period of 5 min in a square (30 cm) closed arena equipped with infrared light-sensitive photocells and values expressed as counts per 5 min. The beams in actophotometer cut by the animal were taken as measure of movements. The apparatus was placed in a darkened, light- and sound- attenuated and ventilated testing room [56]. In ICV colchicine on zero day (previous day to the performing of surgery) and then on 21st day after ICV administration.

RESULTS

Effect of Vinpocetine and Ro 20-1724 on behavioral parameters

Gross behavioral activity

In the present series of experiments, the mean score of gross behavioral activity remained unchanged throughout the period of observation for 21 days in sham-operated, aCSF- and colchicine-injected rats. Chronic administration of vinpocetine (5, 10, 20mg/kg) and per se group of animals (20 mg/kg) as well as Ro 20-1724 (0.5 & 1 mg/kg) was not found to have any effect on gross behavioral activity in experimental animals. However, Ro 20-1724 (2 mg/kg) in colchicine injected animals and per se group of animals (2 mg/kg) did show significant reduction in the locomotor activity. The results are presented in Table.1, Fig.1.

Table: 1. Effect of vinpocetine and Ro 20-1724 on behavioral parameters evaluated in ICV colchicine Model

Groups	Locomotor activity (counts per 5 min.)	MWM (in sec.)		
		IAL	1st RTL	2nd RTL
Sham	356.67 ± 10.904	63.45 ± 5.3	11.08 ± 3.3	12.08 ± 1.5
aCSF	352.33 ± 11.589	66.84 ± 3.1	12.84 ± 2.1	12.43 ± 1.7
ICV COL	344.83 ± 8.677	114.42 ± 5.4[a]	88.45 ± 4.1	85.15 ± 2.6
Vin 5	348.8 ± 14.59	87.12 ± 4.4[b]	62.35 ± 2.8	59.25 ± 2.1
Vin 10	349.67 ± 11.158	70.46 ± 4.1[b]	46.31 ± 2.4	42.41 ± 1.8
Vin 20	355.17 ± 12.38	66.63 ± 3.2[b]	44.19 ± 3.1	39.06 ± 1.4
Vin(per se)	358.2 ± 16.48	47.51 ± 4[a]	11.07 ± 2.6	10.71 ± 2.1
Ro 0.5	351.5 ± 11.21	69.71 ± 3.5[b]	43.84 ± 2.4	40.15 ± 2.4
Ro 1	326 ± 19.85	55.16 ± 2.6[bc]	22.7 ± 1.9	20.24 ± 3.7
Ro 2	254 ± 7.904[bc]	81.85 ± 2.4[a]	83.43 ± 3.4	87.34 ± 4.5
Ro(per se)	247.5 ± 8.457[bc]	78.76 ± 3.07[a]	84.84 ± 1.7	90.64 ± 3.8

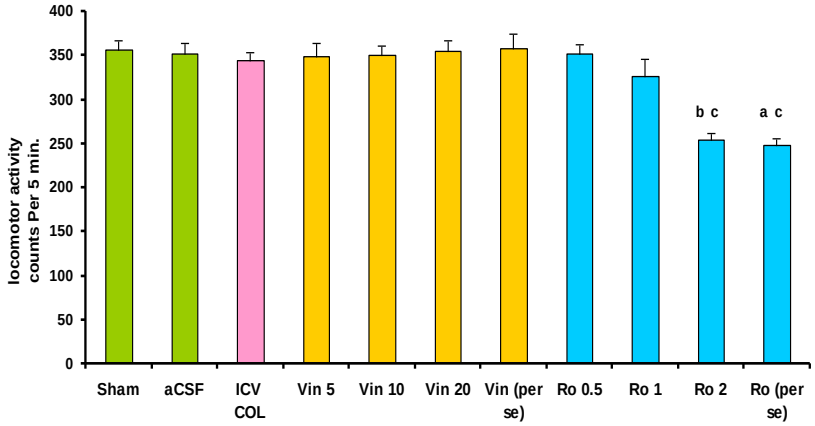

Figure 1. Effect of Vinpocetine and Ro 20-1724 gross behavioral activity in intracerebroventricular colchicine-injected rats. Values are mean ±SEM. a: P < 0.05 as compared to Sham group; b: P < 0.05 as compared to colchicine-injected group; c: P< 0.05 as compared to Vin 20 +Col rats. (One way ANOVA followed by Tukey's test for multiple comparisons).

Spatial navigation task

Sham-operated, aCSF-injected and vinpocetine per se (20 mg/kg) groups of animals quickly learned to swim directly to the platform in Morris water maze on day 13. Colchicine treated rats showed a significant increase in escape latency as compared to sham and aCSF controls but showed improvement in the form of declined escape latency with continued training during the acquisition of spatial navigation task on day 13. Chronic treatment of colchicine treated animals with different doses of vinpocetine (5, 10, 20 mg/kg) showed dose dependent improvement in both acquisition and retention latencies up to 10 mg mg/kg. The effect of 20 mg/kg of vinpocetine was practically equivalent to that of 10 mg/kg.

Vinpocetine per se treatment (20 mg/kg) showed significant improvement in IAL over that of sham & aCSF groups. The other test drug Ro 20-1724 also showed a dose dependent improvement from 0.5 to 1 mg/kg dose. But, like that in BCCAO model, Ro 20-1724 at 2 mg/kg dose impaired both the locomotor activity as well as cognitive function significantly and the same is true with the Ro 20-1724 per se group (2 mg/kg). However, Out of the two drugs investigated in the present study, Ro 20-1724 at 1 mg/kg dose has somewhat better effect w.r.t. the acquisition latency as compared with colchicine injected control rats. Even the retention latencies seem to be significantly improved with this dose as compared to any other dose of Ro 20-1724 or vinpocetine. The retention latencies of 0.5 mg/kg dose of Ro 20-1724 are practically equivalent to that of 10 & 20 mg/kg dose of vinpocetine. The results are presented in Table-1, Fig.2.

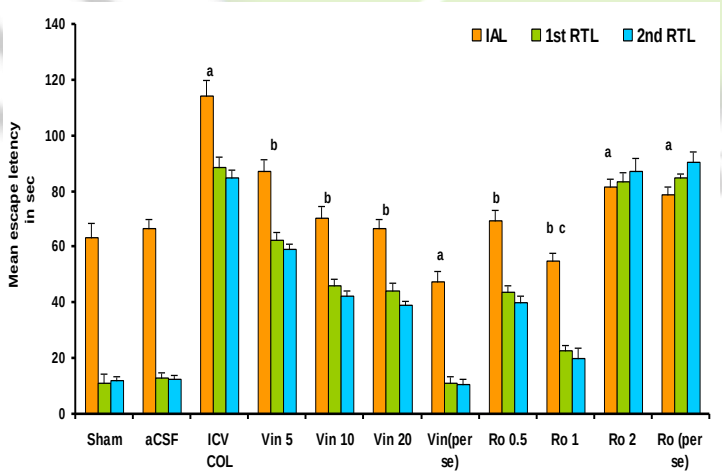

Fig:-2. Effect of vinpocetine and Ro 20-1724 on spatial navigation in intracerebroventricular colchicine-injected rats. Values are mean ±SEM. a: P < 0.05 as compared to sham group; b: P < 0.05 as compared to colchicine-injected group; c: P < 0.05 as compared to Vin 20 +Col rats. (One way ANOVA followed by Tukey's test for multiple comparisons).

Effect of Vinpocetine and Ro 20-1724 on biochemical parameters in brain homogenate

Malondialdehyde (MDA) and glutathione (GSH) levels

Central colchicine administration caused a significant increase in brain MDA levels and depletion of GSH levels as compared to sham & aCSF groups. Chronic treatment (21 days) of colchicine injected animals with vinpocetine (5, 10, 20 mg/kg, i.p) and Ro 20-1724 (0.5, 1, 2 mg/kg, i.p) significantly reduced MDA levels and restored the depleted GSH levels in a dose dependent manner. However, the effect of 10 & 20 mg/kg dose of vinpocetine was found to be practically equivalent w.r.t. MDA levels.The effect of per se groups of vinpocetine (20 mg/kg) and Ro 20-1724 (2 mg/kg) was equivalent to that of sham and aCSF groups w.r.t. both MDA as well as GSH levels. The effect of 1 & 2 mg/kg dose of Ro 20-1724 in colchicine injected rats was significantly better than 10 mg/kg dose of vinpocetine w.r.t. both MDA & GSH levels, though it was equivalent to that of 20 mg/kg dose of vinpocetine w.r.t. restoration of GSH levels. The results are presented in Table- 2, Fig.3 and 4.

Table: 2. Effect of Vinpocetine and Ro 20-1724 on various biochemical parameters evaluated in ICV STZ Model

Groups	MDA (nM/mg protein)	GSH (µM/mg protein)	Nitrite/Nitrate (µM/mg protein)	AChE (nM/mg protein)
Sham	160.167 ± 7.838	11.167 ± 0.510	0.757 ± 0.0528	154.833 ± 6.194
aCSF	157.500 ± 9.895	10.917 ± 0.554	0.760 ± 0.0347	157.667 ± 9.028
ICV COL	165.667 ± 9.982[a]	11.750 ± 0.629[a]	0.885 ± 0.0446[a]	165.667 ± 9.982[a]
Vin 5	169.000 ± 6.050[b]	11.317 ± 0.464[b]	0.757 ± 0.0528[b]	169.000 ± 6.050[b]
Vin 10	499.333 ± 15.517[b]	3.645 ± 0.330[b]	2.862 ± 0.201[b]	410.000 ± 17.677[b]
Vin 20	381.167 ± 12.896[b]	6.430 ± 0.429[b]	1.910 ± 0.0948[b]	309.500 ± 8.831[b]
Vin(per se)	287.167 ± 16.185	8.715 ± 0.232	1.377 ± 0.0999	246.167 ± 15.0443
Ro 0.5	183.167 ± 9.874[b]	11.183 ± 0.533[b]	0.805 ± 0.0300[b]	169.167 ± 6.911[b]
Ro 1	408.000 ± 11.685[bc]	3.893 ± 0.291[b]	2.535 ± 0.151[bc]	394.333 ± 12.852[bc]
Ro 2	358.667 ± 11.176[bc]	6.290 ± 0.497[b]	1.863 ± 0.0356[bc]	301.833 ± 17.215[bc]
Ro(per se)	269.333 ± 15.594	8.617 ± 0.355	1.300 ± 0.0378	242.333 ± 19.503

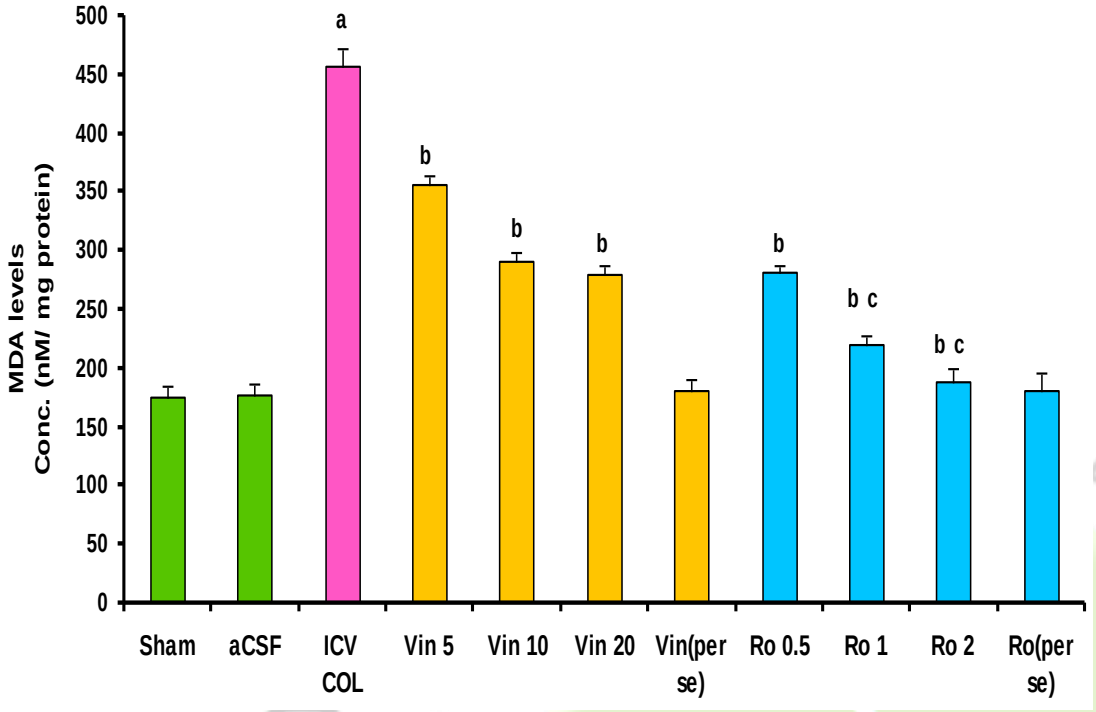

Fig:-3. Effect of vinpocetine and Ro 20-1724 on MDA levels in brain in intracerebroventricular colchicine-injected rats. Values are mean ±SEM. a: P < 0.05 as compared to Sham group; b: P< 0.05 as compared to Colchicine-injected group; c: P < 0.05 as compared to Vin 20 +col rats (One way ANOVA followed by Tukey's test for multiple comparisons).

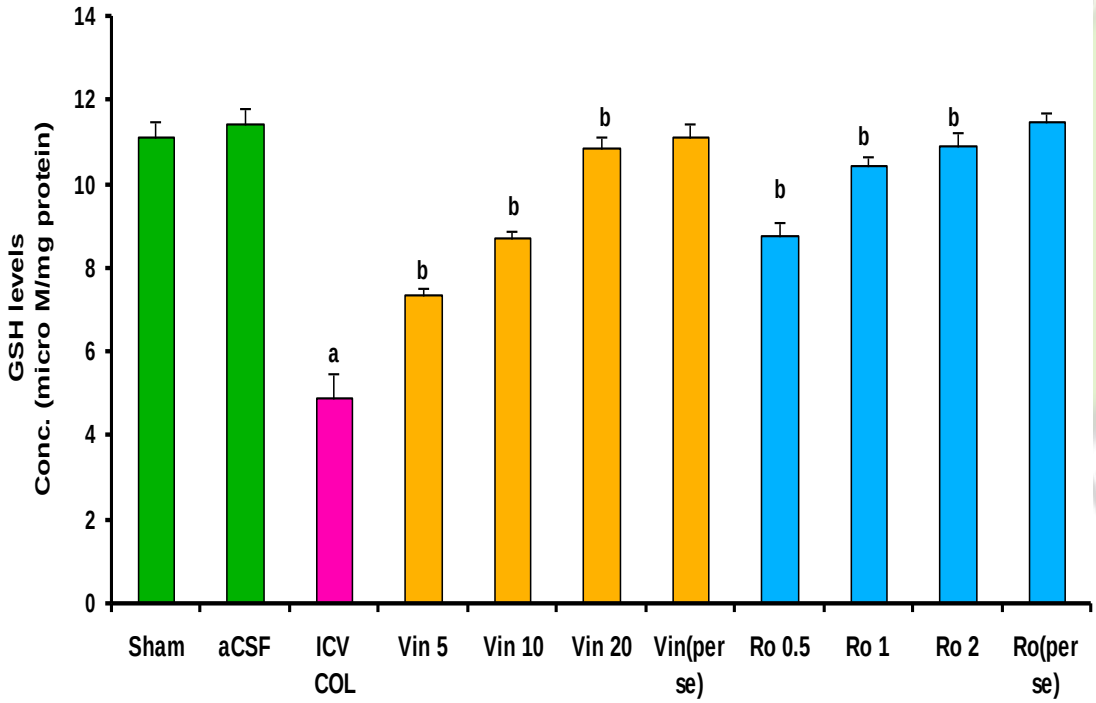

Fig:-4. Effect of vinpocetine and Ro 20-1724 on GSH levels in brain in intracerebroventricular colchicine-injected rats. Values are mean ± SEM. a: P ≤ 0.05 as compared to sham group; b: P ≤ 0.05 as compared to colchicine -injected group; c: P ≤ 0.05 as compared to Vin 20 +Col rats. (One way ANOVA followed by Tukey's test for multiple comparisons).

Nitrite and LDH levels Intracerebroventricular administration of colchicine leads to significant rise in nitrite and LDH levels in brain. Central aCSF injection did not show any change in nitrite and LDH levels as compared to sham-operated rats. Similarly, per se treatment of vinpocetine (20 mg/kg) and Ro 20- 1724(2 mg/kg) did not show significant differences in nitrite and LDH levels compared to that of sham-operated & aCSF rats. However, chronic treatment (21 days) of colchicine injected animals with vinpocetine (5, 10, 20 mg/kg, i.p.) and Ro 20-1724 (0.5, 1, 2 mg/kg, i.p.) significantly reduced the nitrite and LDH levels compared to colchicine control group. No significant difference was observed between 10 & 20 mg/kg doses of vinpocetine and also 1 &2mg/kg doses of Ro 20-1724. However, Ro 20-1724 (1 mg/kg) was found to be more effective than vinpocetine (10 mg/kg) in lowering increased levels of nitrite and LDH (Table- 2, Fig.5 and 6)

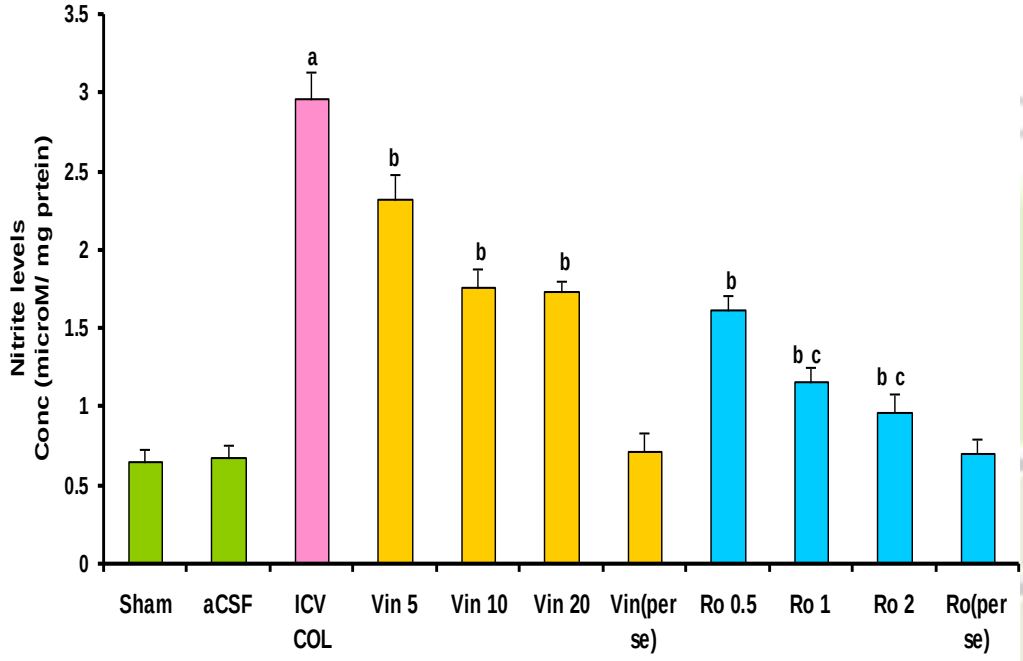

Fig:-5. Effect of vinpocetine and Ro 20-1724 on nitrite levels in brain in intracerebroventricular colchicine-injected rats. Values are mean ± SEM. a: $P \leq 0.05$ as compared to Sham group; b: $P \leq 0.05$ as compared to colchicine -injected group; c: $P \leq 0.05$ as compared to Vin 20 +Col rats. (One way ANOVA followed by Tukey's test for multiple comparisons).

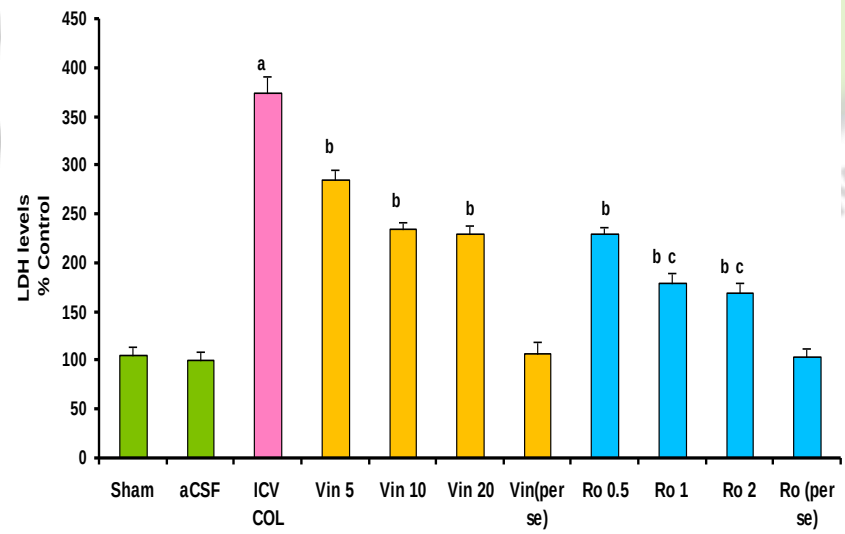

Fig:-6. Effect of vinpocetine and Ro 20-1724 on LDH levels in brain in intracerebroventricular colchicine-injected rats. Values are mean ± SEM. a: $P < 0.05$ as compared to sham group; b: $P < 0.05$ as compared to Colchicine -injected group; c: $P < 0.05$ as compared to Vin 20 +Col rats. (One way ANOVA followed by Tukey's test for multiple comparisons).

Acetylcholinesterase (AChE) levels

Sham operated, aCSF, vinpocetine per se (20 mg/kg) and Ro 20-1724 per se (2 mg/kg) did not show any significant difference in AChE levels in mg/kg doses of Ro 20-1724. But the effect of 1 & 2 mg/kg doses of Ro 20-1724 was significantly better than those of 10 & 20 mg/kg doses of vinpocetine. However, Ro 20-1724 (1 mg/kg) was found to be more effective in restoring the levels of AchE than 10 mg/kg dose of vinpocetine (Table-2, Fig.7).

Myeloperoxidase (MPO) levels

MPO levels were found to be significantly higher in colchicine treated ICV rats compared to sham control group. ICV injection of aCSF did not reveal any change in MPO levels in brain as compared to sham-operated rats. Per se groups of vinpocetine

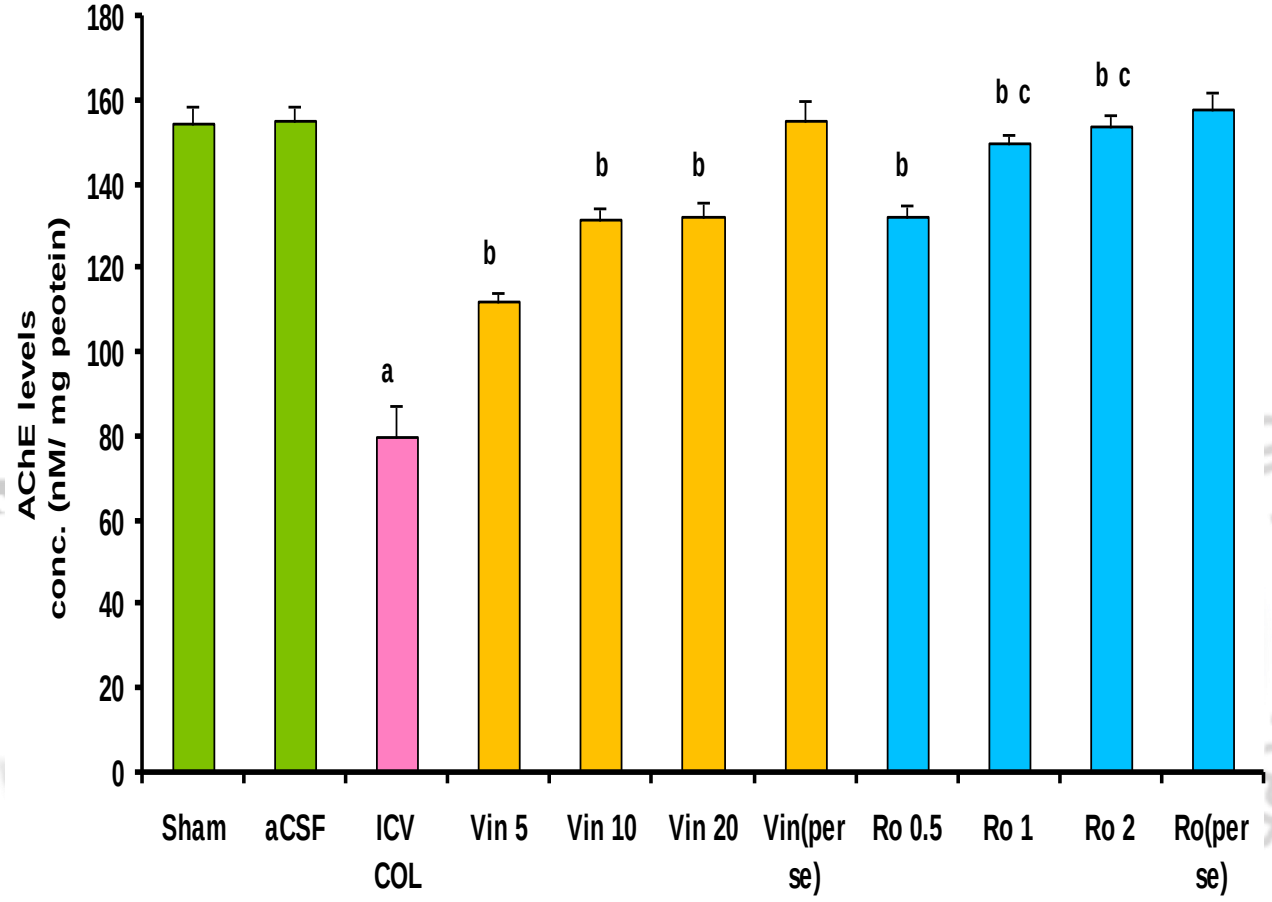

Fig:-7. Effect of vinpocetine and Ro 20-1724 on AChE levels in brain in icv colchicine-injected rats. Values are mean ± SEM. a: $P < 0.05$ as compared to sham group; b: $P < 0.05$ as compared to colchicine-injected group; c: $P < 0.05$ as compared to Vin 20 +Col rats. (One way ANOVA followed by Tukey's test for multiple comparisons).

brain. ICV colchicine administration caused significant decrease in AChE levels in brain compared to sham control rats, which were significantly restored by chronic treatment with vinpocetine (5, 10, 20 mg/kg) and Ro 20-1724 (0.5, 1, 2 mg/kg) in colchicine injected rats. There was no significant difference between the effects of 10 & 20 mg/kg doses of vinpocetine as well as 1 & 2 (20 mg/kg, i.p.) and Ro 20-1724 (2 mg/kg, i.p.) also showed no effect on MPO levels and were similar to that of sham-operated rats. However, chronic administration (21 days) of vinpocetine (5, 10, 20mg/kg, i.p) and Ro 20-1724 (0.5, 1, 2 mg/kg, i.p) significantly reduced the MPO levels in all colchicine injected animals in a dose dependent manner. There was no significant difference between the activities of 10 & 20 mg/kg doses of vinpocetine as well as 1 & 2 mg/kg doses of Ro 20-1724, like that in AChE activity discussed above. The effect of 1 & 2 mg/kg doses of Ro 20-1724 was significantly better than those of 10 & 20 mg/kg doses of vinpocetine while Ro 20-1724 (2 mg/kg) was found to be more effective than 1 mg/kg of Ro 20-1724 (Table- 2, Fig.8).

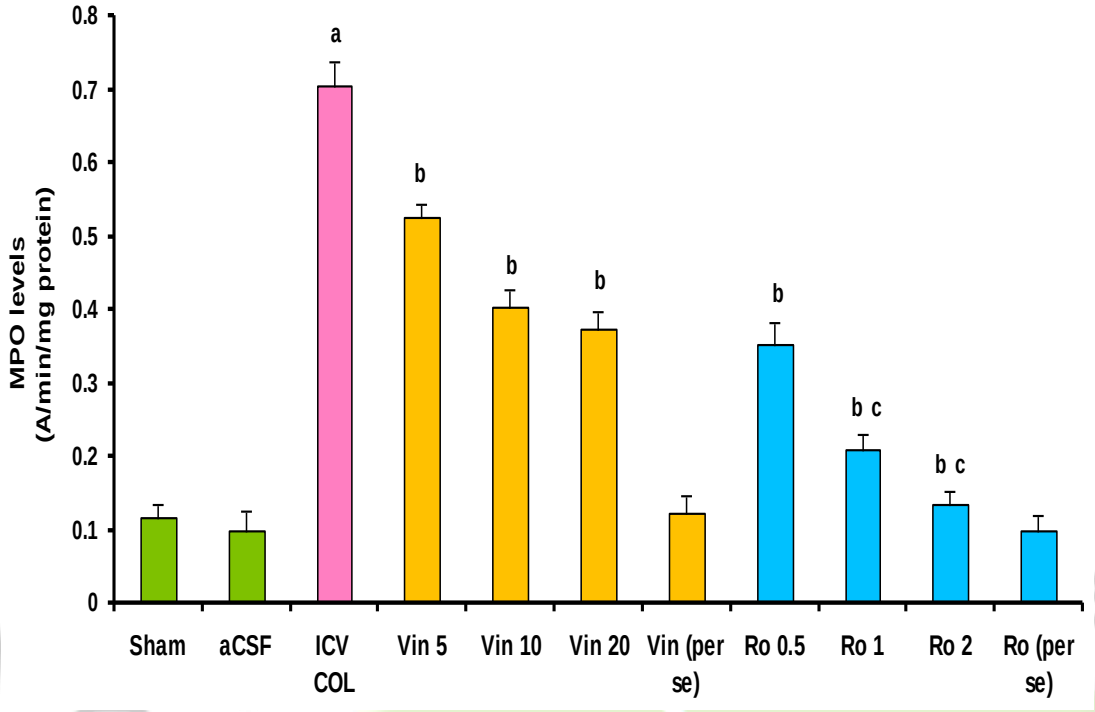

Fig:-8. Effect of vinpocetine and Ro 20-1724 on MPO levels in brain in intracerebroventricular colchicine-injected rats. Values are mean ±SEM. a: P < 0.05 as compared to Sham group; b: P < 0.05 as compared to colchicine-injected group; c: P < 0.05 as compared to Vin 20 +Col rats. (One way ANOVA followed by Tukey's test for multiple comparisons).

DISCUSSION

In ICV colchicine model, the present study was focused on two PDEs viz. PDE-1 and PDE-4 which were identified as plausible relevant targets for improvement in cognitive function based on review of literature. The effect of the selective PDE4-inhibitor (Ro 20-1724) and PDE1-inhibitor (vinpocetine) on cognitive function in rats was investigated using the Morris water maze and other biochemical parameters of oxidative stress and neurotransmitter dysfunction. Furthermore, the present study was designed to investigate the effects of selective PDE-1 and PDE-4 inhibition by vinpocetine and Ro 20-1724, respectively in ICV colchicine induced neuronal dysfunction. As already mentioned, PDE-1 isoenzyme is of non-specific nature & hydrolyzes both cAMP & cGMP, yet it is selectively inhibited by vinpocetine. On the other hand, PDE-4 isoenzyme specifically hydrolyzes cAMP and is selectively inhibited by Ro 20-1724 . Cognitive impairment due to central administration of colchicine has a slow and insidious onset, which takes about 14-21 days. Thus ICV colchicine can be considered as a relevant model to explain sporadic dementia of Alzheimer type (SDAT). It is characterized by a progressive deterioration of cognitive function, microtubular disruption and decrease in AChE and choline-acetyl transferase activity which was observed in the present study that central colchicine induced memory dysfunction was significantly attenuated by treatment with vinpocetine (5, 10, 20 mg/kg) and Ro 20-1724 (0.5, 1 mg/kg) as evidenced by significant decrease in initial acquisition latency (IAL) and retention transfer latency (RTL) indicating procognitive effects. Furthermore, increment in the dose of Ro 20-1724 to 2 mg/kg, though displayed improved biochemical response yet interestingly decreased the cognitive performance. Similar effect was noticed with per se group of Ro 20-1724 at the same dose. This might have been due to already reported lethargic effects of Ro 20-1724 at higher doses. On the other hand, per se groups of vinpocetine showed significant improvement in cognitive performance compared to sham group of animals indicating its procognitive property even in normal animals in confirmation with earlier reports . Spontaneous locomotor activity (SLA) of sham, aCSF, ICV colchicine, vinpocetine (5, 10 & 20 mg/kg) and per se group of vinpocetine and Ro 20-1724 (0.5, 1 mg/kg) did not show any significant difference. But Ro 20-1724 per se and Ro 20-1724 (2 mg/kg) in colchicine treated rats showed significant reduction in their locomotor activity. This behavioral toxic effect of Ro 20-1724 may possibly be attributable to some

mechanism other than that of the involvement of cAMP signaling.

The treatment of ICV colchicine rats with Ro 20-1724 and vinpocetine significantly decreased MDA levels compared to ICV colchicine treated control group indicating attenuation of lipid peroxidation. Furthermore, there was simultaneous significant restoration in GSH levels in both vinpocetine as well as Ro 20-1724 treatment groups. GSH is an essential tripeptide, natural antioxidant found in all animals. This suggests that PDE inhibitory activity of vinpocetine and Ro 20-1724 may be getting supplemented by their antioxidant properties in providing protection against oxidative damage – possibly by increasing endogenous defensive capacity of brain to combat oxidative stress induced by ICV colchicine. A second interrelated mechanism that might account for the increase in oxidative damage is nitric oxide. Colchicine increases the expression of inducible nitric oxide synthase, thereby increasing nitric oxide (NO). In the present study, colchicine when given centrally led to increase in nitrative stress as compared to sham control. Ro 20-1724 and vinpocetine treatment to colchicine administered animals was found to have significant decrease in nitrite levels when compared to ICV colchicine control rats. Thus, the observed beneficial effects of PDE 1 and 4 inhibitors might be due to their inhibitory effect on iNOS activity, thereby decreasing nitric oxide production, free radical formation and neurotoxicity. This further suggests that attenuation of lipid peroxidation could account for the neuroprotective effect at least partly, seen with these agents. The activity of AChE was found to be significantly decreased in ICV colchicine treated rats compared to sham control contributing to cholinergic deficits. The decreased levels of AChE were significantly restored by different doses of both vinpocetine as well as Ro 20-1724. However, vinpocetine showed significant but comparatively lesser effect than Ro 20-1724. These observations suggest the modulation of cholinergic neurotransmission and/or prevention of cholinergic neuronal loss by both the test drugs. The restoration of decreased levels of AChE in ICV colchicine injected rats by Ro 20-1724 and vinpocetine may possibly be due to modulation of cyclic nucleotides by PDE inhibition which may eventually be contributing to this improvement in AChE levels and subsequently cognitive performance. Both a vinpocetine and Ro 20-1724 were able to decrease the inflammatory response as measured by MPO activity which is an enzyme specific to granulocyte lysosomes, is a parameter directly related to the absolute number of polymorphonuclear cells (PMN cells) . Activation and accumulation of PMN cells at the site of injury is one of the initial events of tissue injury which triggers the release of oxygen free radicals, arachidonic acid metabolites and lysosomal proteases, with the progress of tissue injury. Out of the two drugs investigated in the present study, Ro 20-1724 seems to be significantly better than vinpocetine. Lactate dehydrogenase (LDH) is a non specific marker of cell death. ICV colchicine treated rats showed significant rise in LDH levels compared to sham control. However, the same were significantly attenuated by treatment with vinpocetine and Ro 20-1724 in ICV colchicine rats. The attenuation of all these events including LDH activity by the two test drugs employed in this study; perhaps collectively contribute to prevention of cell death.

Out of the two PDE inhibitors investigated presently, Ro 20-1724 seems to be more effective than vinpocetine up to 1 mg/kg dose, though vinpocetine also showed significant effect. Ro 20-1724 at 2 mg/kg dose significantly impaired the locomotor activity of the animals and the latter took longer time to reach the hidden platform which means significant increase in escape latency. It may perhaps thus be safe to conclude that the beneficial effects of the investigated test drugs may be due to their procognitive activity through PDE inhibition and consequent, anti-oxidant, anti-inflammatory activities and modulation of cholinergic activities in these models.

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