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ORIGINAL PAPER

Trans-cinnamaldehyde Modulates Hippocampal Nrf2 Factor and Inhibits Amyloid Beta Aggregation in LPS-Induced Neuroinflammation Mouse Model

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Abstract

Trans-cinnamaldehyde (CNM) has recently drawn attention due to its potent anti-inflammatory and antioxidant properties. The current study explored the memory enhancing effects of CNM against lipopolysaccharide (LPS)-induced neuroinflammation in mice. CNM and curcumin (a reference antioxidant) were administered at a dose of 50 mg/kg i.p. 3 h after a single LPS injection (0.8 mg/kg, i.p.) and continued daily for 7 days. Our results displayed that CNM and curcumin significantly ameliorated the LPS-induced impairment of learning and memory, neuroinflammation, oxidative stress and neuronal apoptosis. Memory functions and locomotor activity were assessed by Morris water maze, object recognition test and open field test. Both CNM and curcumin activated the nuclear factor erythroid 2 related factor 2 (Nrf2) and restored levels of downstream antioxidant enzymes superoxide dismutase and glutathione-*S*-transferase (GST) in the hippocampus. They also attenuated LPS-induced increase in hippocampal contents of interleukin-1β (IL-1β), malondialdehyde and caspase-3. Immunohistochemistry results showed that both CNM and curcumin reduced $A\beta_{1-42}$ protein accumulation in brain of mice. Remarkably CNM's effect on IL-1β was less pronounced than curcumin; however it showed higher GST activity and more potent anti-apoptotic and anti-amylodogenic effect. We conclude that, CNM produces its memory enhancing effects through modulation of Nrf2 antioxidant defense in hippocampus, inhibition of neuroinflammation, apoptosis and amyloid protein burden.

Keywords *Trans*-cinnamaldehyde · Neuroinflammation · Nrf2 · Curcumin · LPS · Amyloid beta

Introduction

Neuroinflammation plays a key role in the progression of neurodegeneration and the resulting neuronal injury is responsible for memory deterioration over time [1]. Neuroinflammation encompasses the inflammatory reactions that accompany neurodegenerative diseases which involve mainly the innate immune system [2]. These inflammatory responses are governed mainly by microglia; they undergo phenotypic switching and consequently, produce free radicals, cytokines and attract neutrophils [3]. Excessive reactive oxygen species (ROS) build-up causes damage to biological macromolecules like lipids and proteins and results in oxidative stress leading to amyloid beta (Aβ) or tau associated neurotoxicity [4]. Moreover, ROS stimulate pro-inflammatory genes transcription and release of cytokines and chemokines, creating a vicious cycle of oxidative stress and inflammation [5]. When lipopolysaccharide (LPS) is administered systemically, it induces neuroinflammation through microglial and astrocytes activation. It elevates the production of inflammatory cytokines through the enhancement of NFκB nuclear translocation after binding to toll-like receptor 4 (TLR4) [6] on microglia and astrocytes, eventually causing cognitive deterioration and sickness behaviour. LPS is used as an experimental model of neuroinflammation that accompany many neurodegenerative diseases [7, 8].

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Cells adapt several mechanisms for protection against oxidative stress associated damage. The nuclear factor erythroid 2 related factor 2 (Nrf2), is an essential transcription factor to counteract oxidative stress and neuroinflammation [9]. Nrf2 activates antioxidant response element (ARE)-dependent gene expression of a number of antioxidant and cytoprotective proteins that include thioredoxins (Trxs), superoxide dismutase-1 (SOD-1), glutathione peroxidase (GPx), glutathione-*S*-transferase (GST), glutathione reductase (GR), heme oxygenase-1 (HO-1) and NAD(P)H quinone oxidoreductase-1 [10]. Beside its important role in the induction of antioxidant proteins, Nrf2 was also found to suppress the NfκB inflammatory pathway emphasising its protective role in neuroinflammation [11]. Thus, Nrf2 signalling pathway has been considered a potential therapeutic target for neuroinflammatory and neurodegenerative disorders.

Trans-cinnamaldehyde (CNM) is the main bioactive oil found in the stem bark of *Cinnamomum cassia* giving cinnamon its pleasant odour. It has been reported to show diverse pharmacological activities like anticancer, anti-diabetic, antimicrobial and anti-coagulant effects [12–15]. It has also been shown to demonstrate powerful anti-inflammatory activities through different pathways like TLR4, NFκB and MAPK and to offer neuroprotection in dopaminergic degeneration [16]. Recent evidence indicates that prophylactic treatment with CNM is able to reduce microglial activation and offer protection against memory impairment in a model of LPS-induced neuroinflammation [17].

Curcumin, a yellow pigment originally isolated from turmeric, is well known for its antioxidant and anti-inflammatory effects. Several studies have also shown that curcumin offers neuroprotection and memory-enhancing properties that can delay or inhibit neurodegenerative diseases, including Alzheimer's disease (AD) [18–20]. Animal studies reported that curcumin may counteract dementia development, because of its antioxidant and anti-inflammatory properties, as well as its significant role in Aβ metabolism [21, 22]. Therefore, curcumin was used as a reference antioxidant in the current study.

Nevertheless, the Nrf2-mediated antioxidant mechanism of CNM and its ability to attenuate already induced neuroinflammation are not yet fully elucidated. The present study explored the effects of CNM on Nrf2 modulation and its potential to reverse neuroinflammation in LPS-treated mice through assessing animal behaviour, and several hippocampal biochemical parameters including: cytoplasmic and nuclear Nrf2, downstream antioxidant proteins like SOD, GST and GPx, IL-1 beta, caspase-3 and deposition of $A\beta_{1-42}$ proteins.

Experimental Procedures

Animals

Adult Swiss albino male mice weighing 25–30 g, 3–4 months old were used. The mice were obtained from the modern veterinary office (Giza, Egypt) and were kept in the animal house at Modern Sciences and Arts University where they were exposed to 12 h/12 h dark/light cycle and had free access to food and water. Animals were treated according to the guidelines of the Ethics Committee at Cairo university [registration number: PT (1565), 2015] and recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 8523, revised 1985). All efforts were made to reduce animals' suffering.

Chemicals

The LPS serotype (055:B5) (Sigma-Aldrich, MO, USA) and CNM were dissolved in phosphate buffered saline (PBS) [23]. Curcumin (Sigma-Aldrich, MO, USA) was dissolved in 0.5 N NaOH in PBS [24].

Experimental Design

Mice were randomly allocated into four groups containing 20 mice each; control, LPS, curcumin and CNM. The control group received a single i.p. injection of PBS on the first day, followed by 0.2 ml 0.5 N NaOH in PBS i.p., daily for 7 days. The LPS group received a single injection of LPS (0.8 mg/kg) i.p. on day 1 followed by 0.2 ml 0.5 N NaOH in PBS i.p. daily for 7 days. The curcumin group received the LPS once followed by curcumin dissolved in NaOH/PBS (50 mg/kg) i.p. 3 h later and repeated daily for 7 days. The CNM group received the LPS once followed by CNM dissolved in PBS (50 mg/kg) i.p. 3 h later and repeated daily for 7 days. The dose of CNM was chosen based on the literature where it was reported to show the best improvement in learning and memory and neuroprotection when given prophylactically before LPS [17]; and the dose of curcumin was chosen accordingly after checking the literature for the safety of the chosen doses [25].

Behavioural Experiments

On the fourth day of the experiment, mice were trained on the Morris water maze task (MWM) for four successive days. Training on the open field and the object recognition task took place on day 7 of the experiment. On the following day (day 8 of the experiment), mice were subjected to

the probe test, open field test (OFT) and object recognition test (ORT). Behaviour of animals was video recorded and analysed off-line by a blinded investigator.

MWM

This test measures the spatial memory of a mouse. Mean escape latency (MEL) was recorded during the 4 days of acquisition phase. The time spent in the target quadrant in relation to other quadrants was calculated in the probe test and expressed as percent quadrant time (Q) [26].

OFT

This was carried out to ensure that the mice motor activity was not affected by the LPS. The mean frequencies of ambulation (number of lines crossings), rearing (number of times an animal stood in a vertical upright position) and grooming (number of face licking of an animal) within 3 min were scored by a blinded investigator using hand-operated counters after being video recorded on day 8 of the experiment for each group [27].

ORT

This test measures the non-spatial memory of a mouse. After training on the OFT, mice were trained on the ORT task by exploration of two identical objects placed at the end of the arena of the open field which had been covered with sawdust, and this was referred to as the sample trial (T1). On the choice trial (T2) 24 h later, one of the familiar objects (F) was replaced with a new different object (N) where object recognition was scored by the number of object sniffs of both the familiar (F) and new (N) objects and preference index (PI), an index of recognition memory, was calculated. PI is the ratio of the number of attempts of exploring the new object in T2 over the total exploratory attempts of both objects in T2 [28].

$$\text{Preference index (PI)} = N/(N + F) \times 100(\%)$$

After completion of behavioural testing, mice were sacrificed, their brains were excised and hippocampi were dissected for subsequent biochemical investigations. Dissection of each brain was performed on ice-cold glass plates for separation of both hippocampi.

Biochemical Experiments

Nuclear and cytoplasmic proteins extraction was done using NE-PER nuclear and cytoplasmic protein extraction kit from Thermo Scientific (catalogue number: 78,833).

Malondialdehyde (MDA)

It was assayed using MDA assay kit (Biodiagnostic, Egypt) according to the manufacturer's procedure [29].

Superoxide Dismutase (SOD) Activity

Enzymatic activity of SOD was determined using SOD assay kit (Biodiagnostic, Egypt) according to the manufacturer's procedure [30].

Glutathione-*S*-Transferase (GST) Activity

Total GST activity was estimated using GST assay kit (Biodiagnostic, Egypt) according to the manufacturer's procedure [31].

Glutathione Peroxidase (GP_x) Activity

Cellular GP_x was assayed using GPx assay kit (Biodiagnostic, Egypt) according to the manufacturer's procedure [30].

Nrf2

This was performed using an ELISA kit with a microtiter plate precoated with a specific mouse-Nrf2 antibody (Aviva systems biology, USA). The concentration of Nrf2 (pg/ml) in the samples was calculated from a constructed standard curve then the protein concentrations were used to estimate the Nrf2 concentration per total gram protein.

IL-1β

This was determined using an ELISA kit provided with a microtiter plate coated with an antibody specific for rat IL-1 beta (Cohesion Biosciences, UK). The concentration of IL-1β (pg/ml) in the samples was calculated from a constructed standard curve then the protein concentrations were used to estimate the concentration of IL-1β per total gram protein.

Caspase-3 Content

This was performed using a microtiter plate precoated with an antibody specific for rat caspase-3 (Cusabio Biotech, China).The concentration of caspase-3 (ng/ml) in the samples was calculated from a constructed standard curve

then the protein concentrations were used to estimate the concentration of caspase-3 per total gram protein.

Immunohistochemistry

Three mice of each group were used for immunohistochemical assay of $A\beta_{1-42}$ peptides. Their brains were dissected and kept in 4% formalin. The 1ry antibody for $A\beta_{1-42}$ was a rabbit polyclonal antibody from Abcam (ab 10148) [8]. The number of stained (positive) cells was averaged across five sections for each mouse. Quantification of $A\beta_{1-42}$ staining was carried out automatically using Image J (NIH) software. The plaque areas and mean plaque intensity were recorded in pixels. The density measured in density units (pixels) was calculated by multiplication of accumulated plaque area by average plaque intensity [32].

Statistical Analysis

Data are expressed as mean ± standard error of mean (SEM). Comparison between means was carried out using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. The level of significance was fixed at $p < 0.05$. Statistical analysis was carried out using Graphpad Prism software, version 5a (GraphPad Software, Inc., USA).

Results

Behavioural Experiments

The Effect of CNM and Curcumin on Spatial Memory

Administration of LPS to mice impaired spatial memory as indicated by MWM task. Comparison between groups was taken on day 7 of the experiment. LPS-treated mice showed an increase in escape latency time on day 7 to 151% compared with control group (Fig. 1a) and a significant decrease in percent quadrant time to reach 55% compared with the control group (Fig. 1b). Both CNM and curcumin treatments significantly reduced escape latency time in the MWM test on day 7 compared with LPS group by 31% and 33% respectively and normalized it to 103% and 102% respectively as compared with control group (Fig. 1a). In addition, CNM and curcumin administration significantly prolonged the time mice spent in the target quadrant in the probe test compared with LPS group by 83% and 70% respectively and normalized it to 101% and 93% respectively as compared with control group (Fig. 1b).

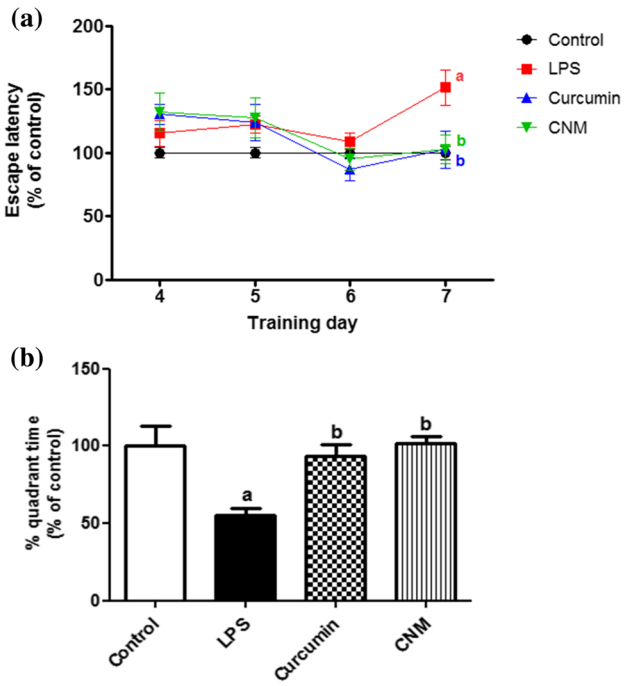

Fig. 1 Effect of CNM and curcumin on escape latency time and percent quadrant time using Morris water maze in LPS-induced neuroinflammation in mice. **a** Escape latency time and **b** the percent quadrant time. **a** A significant difference existed between the LPS group and the control group ($p < 0.05$) on day 7. Both CNM (50 mg/kg/day, i.p.) and curcumin (50 mg/kg/day, i.p.), significantly reduced the escape latency time compared with the LPS group on day 7 ($p < 0.05$). **b** In the probe test a significant difference existed between the LPS and control groups ($p < 0.05$). Both curcumin and CNM significantly increased the time mice spent in the target quadrant compared to the LPS group ($p < 0.05$). Results are expressed as mean ± SEM ($n = 10$ mice). For statistical significance, [a]significantly different from control group at $p < 0.05$, [b]significantly different from LPS group at $p < 0.05$

Effect of CNM and Curcumin on Non-spatial Memory

In the ORT, LPS-treated mice showed a significant reduction in PI to 49% as compared with control group (Fig. 2). Contrarily CNM and curcumin treatment significantly ameliorated PI percent compared to LPS group by 58% and 46% respectively and reduced it to 78% and 72% respectively as compared to control group (Fig. 2).

Effect of CNM and Curcumin on Ambulation, Rearing and Grooming

In the OFT, the LPS group did not show significant difference in ambulation as compared to the control group but rearing and grooming frequencies were significantly increased to 141% and 125% respectively. CNM and curcumin produced a significant fall in frequency of ambulation as compared with LPS group by 24% and 18% respectively and normalized it to 85% and 92% respectively compared

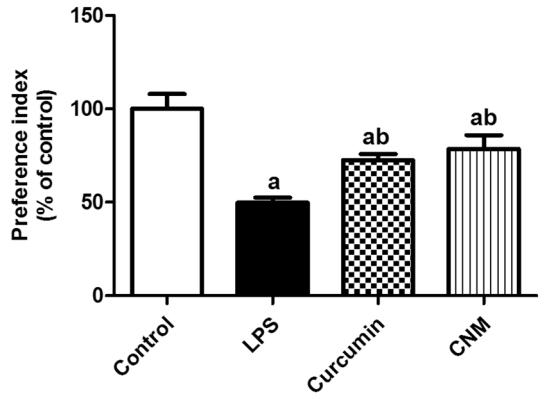

Fig. 2 Effect of CNM and curcumin on preference index using object recognition test in LPS-induced neuroinflammation in mice. A significant difference existed between the LPS and the control group ($p < 0.05$). Both CNM (50 mg/kg/day, i.p.) and curcumin (50 mg/kg/day, i.p.) significantly ameliorated the preference index as compared with the LPS group ($p < 0.05$). Results are expressed as mean ± SEM (n = 10 mice). For statistical significance, [a]significantly different from control group at $p < 0.05$, [b]significantly different from LPS group at $p < 0.05$

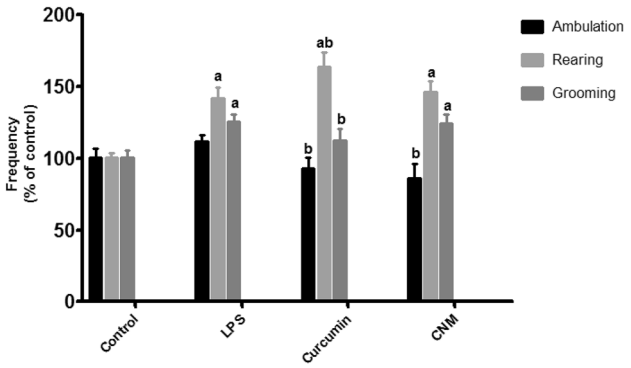

Fig. 3 Effect of CNM and curcumin on ambulation, rearing and grooming using open field test in LPS-induced neuroinflammation in mice. A significant difference was observed between the LPS and the control group in rearing and grooming. CNM (50 mg/kg/day, i.p.) and curcumin (50 mg/kg/day, i.p.) significantly reduced the ambulation frequency as compared with LPS group ($p < 0.05$), while curcumin significantly increased rearing and reduced grooming as compared with LPS group ($p < 0.05$). Results are expressed as mean ± SEM (n = 10 mice). For statistical significance, [a]significantly different from control group at $p < 0.05$, [b]significantly different from LPS group at $p < 0.05$

with control group (Fig. 3). Also, both CNM and curcumin significantly increased rearing frequency to 145% and 163% compared to control group; however, only curcumin significantly increased it by 15% compared to LPS group. Curcumin significantly reduced grooming frequency by 10% as compared to LPS group and normalized it to 111% compared to control group. On the other hand CNM increased grooming frequency to 123% compared to control group but no significant difference existed between CNM and LPS group.

Biochemical Investigations

Effect of CNM and Curcumin on Hippocampal MDA, SOD, GST and GPx

Hippocampal MDA was significantly higher in LPS group reaching 6.58 ± 0.43 nmol/mg protein (146%) compared with control mice. LPS-treated mice showed a marked reduction of hippocampal SOD, GST and GPx activities to 8.69 ± 0.67 U/mg protein (72%), 7.95 ± 0.69 U/mg protein (37%) and 17.93 ± 0.90 U/mg protein (55%), respectively as compared with control group (Table 1). Treatment with CNM and curcumin significantly reduced MDA level by 23% and 25% compared to LPS-treated mice and normalized it reaching 5.07 ± 0.18 nmol/mg protein (112%) and 4.91 ± 0.32 nmol/mg protein (109%) respectively compared to control group (Table 1). Also, both CNM and curcumin significantly ameliorated hippocampal SOD activities by 24% and 35% respectively compared to LPS group and normalized it to control group reaching 10.80 ± 0.34 U/mg protein (89%) and 11.82 ± 0.42 U/mg protein (98%) respectively. Similarly, hippocampal GST was significantly increased compared to LPS group by 139% and 87% by CNM and curcumin respectively; nevertheless CNM had better effect than curcumin as it normalized its level to 19.03 ± 0.27 U/mg protein (88%), whereas curcumin increased it to 14.95 ± 0.90 U/mg protein (70%) as compared to control group (Table 1).

On the contrary, both CNM and curcumin failed to significantly increase hippocampal GPx activity as compared to LPS-treated mice; however both had significantly lower level of GPx compared to control group reaching 20.23 ± 0.83 U/mg protein (62%) and 19.43 ± 1.40 U/mg protein (60%) respectively (Table 1).

Effect of CNM and Curcumin on Hippocampal Nrf2, IL-1β and Caspase-3 Content

Nrf2 activation involves the translocation of Nrf2 from cytoplasm to nucleus [33]. The ratio of nuclear to cytoplasmic Nrf2 content was slightly increased by LPS to 1.99 as compared with control group (Fig. 4). Also, IL-1β and caspase-3 content were significantly increased by LPS injection to 212% and 177%, respectively as compared with control group (Fig. 5).

CNM and curcumin significantly enhanced Nrf2 nuclear translocation, where CNM and curcumin significantly increased relative nuclear to cytoplasmic Nrf2 ratio to

Table 1 Effect of CNM and curcumin on hippocampal MDA, SOD, GST and GPx activities

Groups	Parameters			
	MDA (nmol/mg protein)	SOD (U/mg protein)	GST (U/mg protein)	GPx (U/mg protein)
Control (PBS + 0.5 N NaOH in PBS, i.p.)	4.49 ± 0.12	12.04 ± 0.21	21.53 ± 0.81	31.85 ± 1.40
LPS (0.8 mg/kg, i.p.)	6.58 ± 0.43[a]	8.69 ± 0.67[a]	7.95 ± 0.69[a]	17.93 ± 0.90[a]
LPS + curcumin (50 mg/kg, i.p.)	4.91 ± 0.32[b]	11.82 ± 0.42[b]	14.95 ± 0.90[ab]	19.43 ± 1.40[a]
LPS + CNM (50 mg/kg, i.p.)	5.07 ± 0.18[b]	10.80 ± 0.34[b]	19.03 ± 0.27[bc]	20.23 ± 0.83[a]

Results are expressed as mean ± SEM (n = 20 mice)

[a]Significantly different from control group at $p < 0.05$

[b]Significantly different from LPS group at $p < 0.05$

[c]Significantly different from curcumin group at $p < 0.05$

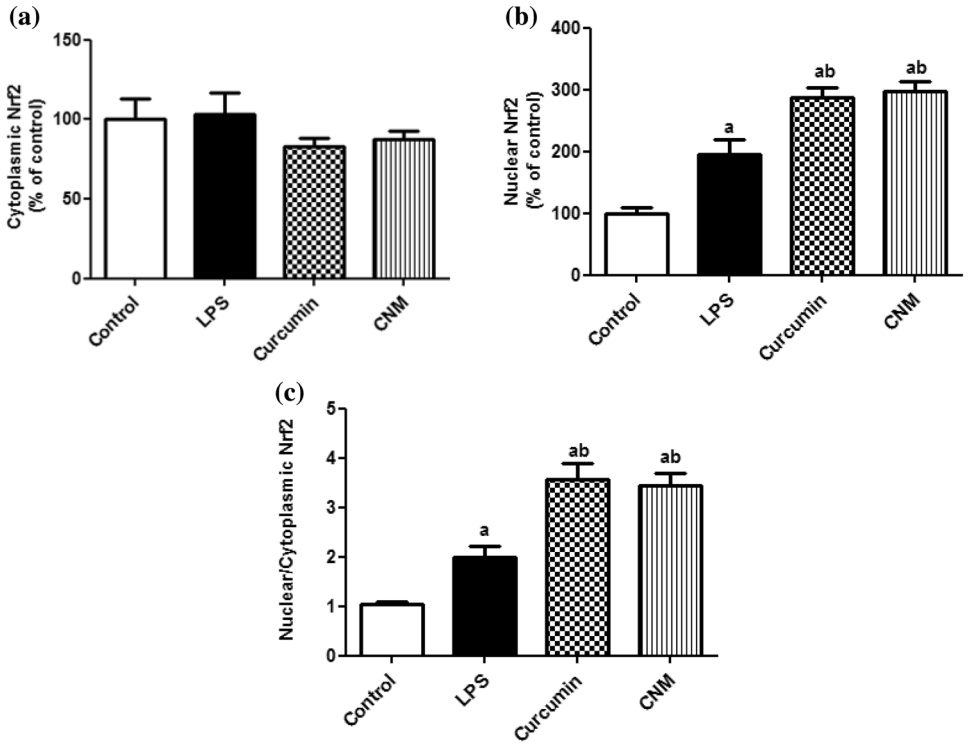

Fig. 4 Effect of CNM and curcumin on hippocampal nuclear and cytoplasmic Nrf2 content in LPS-induced neuroinflammation in mice. **a** Cytoplasmic Nrf2, **b** nuclear Nrf2 and **c** nuclear to cytoplasmic Nrf2 relative content; a significant difference existed between LPS and control group in nuclear and relative nuclear to cytoplasmic Nrf2 content ($p < 0.05$). Both CNM (50 mg/kg/day, i.p.) and curcumin (50 mg/kg/day, i.p.) significantly elevated relative nuclear to cytoplasmic Nrf2 content as compared to LPS group ($p < 0.05$). Results are expressed as mean ± SEM (n = 20 mice). For statistical significance, [a]significantly different from control group at $p < 0.05$, [b]significantly different from LPS group at $p < 0.05$

3.4 and 3.5 respectively compared with LPS-treated mice (Fig. 4).

Both CNM and curcumin were able to significantly attenuate LPS-induced hippocampal rise in IL-1β and caspase-3. IL-1β was less reduced by CNM than curcumin with a 29% and 59% decrease respectively compared with LPS group. Curcumin reversed LPS effect on IL-1β reaching 87% while CNM significantly increased it to 150% compared to control mice. Contrarily, CNM showed significantly better anti-apoptotic effect than curcumin as it reduced caspase-3 by 48% whereas curcumin only reduced it by 31% compared with LPS group. In fact CNM normalized caspase-3 to 96% while curcumin normalized it to 129% compared with control group (Fig. 5).

Effect of CNM and Curcumin on Aβ Plaques Using Immunohistochemistry

Brain sections treated with $A\beta_{1-42}$ antibody and stained demonstrated that the immunoreactivity was localized in the hippocampus. LPS injection induced amyloid plaques deposition in the hippocampus, contrarily to the control group

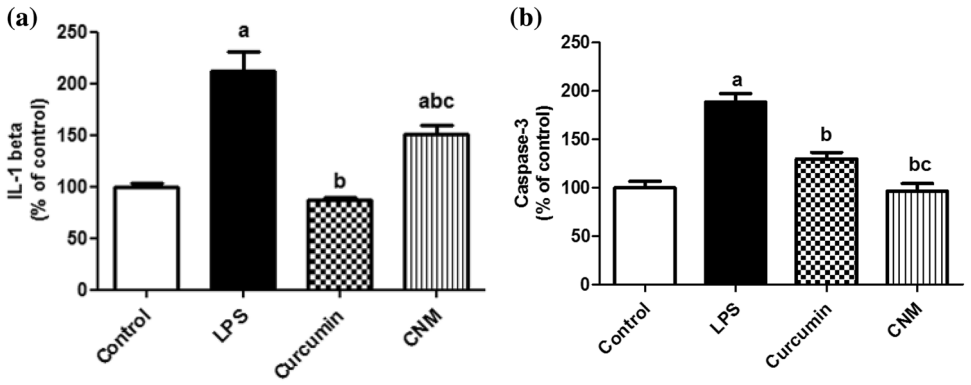

Fig. 5 Effect of CNM and curcumin on hippocampal interleukin-1β and caspase-3 content in LPS-induced neuroinflammation in mice. **a** IL-1β **b** caspase-3 content; a significant difference existed between LPS and control group in IL-1β and caspase-3 hippocampal contents ($p < 0.05$). Both CNM (50 mg/kg/day, i.p.) and curcumin (50 mg/kg/day, i.p.) significantly reduced IL-1β and caspase-3 content as compared to LPS group ($p < 0.05$). A significant difference existed between CNM and curcumin in IL-1β and caspase-3 contents ($p < 0.05$). Results are expressed as mean ± SEM (n = 20 mice). For statistical significance, [a]significantly different from control group at $p < 0.05$, [b]significantly different from LPS group at $p < 0.05$, [c]significantly different from curcumin group at $p < 0.05$

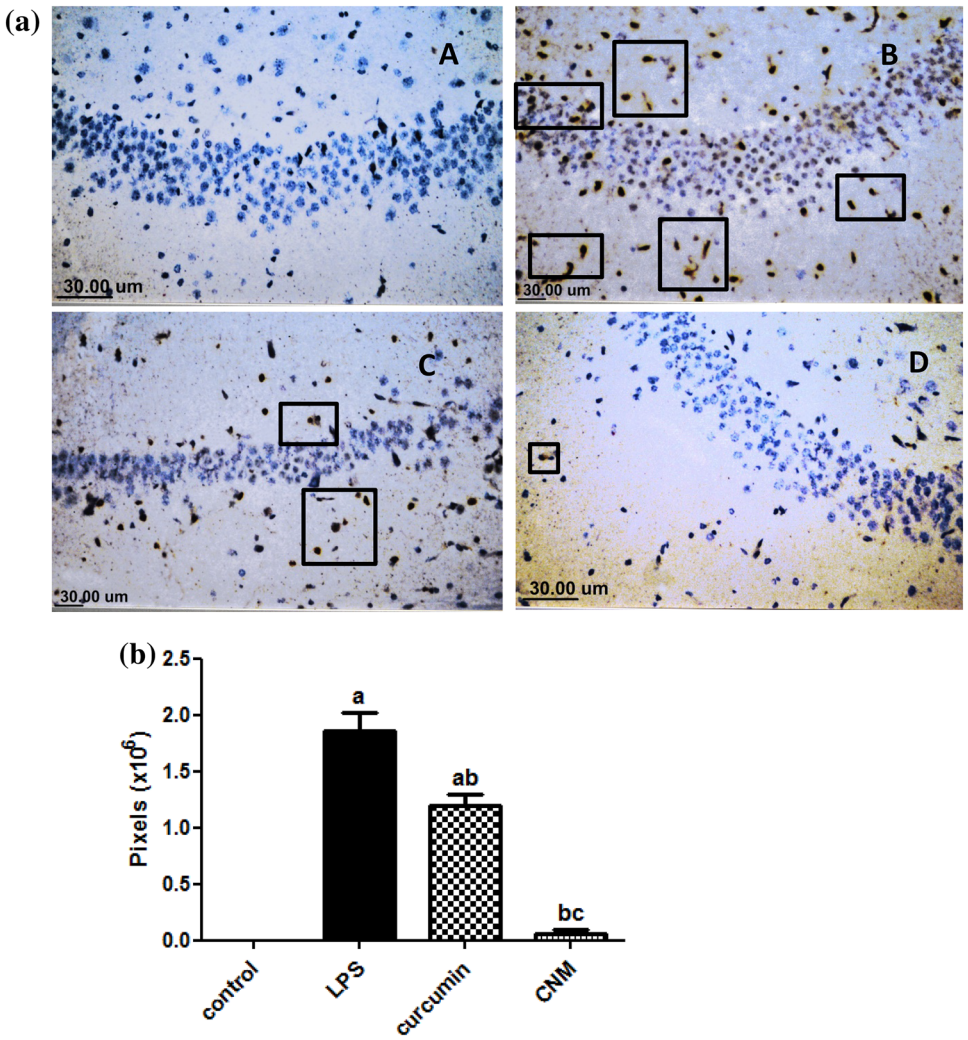

Fig. 6 **a** Immunohistochemistry of $A\beta_{1-42}$ deposition demonstrates that the immunoreactivity was localized in the hippocampi of mice brain. (A) Control group, (B) LPS group, (C) group receiving curcumin, (D) group receiving CNM. Bar = 30 µm. **b** Graph represents the total density of $A\beta_{1-42}$ plaques in different groups. Results are expressed as mean ± SEM of three animals per group. Curcumin and CNM treatments reduced the total plaque density by 35% and 96% respectively compared to LPS group. For statistical significance, [a]significantly different from control group at $p < 0.05$, [b]significantly different from LPS group at $p < 0.05$, [c]significantly different from curcumin group at $p < 0.05$

where no amyloid plaques were detected (Fig. 6a). The mean brain plaque density in hippocampus of LPS-treated mice was $1.85 \pm 0.16 \times 10^6$ pixels (density units) while in curcumin-treated mice was $1.19 \pm 0.09 \times 10^6$ pixels (density units) and in CNM-treated mice was $0.06 \pm 0.02 \times 10^6$ pixels (density units). Curcumin treatment reduced the plaque density by 35% while CNM reduced it by 96% compared to LPS group ($p < 0.05$) (Fig. 6b).

Discussion

In the present study, intraperitoneal administration of LPS provoked neuroinflammation manifested as impairment of both spatial and non-spatial memory, enhancement of Aβ deposition, disruption of oxidative balance and hippocampal apoptosis.

Activation of endogenous antioxidant enzymes is essential to counteract ROS-produced damage [34]. Oxidative stress elicits the transcription of antioxidant enzymes by binding of Nrf2, a redox sensitive transcription factor, to the ARE at the promoter region of various cytoprotective and antioxidant genes such as SOD, GST, GPx and HO-1 [35]. Nrf2 is found sequestered in the cytoplasm bound to a protein called Keap-1 that prevents Nrf2 from translocation to the nucleus and binding to ARE promoters of target genes [36]. LPS-treated mice had increased hippocampal contents of nuclear Nrf2 suggesting a cellular compensatory mechanism to adapt with increased cellular oxidative stress as reflected by increased MDA content. However significant reductions in activity of subsequent downstream antioxidant proteins such as SOD, GST and GPx were evident as they were subject to oxidative damage which corroborates findings of previous studies [37–39].

The current behavioural tests revealed that CNM and curcumin treatments similarly ameliorated spatial and non-spatial memory impairment in both MWM test and ORT respectively which could be attributed to their role in restoration of oxidative balance and anti-inflammatory effects as indicated by MDA content, SOD, GST and IL-1β. This finding strengthen recent finding showing that CNM was able to reverse neuroinflammation and behavioural deficits in diabetic rats [40]. It is worth mentioning that exposing animals to non-interrupted behavioural experiments introduces an additional stress component which may affect behavioural outcomes; however, this error was applicable to all animals including control mice, so they were suffering from the same stress applied to all animals, making this factor negligible in terms of behavioural outcomes of the experiments.

In the present study curcumin-treated mice showed moderate content of aggregated $A\beta_{1-42}$ protein in parallel with reduced hippocampal caspase-3 content. Also, it significantly activated Nrf2 through enhancing its nuclear translocation in hippocampi of mice. These results support previous studies in cellular and animal models [41–44]. CNM reversed LPS-mediated neuroinflammation reflected by reduced IL-1β level in hippocampi of mice; however its anti-inflammatory effect was less pronounced than curcumin. Our results support previous in vivo and in vitro work where CNM showed potent anti-inflammatory effects [12, 17, 23]. In addition, CNM and curcumin similarly promoted nuclear Nrf2 translocation in hippocampi of treated mice. This beneficial effect is further evidenced by significant rise in activity of downstream antioxidant enzymes levels like SOD and GST where CNM-treated mice had significantly better GST activity than curcumin; suggesting that CNM may produce its neuroprotective effects through modulation of Nrf2 pathway. Moreover, our findings showed that CNM guarded mice against neuronal apoptosis induced by LPS and reduced $A\beta_{1-42}$ plaque density significantly better than curcumin. CNM showed few $A\beta_{1-42}$ plaques in the brains of treated mice in parallel with lower caspase-3 content than curcumin in the hippocampus of treated mice. LPS-induced neuroinflammation provoke the formation of Aβ through increasing IL-1β which has an important influence on synaptic plasticity and Aβ formation [45]. It was found that CNM has attenuated this inflammatory mediator in hippocampi of LPS-treated mice.

It was reported that Aβ accumulation induces neuronal loss through stimulation of apoptotic pathways which explain the similarity between the extent of Aβ accumulation and apoptosis marker in treated mice [46, 47]. In addition, Aβ oligomers exacerbate neuroinflammation through stimulation of TLR-4 on microglia thereby increasing NFκB and subsequent IL-1β and TNFα transcription. Microglia is able to phagocytize Aβ which enters the nucleus and causes additional IL-1β transcription [8, 48, 49]. Furthermore, IL-1β was proven to directly activate caspase-3 and initiate apoptosis of neuronal cells [50].

The present findings point out that CNM restored oxidative balance in hippocampi of LPS-treated mice via Nrf2 signalling and reduced $A\beta_{1-42}$ plaque burden; both actions can lead to inhibition of NfκB activation [51, 52] and subsequently its downstream proinflammatory cytokines like IL-1β and mitigate neuroinflammation and memory impairment. Additionally, CNM showed less potent anti-inflammatory effect compared to curcumin, however its anti-apoptotic and anti-amyloidogenic effects were more pronounced than curcumin.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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