

# Neuroprotective Effect of Curcumin against Cholesterol Induced Neuroinflammation in *In-Vitro* and *In-Vivo* Models

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## Abstract

The present study was aimed to investigate the neuroprotective effect of curcumin against cholesterol mediated neurotoxicity in *in-vitro* and *in-vivo* models. In *in-vitro* model, PC12 cell lines were treated with cholesterol (10 & 50 µg/ml) alone or along with curcumin (5 & 10 µg/ml) and simvastatin as a standard control (1 µg/ml). After 48hrs of incubation, cells were studied for morphology, viability and cytokines as a marker. In *in-vivo* model, four groups of rabbits were used where one among them was fed with normal diet served as control; remaining three groups were fed with high fat diet. Curcumin (80mg/kg) and simvastatin (20mg/kg) were administered to rabbits orally from the fourth week to twentieth week. Results reveal that PC12 cell lines treated with curcumin (10µg/ml) and simvastatin (1µg/ml) had significantly reversed the cholesterol induced increased proinflammatory markers such as interleukin (IL-1β) and TNF-α. Further the oral administration of curcumin had shown significant reduction in brain cholesterol level and protects the brain from oxidative stress by reducing oxidative stress and brain antioxidant system. The experimental data results of our study show that curcumin protects PC 12 cell line from cholesterol induced neurotoxicity. Furthermore, curcumin had an influence on attenuating cholesterol induced proinflammatory mediator release and neuronal cell death in high fat diet induced neurotoxicity in *in-vitro* and *in-vivo* models.

**Keywords:** Cholesterol; Curcumin; Cytokines; Neuroinflammation; Oxidative stress; Neurodegeneration

## 1. INTRODUCTION

Utilization of cholesterol in the brain is indispensable for an assortment of key activities such as the neurotransmitters synthesis, storage and secretion. It is also construct the essential barrier such as blood brain barrier (BBB) in order to protect the brain cells from the invading toxins. Reports indicate the neurotoxic effect of cholesterol. Hypercholesterolemia is associated with neuroinflammation and mimics Alzheimer's Diseases (AD) in the CNS despite the fact that cholesterol does not pass the BBB [1, 2, and 3]. On the other hand oxysterols the oxygenated derivatives of cholesterol are able to pass through BBB, which was further substantiated by increased brain level of 27-hydroxycholesterol in AD patients [4]. The onset of neurodegenerative disorder such as Alzheimer's disease has been delayed or prevented by the use of non-steroidal anti-inflammatory drugs [5, 6]. Indeed, epidemiologic studies have also reported that the anti-inflammatory drugs users less likely affected for risk of developing AD [7]. The herbs or natural products have been found made to control neuroinflammation related neurodegeneration through anti-inflammatory effects [8, 9 and 10].

Curcumin an active ingredient isolated from the rhizome part of *Curcuma longa*, (Family: Zingiberaceae) is a potent anti-inflammatory [11, 12] and anti-oxidant compound [13, 14]. Apart from the profuse medicinal uses, turmeric powder is also commonly used as a cookery substance in various countries such as India, China and other Asian countries. The epidemiological studies revealed that the

regular dietary intake of turmeric powder reduce the risk of AD among rural Indian population [15]. It has been shown that various doses of curcumin reduce the amyloid pathology in Alzheimer's transgenic mouse model [16]. In addition, the hypocholesterolemic effect of curcumin in high fat diet fed animals also been reported [17]. However the synergistic effect of curcumin in hypercholesterolemia associated neurodegeneration is not yet investigated. In the present study, we have investigated the effect of curcumin in cholesterol mediated neuroinflammation in both *in-vitro* and *in-vivo* models.

## 2. MATERIALS AND METHODS

### 2.1 Drugs and Chemicals

Curcumin crystals were obtained from Sabinsa Corporation, USA. Simvastatin was obtained as gift sample from Lupin pharmaceuticals, Pune, India. Corboxymethyl cellulose, Cholesterol, Sodium pyrophosphate, n-butanol, Ammonium sulphate, Bovine serum albumin, Periodic acid and Arsenic oxide were purchased from Hi Media. Glycerol trioleate and Tris-HCl procured from Sigma, and Chromotropic acid was provided by NICE.

### 2.2 Cell culture

PC12 cell lines were obtained from the American Type Culture Collection (ATCC) and were cultured in Dulbecco's modified Eagle's Medium containing 10 % of fetal bovine serum, 5 % of horse serum, 1% of glutamine and 1% of Penicillin. Cells were maintained in a humidified atmosphere containing 5% CO<sub>2</sub> at 37°C. Cells (1×10<sup>5</sup>

cells/ml) were incubated with various concentrations of cholesterol (25 & 50 µg/ml) alone or co incubated with various concentration of curcumin (5 & 10 µg/ml) and simvastatin (1 µg/ml) for 24 hrs. Morphological assessment was done by observing the neuronal cells under inverted tissue culture microscope (Olympus IX 70, Japan).

### 2.3 Assessment of cell viability

Viability of cells was determined by the MTT assay. Exponentially growing cells ( $1 \times 10^5$  cells/ml) were plated in 96-well plates and treated with either DMSO as a control group or two different concentrations of cholesterol (25 & 50 µg/ml). Test drug control group were treated with fixed concentration of cholesterol (50 µg/ml) with two different concentration of curcumin (5 & 10 µg/ml). Simvastatin (1 µg/ml) with cholesterol (50 µg/ml) was served as standard control. Incubation was carried out at 37°C for 24 h and (3-(4, 5 dimethyl thiazole-2 yl) - 2,5-diphenyl tetrazolium bromide) MTT solution was added to each well (1.2mg/ml) and incubated for 4h. The reaction results in the reduction of MTT by mitochondrial dehydrogenases of viable cells to form a purple coloured formazan product. The formazan product was dissolved in DMSO and the amount was estimated by measuring absorbance at 570 nm in an ELISA plate reader (Bio-Rad).

### 2.4 Nitrate assay

Nitrites concentrations in the cell supernatants were determined as nitrites by using Griess reagent [18]. 100 µl of cell supernatant were mixed with equal volume of Griess reagent, and the optical density was determined at 540nm in an ELISA reader.

### 2.5 Lactate Dehydrogenase (LDH)

Cell supernatants were assayed for LDH using a LDH kit (Ecoline). 20µl supernatant was mixed with 1ml of 0.1M Tris buffer pH 8.9 containing 50 mM lactate and measuring the absorbance change at 360 nm every minute for 3 min as a measure of NADH consumption [19]. The results are expressed as U/L of LDH activity.

### 2.6 Estimation of cholesterol

Determination of cholesterol was used by cholesterol estimation kits (Ecoline). The supernatant was removed and 1ml of reagent was added. The colour intensity was measured in a UV-VIS Spectrophotometer at 540 nm [20].

### 2.7 Cytokines determination

The cellular debris free cell culture supernatants were assayed in triplicate for IL1β and TNF-α with specific Quantikine ELISA kits according to the manufacturer's instruction, using micro plate reader [21].

### 2.8 Cytokines protein expression studies by Western blot

Brain samples were homogenized with ice-cold lysis buffer with protease inhibitors. The homogenates were centrifuged at 10,000×g for 10 min. The supernatants were collected, and the total protein concentrations of the supernatants were determined by Bradford et al (1976) [22] using bovine serum albumin (BSA) (Bio Rad) as a standard. Protein samples (20µg) were separated by 10% SDS-PAGE (Bio Rad system) and transferred on to a nitrocellulose membrane (Amersham Biosciences). After blocking at room temperature in 10% milk with TBST buffer (10 mM Tris- Hcl, 120 mM Nacl, 0.1% tween-20,

pH7.4) for 1 hr in a shaker, the membrane was incubated with anti mouse TNF and iNOS antibodies (Sigma) at concentrations of 1:1000. Membranes were then washed three times in TBST buffer and incubated with horseradish peroxidase-conjugated secondary antibodies (1:5000) (Sigma) at room temperature for 1 hr, and washed three times. Visualization was carried out using an ECL kit (advanced chemiluminescence, GE healthcare. UK). To serve as a protein loading control, the membrane was stripped and probed for β-actin using its specific antibody (1:5000 Sigma-Aldriches). The films were scanned by an optical densitometer to detect the intensity of protein bands and analyzed.

### 2.9 Animals and Animal grouping and drug administration

Normal, healthy, adult, white New Zealand male rabbits weighing 2.0 to 2.5kg were acquired from the institutional animal breeding house, PSG IMS&R, Coimbatore, Tamil Nadu, India. Animals were maintained at ambient temperature at 18-23 ± 2°C. The animals were housed in large spacious hygienic cages during the course of the experimental period. Experimentation on animals is approved by Institutional Animal Ethical Committee (IAEC), governed by CPCSEA. The approval no is as follows (158/99/ CPCSEA).

Animals are divided into four groups of sixteen male rabbits. Group 1(n=4) rabbits are control group fed with normal diet. Group 2 (n=4) rabbits fed with high fat diet. Group 3(n=4) rabbits are simvastatin (20 mg/kg) treated high fat diet control group and Group 4 (n=4) group rabbits are treated with curcumin (80mg/kg) treated high fat diet control group. The induction of hypercholesterolemia was done by feeding high fatty diet which composed of bengal gram (30%w/w), sucrose (25% w/w), whole milk powder (16%w/w), yeast (1%w/w), hydrogenated groundnut Oil (10%w/w), cholesterol (5%w/w), sodium chloride (5%w/w), shark liver oil (2% w/v) and egg yolk powder (7%w/w).

### 2.10 Blood withdrawal and plasma separation

2ml of blood was withdrawn from the marginal ear vein from rabbit (n=4) at different week intervals (basal, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, 16<sup>th</sup> and 20<sup>th</sup>) into a heparinized vacutainer. Further, blood samples were centrifuged at 4,000 rpm for 5 min and the resultant plasma was subjected to cholesterol estimation using standard cholesterol estimation kit.

### 2.11 Collection of brain

Rabbits (n=4) were sacrificed at the end of the study period by carbon dioxide euthanasia. The skull was cut opened whole brain was removed and homogenized with ice cold phosphate saline (BPS) pH (7.4). The brain homogenate was centrifuged 4000 x g for 10 min, resultant supernatants was separated and stored in -70°C. The cell supernatants were subjected following antioxidant and oxidative marker enzymes given below.

### 2.12 Estimation of superoxide dismutase (SOD)

Superoxide dismutase was assayed by taking 0.05 ml of cell lysates followed by the addition of 0.3 ml of sodium pyrophosphate buffer (0.025 M, pH 8.3), 0.025 ml of PMS (186µM) and 0.075 ml of NBT (300µM in buffer, pH 8.3). Reaction was stated by addition 30°C for 90 seconds, the

reaction stopped by addition of glacial acetic acid (0.025ml). Then the reaction mixture was stirred vigorously and shaken with 2 ml of n-butanol. The mixture was allowed to stand for 10 minutes and centrifuged. 1.5 ml of n-butanol alone served as blank. The color intensity of the chromogen was read at the wave length 560 nm using UV-VIS spectrophotometer [23].

### 2.13 Catalase estimation (CAT)

The activity of Catalase was measured according to the method of Beers and Sizer (1952) [24]; Catalase measurement was done based on the ability of Catalase to inhibit oxidation of hydrogen peroxide ( $H_2O_2$ ). 2.25 ml of potassium buffer (65 mM, pH 7.8) and 100 $\mu$ l of the cell lysates or sucrose (90.32 M) were incubated at 25°C for 30 minutes.  $H_2O_2$  (7.5 mM, 650 $\mu$ l) was added to initiate the reaction. The change in absorbance at 240 nm was measured for 2-3 minutes.

### 2.14 Estimation of glutathione S transferase activity (GST)

Glutathione-S-transferase (GST) activity was measured according by Habig *et al* (1974) [25]. The reaction mixture consisted of 1.425 ml phosphate buffer (0.1 M, pH 7.4), 0.2 ml reduced glutathione (1 mM), 0.025 ml CDNB (1 mM) and 0.3 ml PMS (10%) in a total volume of 2.0 ml. The changes in absorbance were recorded at 340 nm.

### 2.15 Lipid peroxidation assay (TBARS)

Lipid peroxidation was evaluated by measuring the TBARS content according to the TBA test described by Ohkawa *et al* (1979) [26] with slight modification. 0.2 ml of the cell lysates was taken and to this 0.8 ml saline, 0.5 ml of BHT and 3.5 ml TBA reagent (0.8%) were added and incubated at 60°C in a boiling water bath. After cooling, the solution was centrifuged at 2000 rpm for 10 minutes. The absorbance of the supernatant was determined at 532 nm using spectrophotometer.

### 2.16 Histopathological studies

The studied animals brain were excised quickly and fixed in 10% buffered neutral formalin. Paraffin sections 5-10 $\mu$  were prepared, stained with haematoxylin and eosin and mounted in neutral DPX medium.

### 2.17 Statistical analysis

All the data are expressed as Mean  $\pm$  SEM. Data are analysed using Graph Pad Prism version 5.0, USA. One way ANOVA followed by Tukey's Multiple Comparison Test. For analysis of plasma cholesterol at various week intervals two way ANOVA was employed followed by Bonferroni's post test. The level of significance  $p < 0.05$  was considered as significant.

## 3. RESULTS

### *In Vitro* studies

#### 3.1 Morphological Changes

Marked change in the morphology of the neuronal cells was observed with excessive cholesterol (10 $\mu$ g/ml and 50 $\mu$ g/ml) administration. The cholesterol (50 $\mu$ g/ml) produced comparatively more cellular damage and increased vacuolation than the lower dose (10 $\mu$ g/ml). The cells co-incubated with cholesterol showed less neurite outgrowth, increased cellular damage and shrinkage. Treatment of curcumin (10 $\mu$ M) and simvastatin (1 $\mu$ M)

marginally protected the cholesterol effect in the cells and the cells morphology was found to be normal.

#### 3.2 MTT Dye Assay

MTT is converted by mitochondrial dehydrogenase enzymes into a blue color formazan dye. The amount of colour produced is directly proportional to the cell viability. In comparison to solvent treated cell, cells treated with cholesterol (50 $\mu$ M) produced significant increase in dye formation. Curcumin (5 & 50 $\mu$ M) had a protective effect as noted by decreased formazan dye formation (Table 1). Simvastatin (1 $\mu$ M) also significantly protected the cells against cholesterol induced degeneration.

#### 3.3 LDH Assay

Incubation of cells with cholesterol led to a dose-dependent increase in the LDH levels. Significant increase in the LDH was observed with cholesterol (50 $\mu$ g/ml) treated cells. Lower dose of cholesterol not produced any significant alteration in the LDH level. Incubation of curcumin (5 & 10  $\mu$ g/ml) along with cholesterol significantly decreased the LDH level. Similar effect was observed with simvastatin treatment.

#### 3.4 Nitrite Assay

Excessive cholesterol (10 & 50  $\mu$ g/ml) inclusion in the media resulted in increased release of nitrites in PC12 cells in comparison to vehicle treated cells. The effect was found to be dose dependant. Co-incubation of the PC12 cells with curcumin (5 & 10 $\mu$ M) and simvastatin 1 $\mu$ g attenuated the effects cholesterol on nitrite levels (Table 1).

#### 3.5 Cytokines

As shown in Table 1, 48 h incubation of cholesterol (50  $\mu$ g/ml) resulted in increased secretion of IL-1 $\beta$  and TNF- $\alpha$  in PC12 cells. This cholesterol induced increased release of IL-1 $\beta$  and TNF- $\alpha$  was significantly ( $p < 0.001$ ) inhibited with curcumin (10  $\mu$ g/ml) administration. Simvastatin significantly reduced IL- $\beta$  and did not produce any change in TNF- $\alpha$  release induced with cholesterol administration.

#### 3.6 Cholesterol levels in cell supernatants

Cholesterol level in cell supernatants after incubation period was found to be slightly decreased in comparison to its original concentration incubated indicates utilization of cholesterol by the cells. Co-incubation of cultures with curcumin (5 $\mu$ g & 10 $\mu$ g/ml) dose dependently increased the cholesterol utilization and resulted in decreased cholesterol levels. In comparison to curcumin the utilization of cholesterol by the cells treated with simvastatin was found to be higher.

#### 3.7 Western blot analysis

Significant ( $p < 0.01$ ) increase in iNOS and TNF- $\alpha$  expression was observed in cholesterol treated PC12 cell lines as compared to DMSO treated PC12 cell lines. Administration of curcumin (10  $\mu$ g/ml) and simvastatin (1  $\mu$ g/ml) has shown decreased iNOS and TNF- $\alpha$  expression as compared with cholesterol treated PC12 cell lines (Figure 1A&B).

### *In Vivo* studies

#### 3.8 Plasma cholesterol

At the end of the 20<sup>th</sup> week the rabbits fed with high fat diet (HFD) have shown increased body weight. The percentage increase in body weight was found to be 42% in HFD fed

rabbits at end of the 20<sup>th</sup> week (data not shown). HFD administration significantly ( $p < 0.001$ ) increased the plasma cholesterol level in comparison to the rabbits fed with normal diet (Figure 2). Curcumin treatment has shown significant reduction in plasma cholesterol on 8<sup>th</sup> week till the end of the study period as compared with vehicle treated HFD fed rabbits. Similar effect was observed with simvastatin treated rabbits.

### 3.9 Brain cholesterol

Significant ( $p < 0.001$ ) elevation of brain cholesterol level was observed after 20<sup>th</sup> week period in HFD fed rabbits (Table 2). Rabbits treated with curcumin and simvastatin significantly ( $p < 0.01$ ) reduced the brain cholesterol as compared to vehicle treated rabbits. The cholesterol level was measured in whole brain samples.

### 3.10 Superoxide dismutase (SOD)

High fat diet feeding in rabbits for 20 weeks resulted in a significant ( $p < 0.001$ ) depletion of hippocampus superoxide dismutase activity in comparison to normal diet fed rabbits (Table 2). Simultaneous treatment of HFD fed rabbits with curcumin and simvastatin, restored the hippocampus superoxide dismutase activity as indicated by significant ( $p < 0.001$ ) elevation of SOD levels in comparison to HFD alone treated rabbits.

### 3.11 Catalase (CAT)

Catalase level was found to be unaltered in HFD fed rabbits in hippocampus region in comparison to normal diet fed rabbits. Curcumin and simvastatin did not influence the hippocampus CAT activity in comparison to HFD alone treated rabbits was observed.

### 3.12 TBARS activity

TBARS level was found to be significantly ( $p < 0.001$ ) elevated in the brain with HFD consumption in comparison to normal diet fed rabbits (Table 1). TBARS level was found to be decreased with curcumin treated HFD fed rabbits. Simvastatin treatment did not alter the brain TBARS level in HFD fed rabbits.

### 3.13 Glutathione-S-transferase (GST)

The brain hippocampus activity of GST in HFD fed rabbit was found to be significantly ( $p < 0.01$ ) decreased in comparison to normal diet fed rabbits. No significant alteration of GST activity was observed with curcumin and simvastatin treatment.

### 3.14 Histopathology Rabbits treated with high fat diet alone

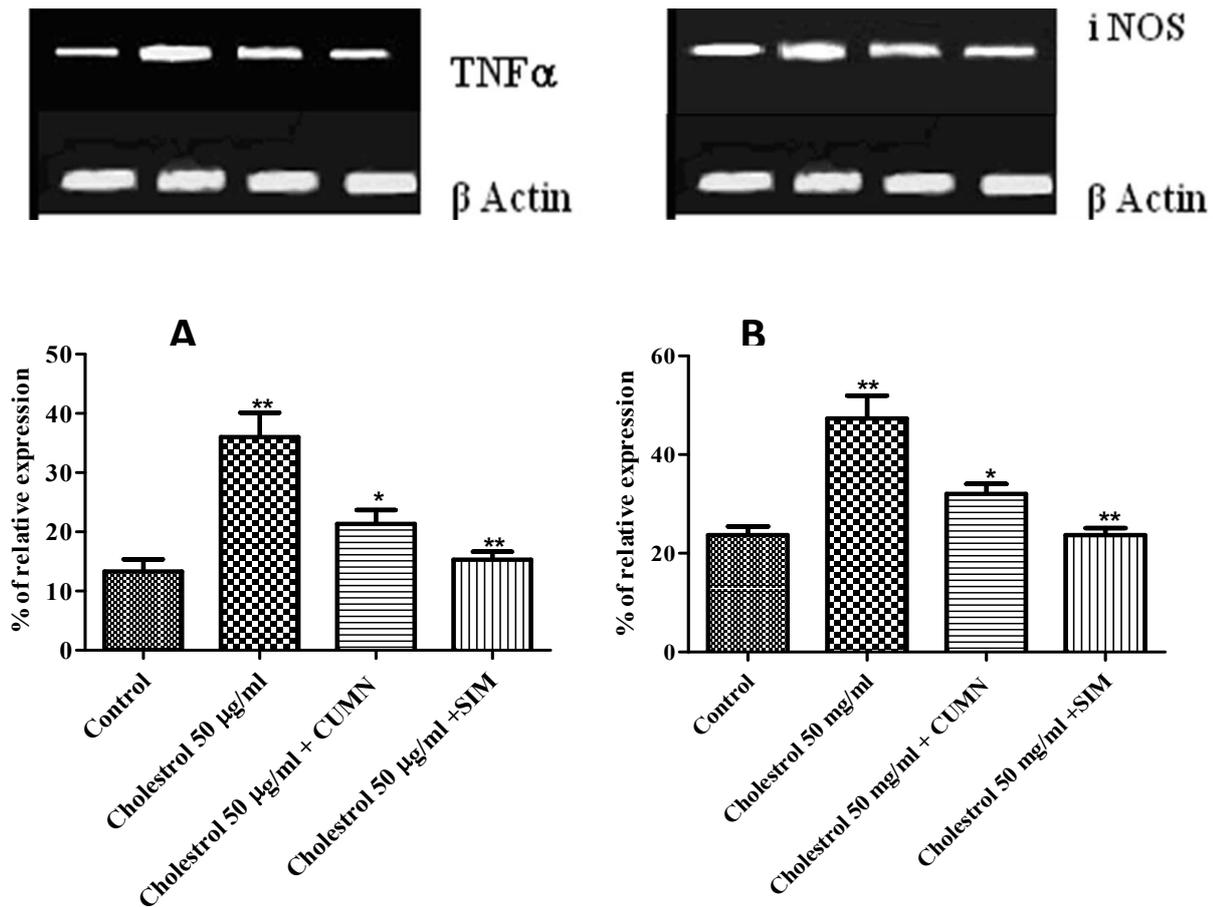
Histopathological examination of cerebral cortex shows neurons, ganglion cells, astrocytes and few microglial cells. The white matter shows predominance of oligodendroglial cells (Figure 3A&B). The blood vessels show perivascular cuffing by mononuclear cells composed chiefly of lymphocytes. This finding is also noted in a few places around the blood vessels traversing the subarachnoid space (Figure 3C&D). Yet the vein cells have also exhibited edema, gliosis and dense lymphocytic infiltration. The treatment of simvastatin and curcumin resulted in intact cerebral cortex neurons, ganglion cells, astrocytes and few microglial cells. The white matter shows predominance of oligodendroglial cells (Figure 3E). There is no evidence of perivascular cuffing of lymphocytes-either within the brain substance (or) in the subarachnoid space vessels. One focal area shows gliosis characterized by increased number of astroglial cells.

**Table 1:** Effects of curcumin on cell viability, cholesterol, nitric oxide, LDH, interleukin-1 $\beta$  and TNF $\alpha$  in cholesterol incubated PC12 cell culture. Values are expressed as mean  $\pm$  S.E. \*\*\* $p < 0.001$  treatments vs control.

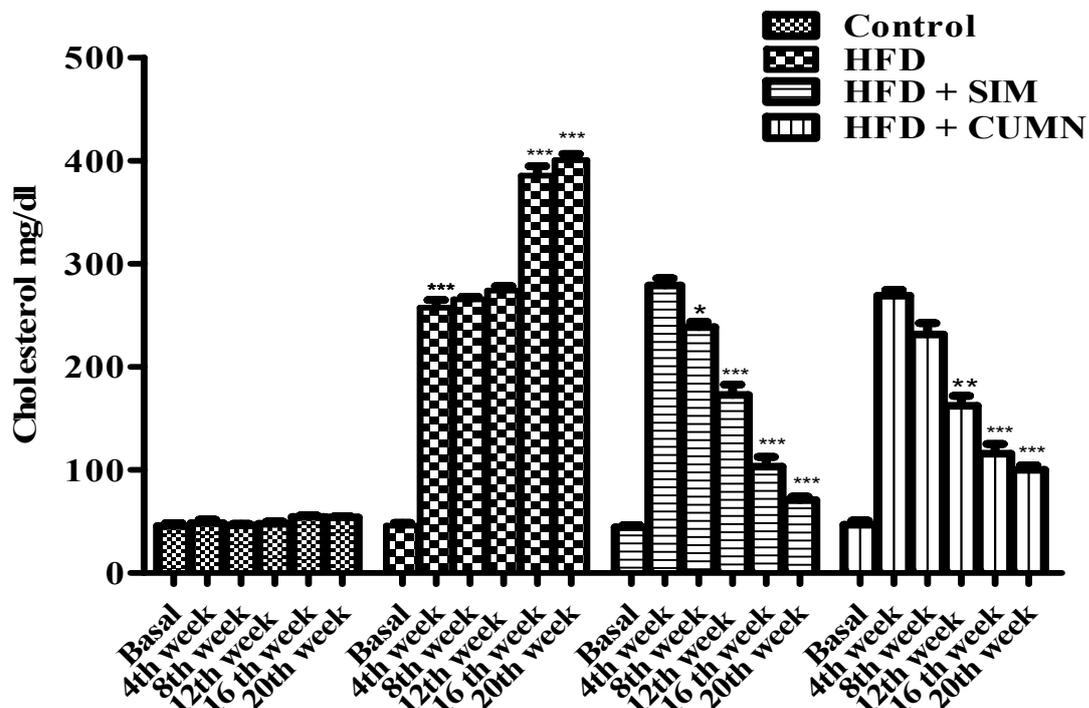
	CTRL	C10	C50	CUR5	CUR10	SIM1
Viability	99.15 $\pm$ 4.11	84.25 $\pm$ 1.85	59.88 $\pm$ 2.67***	75.78 $\pm$ 2.90**	83.45 $\pm$ 1.26***	86.17 $\pm$ 2.99***
CHL	1.28 $\pm$ 0.05	5.43 $\pm$ 0.11	38.93 $\pm$ 1.27***	30.27 $\pm$ 1.80**	17.71 $\pm$ 1.20***	15.59 $\pm$ 1.18***
NO	5.325 $\pm$ 0.17	8.74 $\pm$ 0.09	9.91 $\pm$ 0.15***	7.27 $\pm$ 0.14**	7.96 $\pm$ 0.08***	6.75 $\pm$ 0.24***
LDH	13.75 $\pm$ 0.79	20.47 $\pm$ 0.89	49.00 $\pm$ 0.85***	33.28 $\pm$ 1.48***	22.63 $\pm$ 0.44***	21.83 $\pm$ 0.70***
IL1 $\beta$	84.83 $\pm$ 3.07	91.83 $\pm$ 2.10	182.67 $\pm$ 4.29***	162.50 $\pm$ 1.99***	109.67 $\pm$ 1.86***	163.50 $\pm$ 1.91***
TNF $\alpha$	22.50 $\pm$ 2.18	24.33 $\pm$ 1.43	68.83 $\pm$ 1.33***	62.33 $\pm$ 1.25	31.17 $\pm$ 1.14***	64.33 $\pm$ 1.82

**Table 2:** Effects of curcumin on brain Cholesterol, SOD, CAT, TBARS and GST levels in high fat fed rabbits for 20 weeks. Values are expressed as mean  $\pm$  S.E. \*\*\* $p < 0.001$  treatments vs control.

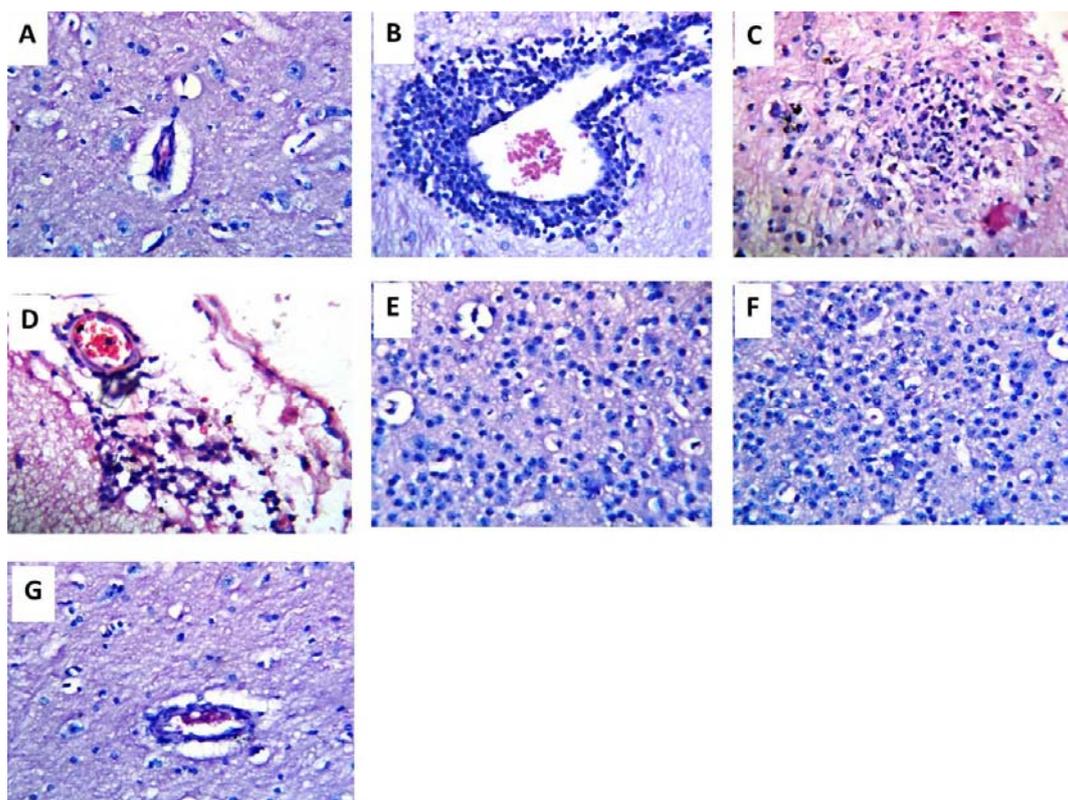
Groups	Brain Cholesterol	SOD	CAT	TBARS nmol/ml	GSTnmol/hr
CON	28.75 $\pm$ 1.76	51.88 $\pm$ 1.23	42.52 $\pm$ 0.81	36.13 $\pm$ 1.06	0.3451 $\pm$ 0.01
HFD	67.25 $\pm$ 4.42***	27.39 $\pm$ 1.47***	42.97 $\pm$ 0.83	49.13 $\pm$ 0.86***	0.2825 $\pm$ 0.01**
SIM	41.5 $\pm$ 2.90**	48.78 $\pm$ 0.70***	41.50 $\pm$ 0.55	46.29 $\pm$ 1.19	0.2950 $\pm$ 0.01
CUR	43.5 $\pm$ 3.42**	49.53 $\pm$ 0.89***	41.19 $\pm$ 0.57	40.70 $\pm$ 0.70***	0.3225 $\pm$ 0.01



**Figure 1:** Effects of curcumin on TNF- $\alpha$  (A) and iNOS (B) gene expression in cholesterol incubated PC12 cells during 48 hours. The intensity of the bands was quantified by scanning densitometry, standardized with respect to  $\beta$ -actin protein and values are expressed as mean  $\pm$  S.E. \* $p < 0.05$  treatments vs control.



**Figure 2:** Effects of curcumin on plasma cholesterol levels in high fat fed rabbits for 20 weeks. Values are expressed as mean  $\pm$  S.E. \*\*\* $p < 0.001$  treatments vs control.



**Figure 3:** Effects of curcumin on rabbit brain histopathological results. (A) Brain tissue showing normal blood vessel in control group animal; (B) Brain tissue showing perivascular cuffing by lymphocytes in cerebral cortex in high fat diet fed animal; (C) Brain tissue showing edema, gliosis and lymphocytic infiltration in high fat diet fed animal; (D) Lymphocytes in subarachnoid space and around blood vessels in in high fat diet fed animal; (E) Brain tissue showing gliosis characterized by proliferation of astroglial cells in simvastatin treated animal; (F) Brain tissue showing gliosis characterized by proliferation of astroglial cells in curcumin treated animal; (G) Brain tissue showing normal blood vessel in curcumin treated animal.

#### 4. DISCUSSIONS AND CONCLUSION

In this study, we demonstrated that the protective effect of curcumin against the neuroinflammation induced by feeding high fat diet in rabbits as well as high cholesterol incubation in cell lines. The present *in-vitro* study highlights the inflammatory effect of cholesterol on PC12 cell lines mediated through nitrosative stress and cytokines expression such as IL- $\beta$ 1 and TNF- $\alpha$ . This is a first kind of report that PC12 cell lines incubated with cholesterol for 48 hrs leads to accumulation of nitrite level as well as cytokine expression *in-vitro*. Report indicated HFD causes increased pro-inflammatory cytokines release in reproductive senescent female rats. However, it has been shown by many investigators that free cholesterol treatment causes impairment of anti-oxidants, mitochondrial dysfunction and NF $\kappa$ B activation and advent cell to apoptosis [27, 28]. For example, 7-ketocholesterol is an oxidation product of cholesterol, has shown apoptosis in PC12 cells by increasing the levels of phospho-I $\kappa$ B-a, NF $\kappa$ B p65 and NF $\kappa$ B p50 with the ROS-dependent NF $\kappa$ B activation [29]. The cholesterol induced neurotoxic effect was attenuated by curcumin as shown by significant reduction in the level of IL-1 $\beta$  and TNF- $\alpha$  in PC12 cells. This finding suggests that the cholesterol as such can trigger cytokine gene expression in PC 12 cell lines and that curcumin, possibly

through its cytokine inhibition property. The various pharmacological activities of curcumin including inhibition of cytokines gene expression are at least partially mediated by inhibition of GSK-3  $\beta$  [30]. Previously we have reported that *per se* curcumin inhibit the GSK-3 $\beta$ , is protein kinase family enzyme in *in-silico* which is involved in gene expression for cytokines [31]. In this investigation we used simvastatin, a HMG-Co-A inhibitor (1 $\mu$ g/ml) used as a standard did not shown any cytokine inhibition property except it does attenuates nitrate and cholesterol level in PC 12 cell lines. This effect was in accordance with the published report on simvastatin against dopaminergic neurotoxin (6-OHDA) mediated nitrosative stress in PC 12 cell lines [32]. This clearly indicates the direct scavenging role of curcumin on cytokines evoked by free cholesterol in PC 12 cell lines.

Ever growing scientific evidence demonstrates that the clear link between hypercholesterolemia and oxidative stress associated neurodegeneration in brain [33, 34]. There are various *in-vivo* rodent high fat diet models mimics the pathology of AD dementia and it was attenuated by lipid lowering drugs [35] as well as neuronal inflammation [36]. It has been shown that cholesterol mediated oxidative stress, increased the expression of inducible nitric oxide synthase (iNOS) produces excessive levels of nitric oxide

in the activated microglia lead to a disruption of neuronal mitochondrial electron transport chain function [37, 38]. Lakshmi et al (2008) [1] has been shown the involvement of cytokines like IL-6, TNF- $\alpha$  and chemokine MCF 1 in mouse brain treated with high fat diet meal. Simvastatin is a HMG-CoA reductase drugs extensively using in the treatment of hyperlipidemia have been implicated in the study of neuroinflammation and neurodegeneration [39, 40]. Farmer et al (2010) [41] have demonstrated that rosuvastatin treatment resulted in significant reduction of neuroinflammation in an animal model of high cholesterol diet induced hypercholesterolemia.

The results from *in-vivo* experiments further confirms the neuroprotective effect of curcumin administered twenty weeks following high fat meal fed rabbits. The significant reduction in plasma and brain cholesterol level was observed in curcumin treatment associated with attenuation of iNOS and TNF- $\alpha$  expression in hippocampus. Further, curcumin treatment restored the brain superoxide dismutase level and decreased the CAT and MDA level in high fat diet treated rabbits. Balusamy et al (2008) [42] have shown that curcumin prevents protein oxidation and protect mitochondrial complex-I by restoring depleted antioxidant glutathione (GSH). They have also suggested that curcumin has vital role for preventing neurodegenerative disorder linked with GSH depletion mediated by oxidative stress. Various doses of curcumin reduce the oxidative stress and amyloid induced neuroinflammation has been implicated in Alzheimer's transgenic mouse model [16]. This study further supports the neuropathological changes observed in high fat fed rabbit brain cortex with focal inflammation associated with lymphocytic infiltration and this effect was reversed by curcumin administration.

It can be concluded that curcumin has shown neuroprotective effect in HFD model of neuronal inflammation and the neuroprotective effect is might be due to the inhibition of brain cytokines, oxidative stress and reduction in cholesterol.

#### COMPETING INTERESTS

None

#### ACKNOWLEDGMENTS

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