Research Article

Characterization of Curcumin di-acetate Aimed at Brain Targeting

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Abstract

In the present study curcumin derivative was synthesized to specifically target to the brain and evaluate the ability of curcumin derivative to cross blood brain barrier compared to the parent compound. The results of the present study showed that curcumin derivative cross the blood brain barrier 10 times more as compared to the parent compound and get converted into the parent compound in brain. Further, the effect of curcumin di-acetate in scopolamine induced experimental dementia of Alzheimer disease in rats was investigated. Rats were treated with scopolamine (1.4 mg/kg, i.p.) alone and with curcumin (25 mg/kg, i.v.), curcumin di-acetate (25 mg/kg, i.v). The changes in behavioral and biochemical parameters were assessed in rats. Scopolamine administered rats showed impaired learning and memory in hippocampus as compared with control. Simultaneous treatment of curcumin and curcumin di-acetate with scopolamine also caused an improvement in the learning and memory activity and ($P < 0.05$) was found to be significant.

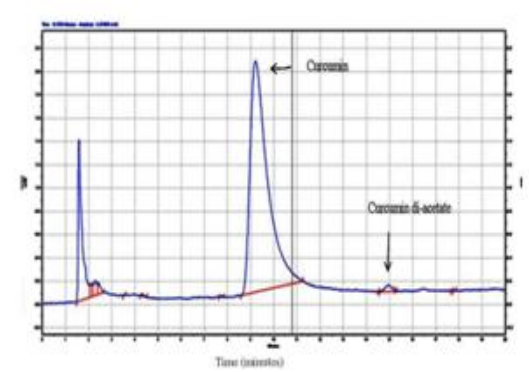

Figure Chromatogram of the brain tissue showing the conversion of curcumin di-acetate to curcumin

Keywords: Curcumin di-acetate, Brain targeting, Drug delivery, Scopolamine

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Introduction

Curcumin, a major yellow pigment obtained from the rhizomes of turmeric, has a wide array of pharmacological and biological activities including antioxidant, anti-inflammatory, anti-infectious and anti-carcinogenic [1-2]. In addition, curcumin has been shown to have the possibility of slowing the progress of Alzheimer's disease (AD) by reducing amyloid β, of delaying the onset of kainic acid-induced seizures and of inhibiting the formation of brain tumors. Apart from the anti-amyloid properties [3-4] and anti-tau hyperphosphorylation properties [5] against the two major pathological changes in AD, curcumin is also able to control the resultant changes that occur during the disease like oxidative stress [6], inflammatory stress [7], and cholesterol regulation [4] which are very beneficial while considering alzheimer's disease therapy.

Alzhiemer's disease is a devastating neurodegenerative brain disorder with progressive losss in memory leads to dementia. It is characterized by the deposition of the senile plaques mainly composed of β- amyloid fragment and neurofibrillary tangles. In the recent past, the plant based molecules attracted attention due to their potential role in dementia. However the efficiency of these herbal compounds also depends on the bioavailability at the right location. Targeting the central nervous system has always been a challenge due to its inaccessibility. Due to the presence of tight junctions within the capillary endothelium severely restricts the delivery of therapeutics to the brain. Enhancing the transport of curcumin has come up as a hopeful alternative to target the brain, by crossing the blood-brain barrier ability. With this background, we have synthesized derivative of curcumin i.e di-acetate to target the brain in the

treatment of neurological diseases and brain tumors. This work will conclude with an analysis of the curcumin di-acetate in brain and its conversion into parent compound.

Curcumin has potential to increase the cholinergic activity of neurons in streptozotocin- induced dementia in rats [8]. Effects of curcumin on the pathophysiology of alzheimer's disease (AD) have been studied and several groups have shown its ability to inhibit Aβ-plaque formation [9-10]. Also, curcumin has been proposed as a potential candidate to treat Parkinson's disease (PD) [11-13]. Chronic stress is a risk factor for the onset of depression that induces neurodegeneration in hippocampal neurons. Curcumin administration can increase hippocampal neurogenesis in chronically stressed rats [14]. Experimental autoimuno encephalomyelitis (EAE) is an animal model for multiple sclerosis (MS) and treatment of EAE rats with curcumin reduced clinical severity of EAE [15]. According to all of these studies, curcumin can act against a wide variety of neurologic diseases.

Curcumin has considerable neuro-protective and anti-cancer properties but is rapidly eliminated from the body. However, the penetration of curcumin in brain is limited due to its rapid systemic elimination [16]. Therefore, it was decided that if a prodrug like mono or di-acetate is prepared it could cross the blood brain barrier exactly the way heroin which is a di-acetate of morphine reaches the brain. On similar perception it was investigated to develop a di-acetate which after crossing the blood brain barrier may get cleaved by same enzyme [17].

Experimental

Materials

Curcumin (purity ≥ 95%) was obtained from Himedia, India. Scopolamine was gifted by Cadila Healthcare Pvt. Ltd, India. All solvents used in this study were High Performance Liquid Chromatography (HPLC) grade. FTIR spectra were recorded using FTIR spectrophotometer (Agilent Cary 630 FTIR Spectrometer). The scanning range was 4000 to 650 cm^{-1} and the resolution was 8 cm^{-1}. A mass spectrum (low resolution) of the synthesized derivative, curcumin di-acetate was recorded at MAT 120. ^{1}H NMR and ^{13}C-NMR spectra were recorded on Bruker Advance II 400MHz NMR spectrometers using $CDCl_3$ as a solvent. Chemical shifts are expressed in δ units; Coupling constants (*J*) are given in Hertz (Hz).

Synthesis of Curcumin di-acetate

Curcumin was added in excess amount of acetic anhydride in the presence of concentrated sulphuric acids. The mixture was stirred for 30 min at room temperature and kept overnight. Next day the aqueous solution was extracted with chloroform followed by the wash of the organic extract with ice cold water. The reaction product was separated by column chromatography (Kieselgel 60, Art 7731, 70-230 mesh, Merk) with dichloromethane as eluting solvents.

Quantification of Curcumin and Curcumin Derivative in Rat Brain

The HPLC system consisted of a chromatographic binary pump (G1312A, Agilent, German), autosampler (G1329A, Agilent, German), diode array detector (G1315D, Agilent, German). For HPLC separation, a reversed-phase C18 column (4.6 × 150 mm, particle size 5µm, Eclipse XDB, Agilent, USA) was used. The mobile phase for curcumin was composed of acetonitrile-10 mM potassium dihydrogen phosphate (pH 4.0 adjusted by acetic acid) (50:50, v/v) at a flow-rate of 0.8 ml/min. The detection wavelength was set at 425 nm. The mobile phase was degassed by sonication (Ultrasonic Cleaner-30A) and filtered (0.45µm Millipore membrane filter Nylon 66) before use. The sample injection volume was 20µl.

Analytical Method Validation

The stock solution of curcumin in acetonitrile (500µg/ml) was diluted with 50% acetonitrile to make serial concentrations of the working standard solutions (0.1, 0.25, 1, 2.5, 10 and 25µg /ml). Calibration standards were

prepared by 5 µl of the working standard solution spiked with 45 µl of blank plasma and organ tissue (brain). Extraction procedures followed the sample preparation as described in Section sample preparation. The calibration curves were given by: (curcumin/curcumin di-acetate peak area for y-axis and curcumin/curcumin acetate concentration for x-axis). The limit of detection (LOD) and the limit of quantification (LOQ) were defined as a signal-to-noise ratio of 3 and the lowest concentration of the linear regression, respectively. The accuracy and precision of intra-day and inter-day for the curcumin and curcumin acetate were assayed. The accuracy (% bias) was calculated as $[(C_{obs}-C_{nom})/C_{nom}] \times 100$, where C_{nom} represented the nominal concentration and Cobs indicated the mean value of the observed concentration. Precision as the relative standard deviation (RSD) was calculated from the observed concentrations as follows: % RSD = [standard deviation (SD)/Cobs] × 100. The % bias and % RSD value for the lowest acceptable reproducibility concentrations was defined as being within ±15%. The recoveries (%) were calculated by comparing the curcumin peak area of the extracted sample with that of the unextracted curcumin standard solution containing the equivalent amount of curcumin in three replicates (at concentrations of 0.1, 0.25 and 2.5 µg/ml).

Animal Study

Albino rats (120 ± 150 g body weight) of either sex were obtained from the Laboratory Animal Center at Shoolini University (Solan, Himachal Pradesh). The experimental protocol was approved by institutional animal ethics committee of Shoolini University (protocol number: **IAEC/SU-PHARM/13/027**). These animals were specifically pathogen-free and had free access to food and water and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each. The Institution Animals Ethics Committee (IAEC) had approved the experimental protocol. The animals (n =24) were divided into four different groups of 6 animals per each group (Table 1). Scopolamine (1.4 mg/kg) as a disease inducer was administered to all groups through intraperitoneal (i.p) route after drugs administration to all the groups except normal control group. The same procedure was carried out for 5 days.

Table 1 Experimental Design

Group-I	Control	Vehicle (0.1% CMC).
Group-II	Disease control	Scopolamine (1.4 mg/kg) i.p.
Group-III	Test-I	Curcumin (25mg/kg) i.v.+ Scopolamine (1.4 mg/kg) i.p.
Group-IV	Test-II	Curcumin di-acetate (25mg/kg) i.v. + Scopolamine (1.4 mg/kg) i.p.

For the quantification of curcumin and curcumin di-acetate in brain, the study was carried out on three new groups comprises of six animals in each group.

Behavioral Tests

Morris Water Maze Test (MWMT)

Morris water maze was used to assess learning and memory in experimental rats. MWM consists of a water tank with 1 cm submerged platform filled with opaque water. The platform was used to train the animals. Trial escape latency time (ELT) i.e time measure to locate the hidden platform was noted as an indicator of acquisition. Four acquisition trials per day were employed for 4 consecutive days. The time spent by the animal, searching for the missing platform in target quadrant Q2 with respect to other quadrant (Q1, Q3, and Q4). On day 5^{th} probe test was conducted, each animal was allowed to travel around the pool for 120 sec on the removal of hidden platform. The mean time spent in

all four quadrants was noted. The mean time spent in the target quadrants in search for the hidden plat form was noted as an index of retrieval or memory.

Locomotor Activity

In both man and animals CNS drugs has a marked effect on locomotion. The locomotion activity can be assessed using actophotometer. Animals are placed individually in the activity cage for 10 min and the activity was monitored. The instrument basically works on the principle of photoelectric cells which are connected in circuit with a counter. The test is done before 30 min and after the drug administration and the count was noted.

Histopathological Study

Brain samples were taken and fixed in 10% formalin for 24 h. Brain samples were then washed with serial dilutions of alcohol (methyl, ethyl and absolute ethyl) were used for dehydration. Specimens were cleared in xylene then embedded in paraffin at 56 °C in hot air oven for 24 h and were observed under microscope using H/E stain.

Preparation of Brain Homogenate

Experimental rats were initially anesthetized by diethyl ether. Then 25 mg/kg of curcumin and curcumin di-acetate in PEG 400 mixture was intravenously injected via the tail vein. The brain tissue was excised out surgically at different time interval i.e. 15, 30 and 60 min after curcumin and curcumin di-acetate administration. The brains were removed and transferred into 50 ml tubes. The brain were weighed and homogenized with 50% aqueous acetonitrile (1:5, w/v). The brain tissue samples were then centrifuged at 6000 rpm for 10 min at 4 °C and the supernatant was collected and preserved at −20 °C before further sample assay. Each biological sample (50 µl) was vortex-mixed with acetonitrile for protein precipitation. After centrifuging at 12,000 rpm for 15 min, 20 and 10 µl of supernatants were collected filtered through syringe filter nylon (0.2 µm) and analyzed by the HPLC.

Results and Discussion

Chemistry

Curcumin di-acetate (Figure 1) was synthesized by using acetic anhydride in the presence of concentrated sulphuric acids. The reaction product was separated by column chromatography with dichloromethane as eluting solvents. The prodrug was obtained in good yields as yellow solids after re-crystallization.
Curcumin di-acetate $C_{25}H_{24}O_{8,}$ *[(1E, 6E)-3,5-Dioxo-1,6-heptadiene-1,7-diyl]bis-2-methoxy-4,1-phenylene di-acetate* **(**Yield 90%; m.p. 143–145 °C)

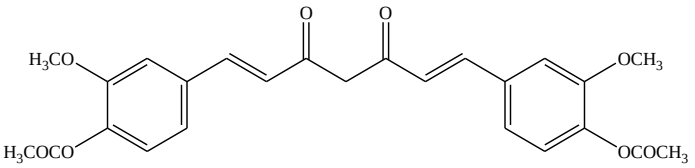

Figure 1 Chemical structure of curcumin di-acetate

IR: 2925, 1767, 1633, 1592, 1510, 1421, 1261, 1194, 1123, 1015,974, 836.

^{1}H NMR(400MHz, $CDCl_3$, δ, TMS=0): δ= 2.307(6-H, s, 4, 4' $OOCCH_3$), 3.816 (6-H, s, 3, 3' OCH_3), 6.50(1H, d, 2-H, *J* =15.81Hz), 7.55(1H, d, 3-H, *J* =15.81Hz), 6.50(1H, d, β-H, *J* =15.81Hz), 7.55(1H, d, γ-H, *J* =15.81Hz), 5.23(1H, s, α-H), 7.007 (2H, d, 2"6"-H, *J* =8.11Hz), 6.51(1H, d, 5"-H, *J*=15.72Hz), 7.007 (2H, d, 2'6'-H, *J* =8.11Hz), 6.99(1H, d, 5'-H, *J*=8.11Hz).

^{13}C-NMR ($CDCl_3$) 183.14, 168.86, 151.42, 141.32, 139.99, 123.31, 121.11, 111.48, 55.96, 29.16.
Mass spectrum showed M^+, 453.1 (M^+ +1) and 475.1(M^+ +23 Na) along with expected fragmentation.

Identification of Curcumin and Curcumin di-acetate in Brain by HPLC

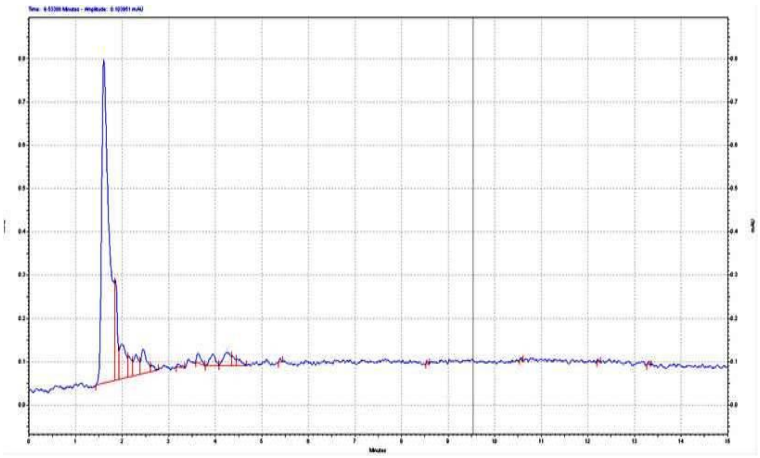

Figure 2 Chromatogram of blank brain

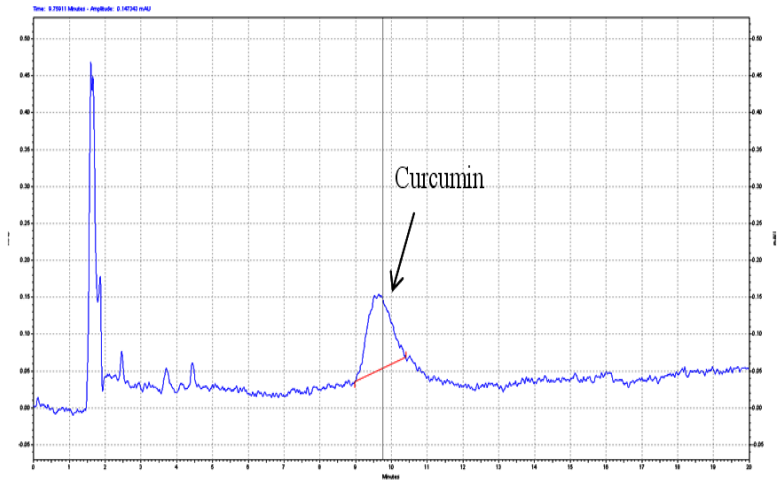

Figure 3 Chromatogram of the brain tissue containing the curcumin after i.v. administration

Figure 2 shows the chromatogram of blank brain. **Figure 3** and **4** shows the chromatogram of the brain tissue containing the curcumin and curcumin di-acetate collected from rats after i.v. administration of both the compound (25 mg/kg). The retention time of curcumin and curcumin di-acetate in chromatogram were 8.1 and 15.6 min, respectively. There was no obvious interference peaks located at the retention times of analyses in the blank chromatogram. These result indicate that Eclipse XDB C18 column with the mobile phase of acetonitrile-10 mM potassium dihydrogen phosphate (pH 4.0 adjusted by acetic acid) provided good separation and selectivity for curcumin in biological matrix with these analytical condition.

Figure 4 shows the chromatogram of the brain tissue collected from rats, after i.v. administration of synthesized derivative (25 mg/kg). From the chromatogram, the two peaks with the two different retention times were observed

which indicate that one peak for curcumin and another peak for curcumin di-acetate. The result clearly indicate that probably curcumin di-acetate convert to curcumin due to enzymatic hydrolysis of curcumin di-acetate in brain.

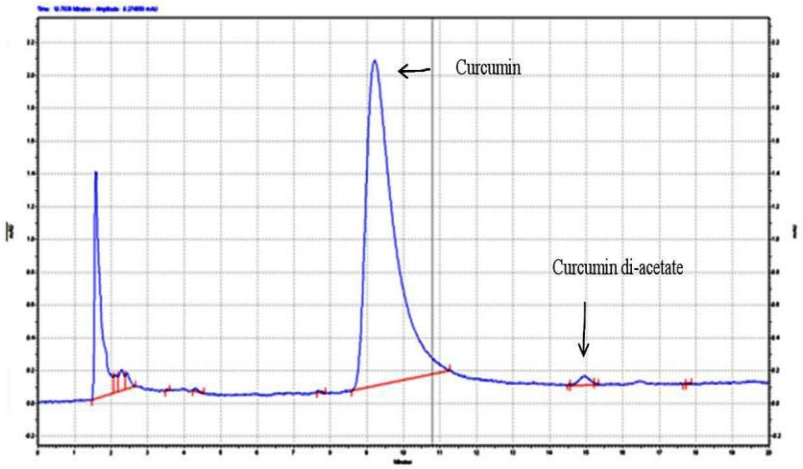

Figure 4 Chromatogram of the brain tissue showing the conversion of curcumin di-acetate to curcumin

Figure 3 and 4 clearly shows that curcumin and curcumin di-acetate can pass through the BBB into brain tissue. After intravenous administration of curcumin di-acetate, the concentration of curcumin di-acetate was significantly more over curcumin with the same dose. The result showed that curcumin di-acetate cross the blood brain barrier 10 times more as compared to the parent compound and gets converted into the parent compound in brain.

Behavioural Tests

MorrisWater Maze Test

Administration of curcumin di-acetate for 5 days significantly decrease escape latency time (ELT) as compared to scopolamine group for 5^{th} days. On 5^{th} day curcumin di–acetate markedly increased time spend in target quadrant (TSTQ) in search of missing platform during retrieval trial, reflecting neuroprotective effect against scopolamine treated rats. Curcumin (5 mg/kg) treated for successive 5 days acts as positive control, possessed significant ($P < 0.05$) decrease in transfer latency when compared to disease control (scopolamine) using Tukey's multiple comparison test. Curcumin di-acetate which is a prodrug of curcumin showed a better effect than curcumin against the parent compound (**Figure 5, Table 2**).

Table 2 Effect on escape latency time in rats following exposure to scopolamine, curcumin, curcumin di- acetate and their simultaneous treatment along with scopolamine

Treatment	Day 1	Day 2	Day 3	Day 4
Control	41.00 ± 0.6325	39.67 ± 0.4216	38.50 ± 0.4282	36.67 ± 0.5578
Scopolamine	52.83 ± 0.6009	56.67 ± 0.6667	58.67 ± 0.8028	60.67 ± 0.7149
Curcumin + Scopolamine	47.17 ± 0.6009	48.17 ± 0.3073	45.67 ± 0.3333	45.33 ± 0.8433
Curcumin di-acetate + Scopolamine	43.50 ±0.4282	43.00 ± 0.3651	42.00 ± 0.5774	41.83 ± 0.7032

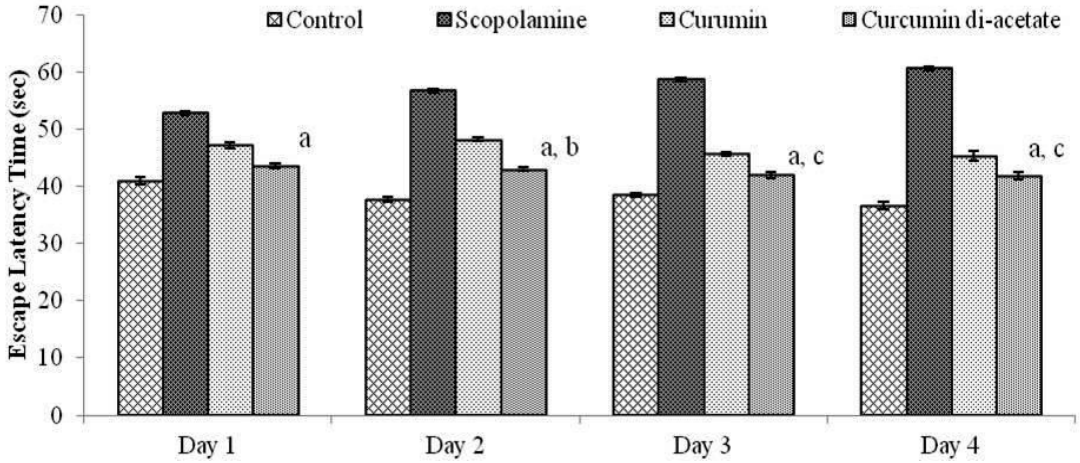

Figure 5 Effect on escape latency time (ELT) in rats following exposure to scopolamine, curcumin, curcumin di-acetate and their simultaneous treatment along with scopolamine values are mean ± SEM of six animals in each group significant difference (*$P < 0.05$) using Tukey's multiple comparison test

a; P < 0.05 versus day 1 ELT in respevtive group,
b; P < 0.05 versus day 1 ELT in vehicle treated group
c; P < 0.05 versus day 4 ELT in scopolamine treated group.

On day 5 probe trial was conducted the plat form was removed and each rat was allowed to explore the pool for 60 seconds. Time spent in all 4 quadrants was noted. The time spend in the target quadrant was more as compare to scopolamine administered rats (**Figure 6, Table 3**).

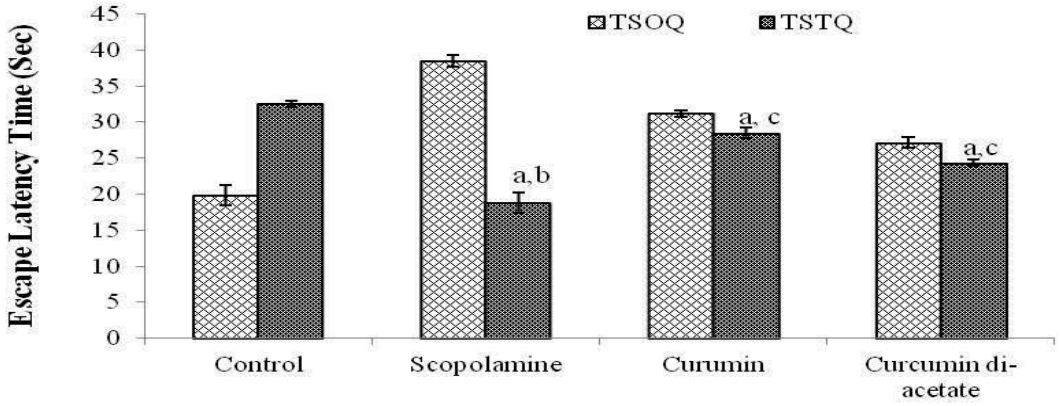

Figure 6 Effect on probe trial method in rats following exposure to scopolamine, curcumin, curcumin di-acetate and their simultaneous treatment along with scopolamine values are mean ± SEM of six animals in each group significant difference (*$P < 0.05$) using Tukey's multiple comparison test.

a; P < 0.05 versus mean time spent in other quadrants in respective group
b; P < 0.05 versus mean TSTQ in vehicle treated group
c; P < 0.05 versus mean TSTQ in scopolamine treated group.

Table 3 Effect on probe trial method in rats following exposure to scopolamine, curcumin, curcumin di-acetate and their simultaneous treatment along with scopolamine

Groups	TSOQ	TSTQ
Control	19.83 ± 1.447	32.5 ± 0.4282
Scopolamine	38.5 ± 0.7638	18.83 ± 1.447
Curumin + Scopolamine	31.17 ± 0.4723	28.5 ± 0.7638
Curcumin di-acetate + Scopolamine	27.17 ± 0.7923	24.33 ± 0.4216

Locomotor Activity

The activity of curcumin and curcumin di-acetate was evaluated using photoactometer. The rats showed significant transfer latency on 5th day as compared to the 1st day treatment in all groups except scopolamine administered rats. The curcumin di-acetate treatment showed significant (*P < 0.05) increase in transfer latency as compared to curcumin treated rats (**Table 4**).

Table 4 Effect of the curcumin di–acetate on the locomotor activity in scopolamine induced memory impairment in rats. Values are expressed as mean ± SEM at n=6; the differences in mean were not significant at *P < 0.05; one way ANOVA followed by Tukey's test

Treatment groups	Activity Score	
	3 Day	5 Day
Control	151.8±4.9	174 ± 3.41
Scopolamine	150.8±4.17	158.7 ± 1.256
Curcumin + Scopolamine	141 ± 4.2	139 ± 3.99
Curcumin di- acetate + Scopolamine	166 ± 2.3	176 ± 6.0

Histological evaluation of brain

Histological study revealed that the rats receiving scopolamine significantly produced histological alterations in brain with necrosis showed when compared with normal control rats. However, rats receiving pretreatment of curcumin di-acetate (25 mg/kg, *i.v.*) showed comparatively better protection of the rat brain indicated by reduction in necrosis (**Figure 7**). Curcumin di-acetate (25 mg/kg, *i.v.*) was found more significant against curcumin (25 mg/kg, *i.v.*).

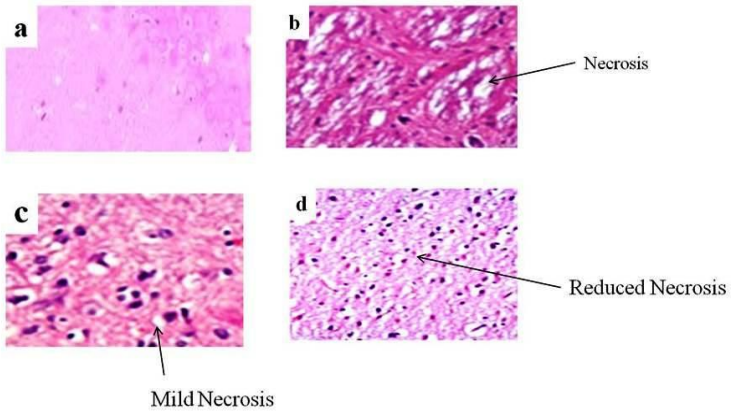

Figure 7 Histopathological studies. Figures a, b, c and d are normal Control, scopolamine (disease control), curcumin, curcumin di-acetate respectively, representing the histological sections of the brain tissue showing neurological necrosis

Conclusions

The present study demonstrated that curcumin and curcumin di-acetate has shown promising memory enhancing effects individually but di-acetate form of curcumin has shown the more potential effect as a curcumin against a scopolamine induced cognitive dysfunction in rats. Since ancient times, curcumin has been used in Asian countries against human ailments. Several studies over the earlier period decade have indicated the safety and usefulness of this polyphenol. Improved bioavailability of curcumin in the near future is likely to bring this promising natural product to the forefront of therapeutic agents for the treatment of human disease.

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Conflict of interest

The authors have reported no conflict of interest.

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