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Neuroprotective effect of carvedilol, an adrenergic antagonist against colchicine induced cognitive impairment and oxidative damage in rat

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ABSTRACT

Cognitive impairment and weak intellectual capacity is a gradually progressive neurodegenerative problem. Growing evidences indicate that oxidants and antioxidant defenses interact in a vicious cycle, which plays a critical role in the pathogenesis of cognitive dysfunction. The present study was carried out to elucidate the neuroprotective effect of carvedilol against the colchicine-induced cognitive impairment and oxidative damage in rats. Colchicine (15 μg/5 μl), a microtubule disrupting agent when administered intracerebroventricularly in rats resulted in poor memory retention in both Morris water maze, elevated plus maze task paradigms and caused marked oxidative stress as indicated by significant increase in malondialdehyde, nitrite levels, depletion of SOD, catalase, glutathione-S-transferase activity and reduced glutathione levels. It also caused a significant decrease in the acetylcholinesterase activity. Chronic administration of carvedilol (2.5 and 5.0 mg/kg; p.o.) for a period of 25 days, starting 4 days prior to colchicine administration resulted in an improvement in memory retention, attenuation of oxidative damage and restoration of acetylcholinesterase activity. Present study demonstrates a neuroprotective effect of carvedilol against colchicine-induced cognitive impairment and associated oxidative damage.

1. Introduction

Memory dysfunction is a progressive neurological disorder that leads to cognitive deterioration. Neuropathological hallmarks of this disease include neurofibrillary tangles, neurite (senile) plaques, amyloid angiopathy, granulovacuolar degeneration and Hirano bodies (Calabrese et al., 2006). Brain oxidative stress associated with cognitive impairment exerts an important role in cognitive dysfunction. Accumulation of reactive oxygen and nitrogen species induces lipid peroxidation, tyrosine nitrosylation, DNA damage and neuronal disruption, which are the common characteristics of Alzheimer brain (Medeiros et al., 2007). Sporadic Dementia of Alzheimer's type (SDAT) is characterized by microtubule dysfunction, cytoskeletal abnormalities and cognitive impairment. It has been demonstrated experimentally in animals, that central administration of cytoskeletal poisons causes cognitive impairment and microtubule dysfunction which mimics the changes seen in SDAT (Flaherty et al., 1989; Sofroniew et al., 1986). Central administration of colchicines causes cognitive dysfunction and oxidative stress in animals (Kumar et al., 2007). Colchicine inhibits microtubule polymerization by binding to tubulin, one of the main constituents of microtubules. It has been used to treat anti-inflammatory disorders, but has a relatively low therapeutic index which causes alterations in the axonal transport, intracellular trafficking of mitochondria and synaptic loss by disrupting microtubule assembly (Muller et al., 2006; McClure, 1972; Mandelkow et al., 2003). It also causes cholinotoxicity resulting in decreased acetylcholine transferase activity (Kevin et al., 1989). Evidence also indicates that the hippocampal infusion of colchicine causes an increase in glutamate/GABA ratio in the cortex of mice brain (Yu et al., 1997) and NO levels in hypothalamus of guinea pig (Laurence et al., 2000). These findings suggest that central colchicine administration causes oxidative stress which consequently leads to cognitive impairment (Kumar and Gupta, 2002).

Oxidative stress has been implicated in the molecular etiopathogenesis of cognitive dysfunction. Therefore, drugs which can modulate reactive oxygen species may be potentially useful in the management of memory dysfunction. Carvedilol is a nonselective β-adrenoceptor blocker with multiple pleiotropic actions like antioxidant, α_1-adrenoceptor blocking effect, vasodilatation, inhibition of apoptosis (Savitz et al., 2000), anti-inflammatory (Yaoita et al., 2002), mitochondrial protective (Abreu et al., 2000), non-competitive inhibitor of NMDA receptor and calcium channel blocker (Lysko et al., 1992). Carvedilol has been shown to exert neuroprotective effect in several models of transient focal stroke (Savitz et al., 2000) and tardive dyskinesia (Naidu et al., 2002), cardioprotective effect in several models of cardiovascular ischemia and reperfusion (Ma et al., 1996) and nephroprotective effect (Padi and Chopra, 2002). These effects have been attributed in part to the free radical scavenging and metal chelating properties of carvedilol (Oettl et al., 2001). In fact, Suzuki et al. (2003), have also shown that carvedilol suppresses lipid autooxidation and protein carbonyl formation in brain homogenate in a

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dose dependent manner. The antioxidant activity of carvedilol emanates from the carbazole moiety (Yue et al., 1994). Studies have also shown that carvedilol prevents the conversion of Aβ into the biologically active form thereby acting as a novel anti-fibrillar agent (Howlett et al., 1999). Based on this background, the present study was carried out to investigate the neuroprotective effect of carvedilol against colchicine-induced cognitive impairment and oxidative stress in rats.

2. Materials and methods

2.1. Animals

Male wistar rats 2–3 months old (Central Animal House, Panjab University, Chandigarh) and weighing 180–200 g at the start of the study were used. Animals were acclimatized to laboratory conditions at room temperature prior to experimentation. Following surgery, animals were kept under standard conditions of a 12 hour light/dark cycle with food and water ad libitum in groups of 2 in plastic cages with soft bedding. All the experiments were carried out between 9.00 and 15.00 h. We further state that experimental protocol has been approved by an Institutional Review Committee for the use of Human or Animal Subjects or that procedures are in compliance with at least the Declaration of Helsinki for human subjects, or the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85–23, revised 1985), the UK Animals Scientific Procedures Act 1986 or the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Surgery and intracerebroventricular administration of colchicines

Surgery was performed as per the previously described protocol (Kumar and Gupta, 2002). All animals were anesthetized with thiopental sodium (45 mg/kg, i.p.) and positioned in a stereotaxic apparatus. The head was positioned in a frame and a midline sagittal incision was made in the scalp. Two holes were drilled in the skull for the placement of the injection cannula into the lateral cerebral ventricle. Co-ordinates for the intracerebroventricular (i.c.v.) cannula implantation were 0.8 mm posterior to bregma, 1.8 mm lateral to the sagittal suture and 3.6 mm beneath the cortical surface. The scalp was then closed with a suture. After surgery, all animals received gentamicin (5 mg/kg, i.p.) to prevent sepsis. Rats were infused i.c.v. with either artificial cerebrospinal fluid (ACSF; in mmol/l: 147 NaCl, 2.9 KCl, 1.6 $MgCl_2$, 1.7 $CaCl_2$ and 2.2 dextrose) or 15 μg colchicine dissolved in ACSF. Solution (5 μl) was injected using a Hamilton micro syringe positioned in the injection cannula. In the sham-operated rats, the surgery was identical except for drilling of holes and placement of the cannula. To promote the diffusion the micro syringe was left in place for a period of 2 min following injection. Special care of the animals was taken during the post-operative period.

2.3. Drugs and treatment schedule

Colchicine (Sigma Chemicals Co., St. Louis, Mo, USA) and carvedilol solutions were made freshly at the beginning of each experiment. Colchicine was prepared in ACSF such that a 15 μg dose was delivered in a 5 μl injection volume for i.c.v. administration. For oral administration, carvedilol was dissolved in 0.5% carboxymethyl cellulose and administered in a dose of 1 ml/100 g body weight. Animals were divided randomly based on their body weights into seven groups of 8 animals in each. The groups were as follows:

Group 1 — sham-operated (received vehicle for carvedilol)
Group 2 — ACSF (5 μl i.c.v.)+vehicle for carvedilol
Group 3 — colchicine treated group (15 μg/5 μl i.c.v.)+vehicle for carvedilol
Group 4 — carvedilol (2.5 mg/kg, p.o.)+ACSF (5 μl i.c.v.)
Group 5 — carvedilol (5.0 mg/kg, p.o.)+ACSF (5 μl i.c.v.)
Group 6 — carvedilol (2.5 mg/kg, p.o.)+colchicine (15 μg/5 μl i.c.v.)
Group 7 — carvedilol (5.0 mg/kg, p.o.)+colchicine (15 μg/5 μl i.c.v.).

The doses of carvedilol were selected based on the previous studies in the laboratory and those reported in the literature.

2.4. Behavioral assessment

2.4.1. Assessment of cognitive performance

2.4.1.1. Elevated plus maze paradigm. The elevated plus maze consisted of two opposite black open arms (50×10 cm), crossed with two closed walls of the same dimensions with 40 cm high walls. The arms were connected with a central square of dimensions 10×10 cm. the entire maze was elevated at a height of 50 cm from the floor. Acquisition of memory was tested on day 13 after colchicine administration. Rats were placed individually at one end of the open arm facing away from the central square. The time taken by the animal to move from the open arm to the closed arm was recorded as the initial transfer latency (ITL). Animals were allowed to explore the maze for 20 s after recording the ITL and were then returned to the home cage. If the animal did not enter the enclosed arm within 90 s, it was pushed on the back into one of the enclosed arm and the ITL was given as 90 s. Retention of memory was assessed by placing the rat in an open arm and the retention latency was noted on day 14 and day 21 of ITL and was termed as the first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL), respectively (Sharma and Kulkarni, 1992).

2.4.1.2. Spatial navigation task. The acquisition and retention of a spatial navigation task was using modified method of Morris water maze (Frautschy et al., 2001). Animals were trained to swim to a visible platform in a circular pool (180 cm in diameter and 60 cm in height) located in a test room. In principle rats can escape from swimming by climbing onto the platform and over time the rats apparently learn the spatial location of the platform from any starting position at the circumference of the pool. Thus the platform offers no local cues to guide the escape behavior of the rats. The only spatial cues are those outside of the tank primarily the visual cues. The pool was filled with water (28±2 °C) to a height of 40 cm. a movable circular platform (9 cm diameter), mounted on a column was placed in a pool 2 cm above the water level during the acquisition phase. A similar platform was placed in the pool 2 cm below the water level for the maze retention phase. During both the phases the platform was placed in the centre of one of the quadrants. The water was made opaque by adding a non-toxic dye. Four equally spaced locations around the edge of the pool (N, S, E, and W) were used as starting points and this divided the pool into four equal quadrants.

1. Maze acquisition phase (training)—Animals received a training session consisting of 4 trials on day 13. In all 4 trials, the starting position was different. A trial began by releasing the animal into the maze facing towards the wall of the pool. The latency to find the escape platform was recorded to a maximum of 90 s. If the rat did not escape onto the platform within this time it was guided to the platform and was allowed to remain there for 20 s. The time taken by rat to reach the platform was taken as the initial acquisition latency (IAL). At the end of the trial the rats were returned to their home cages and a 5 min gap was given between the subsequent trials.
2. Maze retention phase (Testing for retrieval of the learned task)—Following 24 hour (day 14) and 8 days (day 21) after IAL, rat was released randomly at one of the edges facing the wall of the pool and tested for retention of response. The time taken to find the

hidden platform on day 14 and day 21 following central administration of colchicine was recorded and termed as first retrieval latency (1st RL) and second retrieval latency (2nd RL) respectively.

2.4.2. Assessment of gross behavioral activity

Gross behavioral activity was observed on day 1, 7, 14 and 21 following i.c.v. colchicine injection. Each animal was placed in a square (30 cm) closed arena equipped with infra-red light sensitive photocells using digital photoactometer. The animals were observed for a period of 5 min and the values were expressed as counts/5 min. The apparatus was placed in a darkened, light and sound attenuated and ventilated test room (Reddy and Kulkarni, 1998).

2.4.3. Biochemical assessments

Biochemical tests were conducted 24 h after the last behavioral test. The animals were sacrificed by decapitation. Brains were removed and rinsed with ice-cold isotonic saline. Brains were then homogenized with ice-cold 0.1 mmol/L phosphate buffer (pH 7.4). The homogenate (10%w/v) was then centrifuged at 10,000 g for 15 min and the supernatant so formed was used for the biochemical estimations.

2.4.3.1. Measurement of lipid peroxidation. The extent of lipid peroxidation in the brain was determined quantitatively by performing the method as described by Wills (1966). The amount of malondialdehyde (MDA) was measured by reaction with thiobarbituric acid at 532 nm using Perkin Elmer Lambda 20 spectrophotometer. The values were calculated using the molar extinction co-efficient of chromophore (1.56×10^5 $(mol/l)^{-1}$ cm^{-1}).

2.4.3.2. Estimation of reduced glutathione. Reduced glutathione was estimated according to the method described by Ellman (Ellman, 1959). A 1 ml supernatant was precipitated with 1 ml of 4% sulphosalicylic acid and cold digested for 1 h at 4 °C. The samples were then centrifuged at 1200 g for 15 min at 4 °C. To 1 ml of the supernatant obtained, 2.7 ml of phosphate buffer (0.1 mmol/L, pH 8) and 0.2 ml of 5, 5' dithio-bis (2-nitrobenzoic acid) (DTNB) was added. The yellow color that developed was measured at 412 nm using Perkin Elmer Lambda 20 spectrophotometer. Results were calculated using the molar extinction co-efficient of the chromophore (1.36×10^4 $(mol/l)^{-1}$ cm^{-1}).

2.4.3.3. Estimation of nitrite. The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide was determined by a colorimetric assay with Greiss reagent (0.1% N-(1-napththyl) ethylene diamine dihydrochloride, 1% sulphanilamide and 5% phosphoric acid.) according to Green et al. (1982). Equal volumes of the supernatant and the Greiss reagent were mixed and the mixture was incubated for 10 min at room temperature in the dark. The absorbance was measured at 540 nm using Perkin Elmer Lambda 20 spectrophotometer. The concentration of nitrite in the supernatant was determined from sodium nitrite standard curve.

2.4.3.4. Superoxide dismutase activity. SOD activity was assayed by the method of Kono (1978). The assay system consisted of EDTA 0.1 mM, sodium carbonate 50 mM and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of the above mixture, 0.05 ml of hydroxylamine and 0.05 ml of the supernatant was added and the auto-oxidation of hydroxylamine was measured for 2 min at 30 s interval by measuring the absorbance at 560 nm using Perkin Elmer Lambda 20 spectrophotometer.

2.4.3.5. Catalase activity. Catalase activity was assessed by the method of Luck (1971), wherein the breakdown of hydrogen peroxide is measured. Briefly, the assay mixture consisted of 3 ml of H_2O_2 phosphate buffer and 0.05 ml of the supernatant of the tissue homogenate. The change in absorbance was recorded for 2 min at 30 s interval at 240 nm using Perkin Elmer Lambda 20 spectrophotometer. The results were expressed as micromoles of hydrogen peroxide decomposed per min per mg protein.

2.4.3.6. Glutathione-S-transferase activity. The glutathione-S-transferase activity was assayed by the method of Habig and Jakoby (1981). Briefly, the assay mixture consisted of 2.7 ml of phosphate buffer, 0.1 ml of reduced glutathione, 0.1 ml of 1-chloro-2, 4-dinitrobenzene (CDNB) as substrate and 0.1 ml of supernatant. The increase in the absorbance was recorded at 340 nm for 5 min at 1 min interval using Perkin Elmer Lambda 20 spectrophotometer. The results were expressed as nmols of CDNB conjugated/min/mg protein.

2.4.3.7. Acetyl cholinesterase (AchE) activity. AchE is a marker of extensive loss of cholinergic neurons in the forebrain. The AchE activity was assessed by the Ellman method (Ellman et al., 1961). The assay mixture contained 0.05 ml of supernatant, 3 ml of sodium phosphate buffer (pH 8), 0.1 ml of acetylthiocholine iodide and 0.1 ml of DTNB (Ellman reagent). The change in absorbance was measured for 2 min at 30 s interval at 412 nm using Perkin Elmer Lambda 20 spectrophotometer. Results were expressed as micromoles of acetylthiocholine iodide hydrolyzed per min per mg protein.

2.4.3.8. Protein estimation. The protein content was estimated by the Biuret method (Gornall et al., 1949) using bovine serum albumin as a standard.

2.4.4. Statistical analysis

Values are expressed as mean ± SEM. The behavioral assessment data were analyzed by a repeated measures two-way analysis of variance (ANOVA) with drug-treated groups as between and sessions as the within-subjects factors. The interaction drug treatment × session was considered to test for drug effect on retention. The biochemical estimations were separately analyzed by one-way ANOVA. Post hoc comparisons between groups were made using Tukey's test. $P<0.05$ was considered significant.

3. Results

3.1. Effect of carvedilol on memory performance in elevated plus maze paradigm in colchicine-injected rats

In the present experiment, mean ITL on day 13 for each rat was relatively stable and showed no significant variation. All the rats entered the closed arm within 90 s. Following training, sham-operated, ACSF-injected and carvedilol-treated (2.5 and 5.0 mg/kg) rats entered closed arm quickly and mean retrieval transfer latencies (1st RTL and 2nd RTL) to enter closed arm on days 14 and 21 were shorter as compared to ITL on day 13 of each group respectively. In contrast, colchicine-injected rats performed poorly throughout the experiment and did not show any change in the mean retention transfer latencies on day 14 and 21 as compared to pre-training latency on day 13, demonstrating that colchicine-induced marked memory impairment. Chronic administration of carvedilol (2.5 and 5.0 mg/kg) beginning prior to colchicine injection significantly decreased the mean retention latencies on days 14 and 21 following colchicine injection ($P<0.05$ vs i.c.v. colchicine group) (Fig. 1). The mean transfer latencies of carvedilol (2.5 and 5.0 mg/kg, p.o.) treated i.c.v. colchicine-injected groups were significantly different from that of carvedilol *per se* groups on days 14 and 21 ($P<0.05$) (Table 1).

3.2. Effect of carvedilol on spatial navigation task in colchicine-injected rats

Sham-operated, ACSF-injected, and carvedilol *per se* (2.5 and 5.0 mg/kg, p.o.) group of animals quickly learned to swim directly to

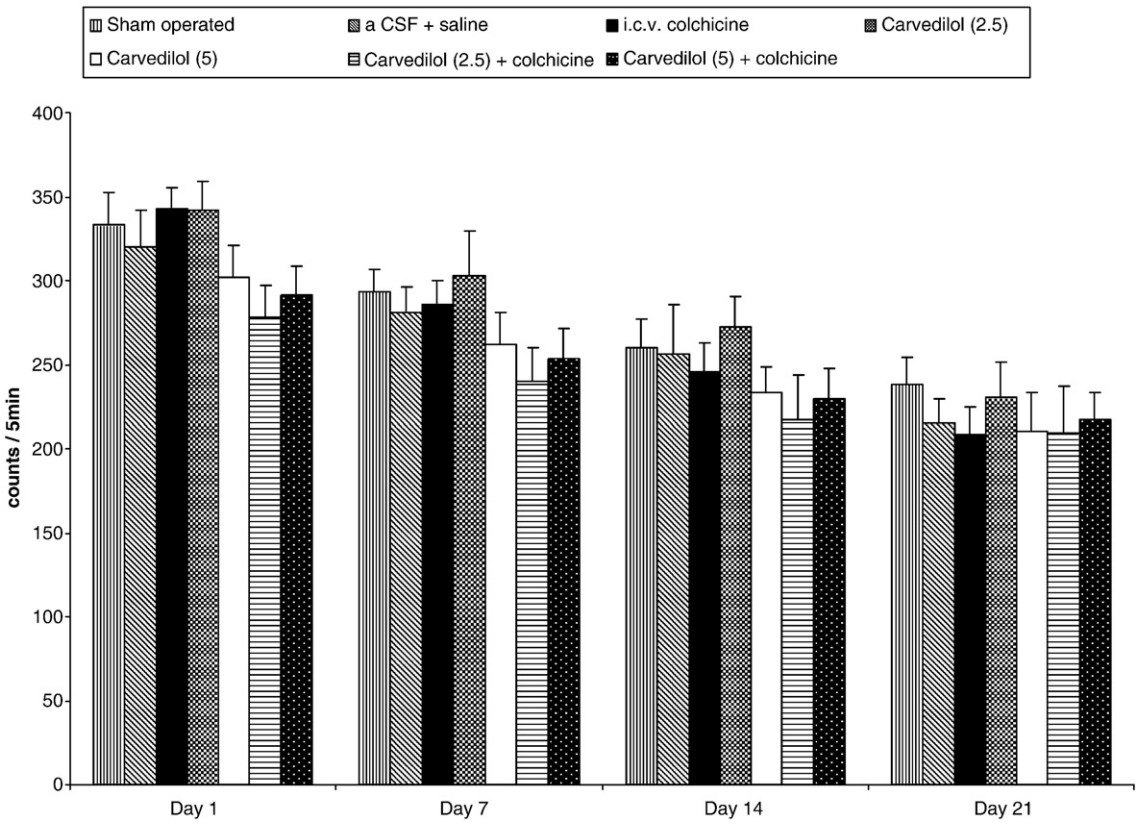

Fig. 1. Effect of carvedilol (CAR; 2.5 and 5.0 mg/kg, p.o.) on locomotor activity in intracerebroventricular colchicine (COL)-injected rats. Values are expressed in mean ± S.E.M. Data was analyzed by two-way ANOVA. (n = 8 in each group).

the platform in the Morris water maze on day 13. Colchicine-injected rats showed an initial increase in escape latency, which declined with continued training during the acquisition of a spatial navigation task on day 13. Carvedilol (2.5 and 5.0 mg/kg, p.o.) group of rats was also performed similarly during the acquisition of a spatial navigation task on day 13 (vs ACSF-injected group). There was a significant difference in the mean IAL of colchicine-injected group compared to ACSF-injected group on day 13 indicating colchicine-induced impaired acquisition of spatial navigation task (P<0.05). In contrast, carvedilol (2.5 and 5.0 mg/kg, p.o.) treatment significantly decreased the IAL to reach the platform in the pre-trained rats as compared to colchicine-injected rats on day 13 following colchicine injection (Table 2).

Following training, the mean retention latencies (1st and 2nd RL) to escape onto the hidden platform was significantly decreased in sham-operated and ACSF-injected rats on days 14 and 21, respectively as compared to IAL on day13 following colchicine injection. On the contrary, the performance in the colchicine-injected rats was changed after initial training in the water maze on days 14 and 21, with significant increase in mean retention latencies compared to IAL on day 13. The results suggest that colchicine caused significant cognitive impairment. However, chronic carvedilol treatment (2.5 and 5.0 mg/kg, p.o.) starting before colchicine injection showed a significant decline in the 1st and 2nd RL as compared to colchicine-injected rats on days 14 and 21, respectively following colchicine injection (Table 2) and improved the retention performance of the spatial navigation task.

Table 1
Effect of carvedilol (CAR; 2.5 and 5.0 mg/kg, p.o.) on memory performance in elevated plus maze paradigm in intracerebroventricular colchicine (COL)-injected rats

Treatment (mg/kg)	Mean transfer latency (in s)		
	ITL	1st RTL	2nd RTL
Sham	58.16±1.79	20.5±4.28	17.0±5.16
ACSF	61.16±1.6	15.66±1.66	12.60±1.49
COL	66.66±1.53[a]	79.33±1.33 [a]	72.33±1.20[a]
CAR (2.5)	62.16±1.51	14.16±1.945	10.16±1.16
CAR (5.0)	60.8±1.077	13.16±1.79	9.86±0.844
CAR (2.5)+COL	62.3±1.470	40.33±0.881[b]	36.8±0.478[b]
CAR (5.0)+COL	61.8±1.238	29.8±0.577[b,c]	24.66±0.958[b,c]

The initial transfer latencies (ITL) on day 13 and retrieval transfer latencies on days 14 (1st RTL) and 21 (2nd RTL) following colchicine injection were observed. Values are expressed in mean ± S.E.M.
ACSF: artificial cerebrospinal fluid; COL: colchicine; CAR: carvedilol.
[a] P<0.05 as compared to artificial cerebrospinal fluid (ACSF)-injected group.
[b] P<0.05 as compared to colchicine-injected group.
[c] P<0.05 as compared to CAR (2.5)+COL group, (repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons).

Table 2
Effect of carvedilol (CAR; 2.5 and 5.0 mg/kg, p.o.) on spatial navigation task in intracerebroventricular colchicine (COL)-injected rats

Treatment (mg/kg)	Mean latency (in s)		
	IAL	1st RL	2nd RL
Sham	43.5±1.29	12.33±1.4	9.16±2.16
ACSF	55.33±1.7	14.33±1.66	11.8±1.49
COL	88.0±1.93[a]	75.0±1.43[a]	62.33±1.80[a]
CAR (2.5)	62.8±1.51	15.55±1.945	11.83±2.16
CAR (5.0)	61.8±1.077	13.33±2.09	9.86±1.421
CAR (2.5)+COL	73.6±0.470[b]	40.5±1.577[b]	36.15±0.475[b]
CAR (5.0)+COL	67.66±0.438[b,c]	28.76±0.569[b,c]	24.5±1.576[b,c]

The initial acquisition latencies (IAL) on day 13 and retrieval latencies on days 14 (1st RL) and 21 (2nd RL) following colchicine injection were observed in Morris water maze. Values are expressed in mean ± S.E.M.
ACSF: artificial cerebrospinal fluid; COL: colchicine; CAR: carvedilol.
[a] P<0.05 as compared to artificial cerebrospinal fluid (ACSF)-injected group.
[b] P<0.05 as compared to colchicine-injected group.
[c] P<0.05 as compared to CAR (2.5)+COL group, (repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons).

Table 3
Effect of carvedilol (CAR; 2.5 and 5.0 mg/kg, p.o.) on colchicines-induced oxidative stress parameters in rat brain

Treatment (mg/kg)	MDA levels nmol MDA/mg protein (% of control)	Nitrite levels μmol/mg protein (% of control)	Reduced glutathione nmol/mg protein (% of control)	Catalase μmol of hydrogen peroxide decomposed/min/mg protein (% of control)	Superoxide dismutase units/mg protein (% of control)	Glutathione-S-transferase nmol of CDNB conjugated/min/mg protein (% of control)
Sham	100±10	100±12	100±10	100±12	100±14	100±12
ACSF	114.28±11	109.33±11	103.33±11	97.05±10	97.52±15	98.66±16
COL	339.88±32[a]	298.39±15[a]	23.38±10[a]	17.98±8[a]	13.47±3[a]	18.67±7[a]
CAR (2.5)	111.7±25	119.29±16	94.35±17	94.9±13.7	98.17±17	97.64±14
CAR (5.0)	100.4±26	105.44±26	96.77±15	98.8±10.8	98.16±16	98.65±13
CAR (2.5)+COL	262.81±27[b]	236.91±8[b]	50.45±8[b]	36.96±6[b]	32.05±8[b]	30.65±8[b]
CAR (5.0) + COL	157.26±6[b,c]	178.98±7.9[b,c]	70.61± 7.6[b,c]	63.71±5[b,c]	63.52±5[b,c]	58.05±6[b,c]

Values are expressed in mean±S.E.M.
ACSF: artificial cerebrospinal fluid; COL: colchicine; CAR: carvedilol.
[a] $P<0.05$ as compared to artificial cerebrospinal fluid (ACSF)-injected group.
[b] $P<0.05$ as compared to colchicine-injected group.
[c] $P<0.05$ as compared to CAR (2.5)+COL group, (repeated measures two-way ANOVA followed by Tukey's test for multiple comparisons).

3.3. Effect of carvedilol on locomotor activity in colchicine-injected rats

In the present series of experiments, the mean scores of locomotor activity for each rat were relatively stable and showed no significant variation. The mean scores in sham-operated, ACSF- and colchicine-injected rats remained unchanged. Chronic administration of carvedilol (2.5 and 5.0 mg/kg, p.o.) had no effect on the locomotor activity as compared to ACSF-injected rats on day 14 and 21 (Fig. 1). Further, both the dose of carvedilol (2.5 and 5.0 mg/kg, p.o.) in colchicine-injected rats did not cause any alteration in the locomotor activity as compared to colchicine-injected rats on day 14 and 21 (Fig. 1).

3.4. Effect of carvedilol on brain lipid peroxidation, nitrite and reduced glutathione levels, glutathione-S-transferase, superoxide dismutase, catalase activities in colchicine-injected rats

Intracerebroventricular administration of ACSF had no effect on brain MDA, nitrite levels, reduced GSH, glutathione-S-transferase, superoxide dismutase and catalase levels as compared to sham-operated rats. Central colchicine administration caused marked increase in free radical generation and significant rise in brain MDA, nitrite levels, depletion of reduced GSH, glutathione-S-transferase, superoxide dismutase and catalase levels as compared to ACSF injected rats. Further, there were no alteration in the brain MDA levels, nitrite level, reduced GSH, reduced glutathione-S-transferase, superoxide and catalase levels due to carvedilol (2.5 and 5.0 mg/kg, p.o.) *per se* treatment as compared to ACSF injected rats. However, chronic carvedilol (2.5 and 5.0 mg/kg, p.o.) administration significantly prevented the increase in MDA, nitrite levels and depletion of reduced GSH (Table 3). It also caused a significant increase in the levels of glutathione-S-transferase, superoxide dismutase and catalase (Table 3).

3.5. Effect of carvedilol on brain acetylcholinesterase levels in colchicine-injected rats

Intracerebroventricular administration of ACSF had no effect on brain acetylcholinesterase levels as compared to sham-operated rats. In contrast, central colchicine injection showed significant decline in the brain AChE activity as compared to ACSF-injected rats. However, chronic oral administration of carvedilol (2.5 mg/kg and 5.0 mg/kg, p.o.) significantly ameliorated the reduction in AChE activity compared to colchicine-injected group (Fig. 2).

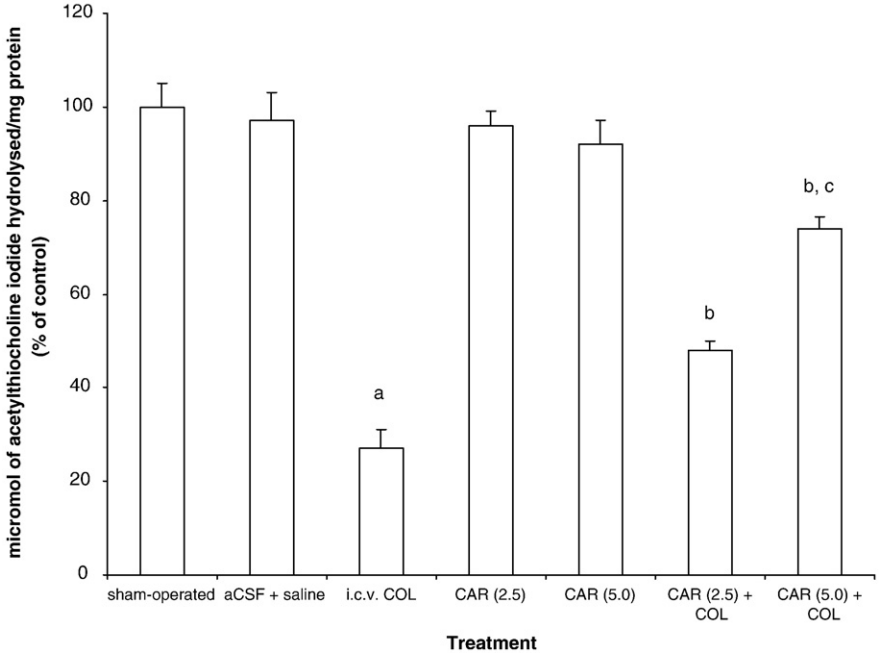

Fig. 2. Effect of carvedilol (CAR; 2.5 and 5.0 mg/kg, p.o.) on acetyl cholinesterase activity in Intracerebroventricular colchicine (COL)-injected rats. Values are mean±S.E.M. [a]$P<0.05$ as compared to artificial cerebrospinal fluid (ACSF)-injected group, [b]$P<0.05$ as compared to colchicine-injected group, [c]$P<0.05$ as compared to CAR (2.5)+COL group; (Repeated measures two-way ANOVA followed by Turkey's test for multiple comparisons). Note: ACSF: artificial cerebrospinal fluid; COL: colchicine; CAR: carvedilol.

4. Discussion

The salient findings of this study indicated that chronic treatment with carvedilol caused a significant improvement in the memory performance tasks and attenuation of oxidative stress as indicated by a decrease in the lipid peroxidation, nitrite levels, restoration of the glutathione levels, catalase, superoxide dismutase and glutathione-S-transferase activities. I.C.V. colchicine administration decreased acetylcholinesterase activity which was ameliorated by carvedilol chronic treatment. These findings suggest that the central administration of colchicine causes progressive deterioration of cognition, microtubule disruption and decrease in cholineacetyl transferase activity (Bensimon and Chermat, 1991). These effects of colchicine are attributed to its ability to cause apoptosis in selected neuronal populations like cerebellar granule cells and basal forebrain cholinergic neurons by activating the c-Jun N-terminal kinase (JNK) pathway (Muller et al., 2006).

Chronic administration of carvedilol was found to improve not only the memory retention but also reduced oxidative damage induced by central colchicine administration. Carvedilol per se had no effect on oxidative stress in the brain of normal animals but it significantly attenuate colchicine-induced oxidative stress. This may be attributed to the antioxidant effect of carvedilol. Carvedilol has been reported to scavenge free radicals and inhibit lipid peroxidation in swine ventricular membranes and rat brain homogenates (Yue et al., 1992a, b). It has also been shown to inhibit superoxide ion release from activated neutrophils (Mačičkova et al., 2005). Carvedilol also has been shown to preserve the endogenous antioxidant system i.e. vitamin E and reduced glutathione which are normally consumed when tissues or cells are exposed to oxidative stress (Lysko et al., 1995; Feuerstein et al., 1997). This may explain the fact that carvedilol treatment was able to restore the levels of reduced glutathione and glutathione-S-transferase activity in the colchicine treated rats.

Colchicine also causes an increase in expression of inducible nitric oxide synthase (Kumar et al., 2006) resulting in increase nitric oxide levels which acts as a precursor for the peroxynitrite free radical (Beal, 1995; Bondy, 1995). Overproduction of nitric oxide causes neurotoxicity to cholinergic neurons (Dawson et al., 1995; Fass et al., 2003). This explains that centrally administered colchicine caused a significant increase in nitrite levels in the brain and carvedilol treatment was able to decrease the nitrite levels. In fact it has been reported in literature that carvedilol acts as a NO quenching agent in vascular endothelial cells and cell-free system (Yoshioka et al., 2000). Carvedilol is approximately 10 times more potent than vitamin E as an antioxidant and being lipophilic in nature it easily crosses the blood brain barrier. Carvedilol is an ortho-substituted phenoxythylamine derivative that contains carbazole moiety which is primarily responsible for antioxidant activity, making it structurally distinct from other β-blockers in common use such as propranolol, and atenolol (Fig. 3) (Stolc, 1999).

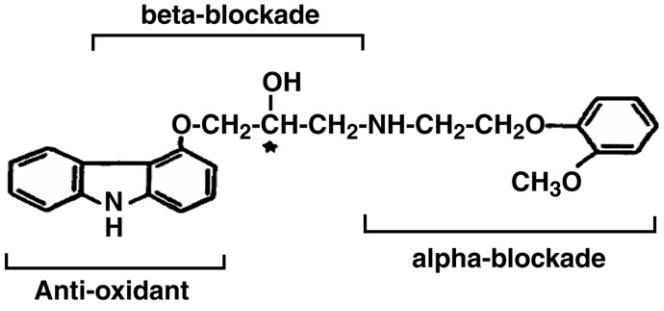

Fig. 3. Chemical structure of carvedilol in which the carbazole component, which is primarily responsible for antioxidant activity, and the regions responsible for β-adrenergic and α-adrenergic blockade.

Apart from the parent compound, even its metabolites namely, SB 211475, BM 910228 and SB 209995 are powerful antioxidants and may be responsible for carvedilol's neuroprotective effect (Savitz et al., 2000). Besides, nebivolol, timolol, betaxolol, cartelol, nipradilol, well known beta blockers shows antioxidant like activity and has been shown to affect reactive oxygen species under oxidative stress (Garbin et al., 2008; Yu et al., 2007).

Alzheimer brains are also associated with reactive gliosis, microglia activation and inflammation (Firuzi et al., 2006). Central administration of colchicine induces a direct inflammatory response in the CNS (Kumar et al., 2006) which causes cholinotoxicity. Carvedilol has been shown to act as an anti-inflammatory agent by suppressing the mRNA expression of inflammatory cytokines and interleukin 1β. It has also been reported that it may inhibit the activator signal transduction pathway of NF-κB (Yuan et al., 2004). All these effects may contribute to the neuroprotective effect of carvedilol. The cholinergic system is responsible for storage and retrieval of memory is by far the most affected in AD. Central administration of colchicine results in death of dentate granule cells and cerebellar granule cells by activation of caspase-3 (Bonfoco et al., 1995) and upregulation of cyclooxygenase-2 expression (Ho et al., 1998), thereby causing cholinergic deficit. In the present study, colchicine caused a significant decrease in the acetylcholinesterase activity which leads to memory deficits. Carvedilol was able to restore the colchicine induced decrease in the AchE activity.

Though the antioxidant action of carvedilol has been attributed to its radical scavenging and iron chelating property but the mechanistic dynamics that underlie theses effects are not yet known. This study warrants further investigation to elucidate the neurochemical and molecular mechanisms involved in the neuroprotective effect of carvedilol. Our results confirm that carvedilol has a protective effect against colchicine-induced cognitive impairment and oxidative stress in rats and it represents a valid rational for the use of carvedilol in the prevention of memory dysfunction and related disorders.

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