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ORIGINAL ARTICLE

Bioavailable Curcumin Alleviates Lipopolysaccharide-Induced Neuroinflammation and Improves Cognition in Experimental Animals

Anu Sunny, Kannan Ramalingam, S. Syam Das[1], Balu Maliakel[1], I. M. Krishnakumar[1], Sibi Ittiyavirah

Department of Pharmacology, School of Pharmacy, Mahatma Gandhi University, Kottayam, [1]R and D Centre, Akay Flavours and Aromatics Pvt. Ltd., Ernakulam, Kerala, India

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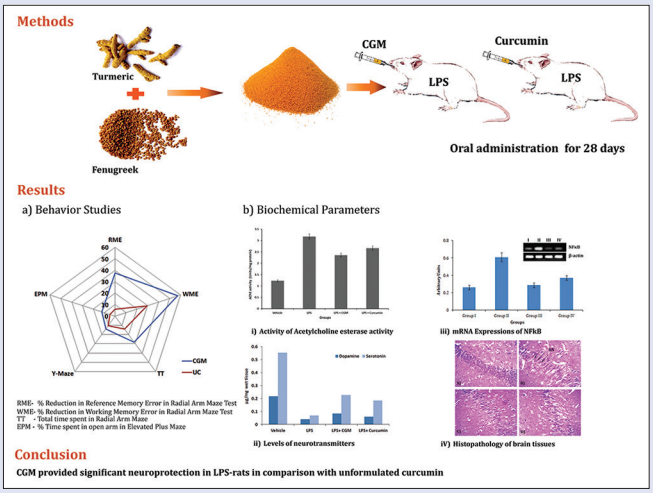
ABSTRACT

Background: Healthy neurons and neurotransmitter levels are necessary for the survival of an organism. Considering the fact that the global incidence of neurological disorders are increasing at an alarming rate; there is a global move toward the development of cost-effective natural neuroprotective agents. **Objective:** In the present contribution, we hypothesized that the formulations of curcumin capable of delivering curcuminoids in the brain would provide enhanced cognitive effects. In this regard, we investigated the relative efficacy of unformulated curcumin (UC) in comparison with "curcumin-galactomannan complex (CGM), an enhanced bioavailable formulation of curcumin that has been reported to possess improved blood-brain-barrier permeability and tissue distribution (Trademarked as "CurQfen®"). **Materials and Methods:** Lipopolysaccharide (LPS)-induced neuro-inflammatory animal model was employed for the study. Wistar rats of 180–200 g body weight (aged 3–4 weeks) were grouped as Group I: Vehicle control, Group II: LPS treated (250 µg/kg b.wt.), Group III: CGM (200 mg/kg b.wt.) + LPS (i.p.250 µg/kg b.wt.), and Group IV: UC (200 mg/kg b.wt.) + LPS (i.p.250 µg/kg b.wt.) and treated for 28 days. **Results:** Behavioral studies (elevated plus maze, radial arm maze, and Y-maze), neurotransmitter levels, and histopathology revealed a statistically significant ($P \leq 0.001$) cognitive improvement and reduced inflammation among CGM treated rats as compared to UC treated groups. **Conclusion:** CGM possesses significant enhanced cognitive effects than UC (IEAC No: DPS/12/2015).

Key words: Cognition, curcumin, curcumin-galactomannan complex, curQfen, lipopolysaccharide, neuroprotective

SUMMARY

- Curcumin-galactomannan complex (CGM) significantly reduced lipopolysaccharide-induced neuroinflammation as compared to unformulated curcumin
- CGM supplementation significantly reduced reference memory errors and improved cognitive functions
- CGM supplementation significantly improved neurotransmitter levels and downregulated nuclear factor-κB expression.



Abbreviations used: UC: Unformulated curcumin; CGM: Curcumin-galactomannan complex; LPS: Lipopolysaccharide; AD: Alzheimer's disease; Ach: Acetylcholine; Aβ: Amyloid-beta; AChE: Acetylcholine esterase; DMC: Demethoxy curcumin; BDMC: Bisdemethoxycurcumin; BBB: Blood-brain-barrier; EPM: Elevated plus maze; RAM: Radial arm maze; RME: Reference memory error; WME: Working memory error.

Correspondence:

Dr. Sibi Ittiyavirah,
Department of Pharmacology, School of Pharmacy,
Mahatma Gandhi University,
Kottayam - 686 631, Kerala, India.
E-mail: sibitho@gmail.com
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INTRODUCTION

Neurodegeneration and neuroinflammation are characteristics of neurological disorders resulting in various degrees of disability and loss of productive lifespan.[1] Dementia is identified as one of the major neurological disorders whose incidence and prevalence are challenging.[2] According to the World Health Organization,[3] 24.3 million people were reported to have dementia in 2006 with almost 4.6 million new cases annually and may grow to reach 130 million by 2050. The highest prevalence of dementia is reported in the United States[1] followed by Africa and the Middle East.

Alzheimer's disease (AD) is identified as the second largest contributor of deaths from neurological disorders.[1] AD is characterized by the deposition of Amyloid beta (Aβ) plaques and tau protein tangles in the nervous tissue leading to neurodegeneration and a decrease in acetylcholine (ACh).[4] Current

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therapeutic treatment modalities for AD include ACh esterase (AChE) inhibitors to enhance ACh levels (e.g. donepezil, rivastigmine) and glutamate inhibitors (e. g., memantine) to suppress excitotoxicity through glutamatergic overstimulation.[5] However, relatively high cost and the side effects associated with these drugs are major limitations for their extensive usage for the purpose of management of dementia.[6]

Development of natural and safe neuroprotective agents has been researched exhaustively for the prevention and control of neurodegenerative disorders. Ginkgolides, derived from *Ginkgo biloba*, were reported to have neuroprotective effects against animal models of AD.[6] Although a large number of nutraceuticals-containing Ginkgolides are aggressively marketing globally, human intervention trials in either the healthy or the AD patients have failed to prove its efficacy in cognitive improvement.[7] *Bacopa monnieri*, a medicinal plant used in Ayurveda, is also studied for its potency in AD.[8] *In vitro* studies[9] showed the inhibitory effect of *Withania somnifera* on Aβ formation.

Curcumin, the yellow pigment of the curry spice turmeric (*Curcuma longa* L) has been extensively studied *(in vitro)* for its neuroprotective effects.[10] Curcumin has been shown to inhibit AchE,[11] inhibit the synthesis of Aβ oligomers,[12] and desegregate amyloid fibrils. However, the clinical significance of the neuroprotective effects of curcumin still remains as a major limitation due to its poor oral bioavailability and lack of brain tissue distribution of the bioactive forms.[13,14] Moreover, recent studies[15,16] have revealed relatively low and very weak antioxidant, anti-inflammatory and antiproliferative effects of curcuminglucuronides, the major metabolites of curcumin.

In the present study, we employed a formulation of curcumin using fenugreek (*Trigonella foenum-graecum*)-derived soluble dietary fiber as curcumin-galactomannan complex (CGM) that has already been shown to have enhanced bioavailability of free (unconjugated) curcuminoids (curcumin, demethoxy curcumin [DMC] and bisdemethoxycurcumin [BDMC]) over curcuminglucuronides[17] CGM was also shown to possess improved blood-brain-barrier (BBB) permeability on oral administration to rats.[18] Hence, we hypothesized that CGM would have enhanced *in vivo* neuroprotective effects and will be able to ameliorate neuroinflammation and dementia symptoms. Lipopolysaccharide (LPS)-induced neuroinflammatory model of rats, a widely used and validated *in vivo* model for the initial screening of neuroprotective and anti-neuroinflammatory effects,[19] has been employed for the study.

MATERIALS AND METHODS

General

Healthy adult male Wistar albino rats (150–200 g b.wt.) were obtained from animal house facility at the Department of Pharmaceutical Sciences, Mahatma Gandhi, University, Kottayam, Kerala. The animals were housed in polypropylene cages in the room where the congenial temperature 27°C ± 1°C, 30%–60% relative humidity and 12-h light and dark cycles were maintained. They were fed with standard pellet diet collected from Hindustan Lever Limited, Bangalore and water given *ad libitum*. All procedures and experiments were conducted in daytime according to the specification of the Indian National Science Academy. The experiments were carried out after obtaining the permission of the Institutional Animal Ethics Committee, Department of Pharmaceutical Sciences [IEAC No: DPS/12/2015].

Characterization of curcumin-galactomannan complex

Modified method of Jadhav *et al*., 2007[20] was used for the high-pressure thin layer chromatography (HPTLC) and HPLC analysis for the identification, confirmation, and quantification of curcuminoids In both unformulated standard curcumin with >95% purity (UC) and CGM. The mobile phase for HPLC consisted of 43:57 (*v*/v) of acetonitrile: water containing 0.2% phosphoric acid and that for HPTLC was chloroform: methanol (48:2) (*v*/v). HPTLC measurements were carried out in the HPTLC system (Camag, Muttenz, Switzerland) consisting of a development chamber with twin trough chamber (10 cm × 20 cm) and visualized using CAMAG TLC Scanner (Visualizer-171217). Densitometric analysis of the data obtained was carried out using winCATS software (Camag, Muttenz, Switzerland). HPLC analysis was carried out in a Shimadzu M20 model fitted with photodiode array detector (Shimadzu Analytical India Pvt. Ltd, Mumbai, India) and reverse-phase C_{18} column (250 mm × 4.6 mm, 3 µm) (Phenomenex, Hyderabad, India) operated under 160 kgf/cm^2. Analytical reference standards of curcumin (CAS# 458-37-7; Batch No: FOH127; purity >98%), DMC (CAS# 22608-11-3; Batch No: FOH153; purity >98%) and BDMC (CAS#33171-05-0; Batch No: FOH152; purity >95%) were obtained from Sigma-Aldrich, Bangalore, India. All solvents were of HPLC grade.

Lipopolysaccharide model

In this model, bacterial LPS was used to induce a neuroinflammatory response as per the method of Lee *et al*.[21] Male Wistar rats were divided into four groups with six animals/group as shown below.

- Group I : Vehicle control
- Group II : LPS treated (250 µg/kg b.wt.)
- Group III : CGM (200 mg/kg b.wt.) + LPS (250 µg/kg b.wt.)
- Group IV : Curcumin (200 mg/kg b.wt.) + LPS (250 µg/kg b.wt.).

Standard curcumin UC and CGM were administered orally through gastric intubation and LPS was administered intraperitoneally, 4 h before the conduct of behavioral tests on the 14th day. Further, animals were anesthetized with Chloral hydrate; brain tissues were removed, and subcortical region (including the striatum) was separated, cleaned with ice-cold saline, blotted dry, and transferred to ice-cold containers for various biochemical analyses.

Behavioral studies

Elevated plus maze (EPM), radial arm maze (RAM), and Y-maze experiments were conducted as the behavioral tests to measure spatial learning and memory errors. EPM tests were conducted as per the standardized protocol.[22] RAM test was based on the protocol of Olton *et al*.[23] and Y-maze was done according to the protocol described by Dellu *et al*.[24]

Estimation of neurotransmitters

Tissue samples for the estimation of neurotransmitters were prepared according to the method of Persky and Reese.[25] AchE was estimated as per the procedure of Ellman *et al*.[26] The concentration of Glutamate was determined by the method of Subaraja and Vanisree.[27] Dopamine was estimated according to the procedure of Jacobowitz *et al*.[28] and Serotonin was estimated by the method of Curzon and Green.[29]

Nuclear factor-κB Expression study

Total RNA was isolated from the brain tissues using TRI reagent (Sigma-Aldrich, Bangalore, India) by the method described by Chomczynski and Sacchi.[30] Total RNA was reverse transcribed, and polymerase chain reaction (PCR) was performed using Eppendorf reverse transcription-PCR (RT-PCR) kit with gene-specific primers. The sequence of the primers is given in Table 1. PCR mixture was resolved on 2% agarose gel-containing ethidium bromide and the gels were

Table 1: Primer sequence used

Genes	Primer sequence	Accession number
β-actin	Forward 5' ACCCGCGAGTACAACCTTCT3' Reverse 5' ATGGCTACGTACATGGCTGG3'	NM_031144.3
NFκB	Forward 5'- GATCCTTTCGGAACTGGGCA -3' Reverse 5'-CCATCTGTTGACAGTGGTATATCT-3'	NM_001276711.1

subjected to densitometric scanning (Bio-Rad Gel Doc, California, USA) to determine the optical density and then normalized against an internal control, β-actin, using Quantity One™ imaging Software (Bio-Rad, California, USA).

Histopathological studies

The histopathological studies were carried out according to the method of Gurr.[31] Brain tissues were kept in 10% formalin for 2 weeks, further dehydrated and embedded in paraffin blocks. Each paraffin block was sectioned into 5-µm thickness and stained with hematoxylin and eosin (H and E), and evaluated under a light microscope and an image analyzer (Leica Application Suit, Leica Microsystems, India). Each tissue section was assessed for histological changes such as neurodegeneration, neuroinflammation and for the presence of reactive astrocytes.

Statistical analysis

The results were analyzed using a statistical program SPSS for Windows, Version 17.0 (IBM, Chicago, USA). A one-way ANOVA was employed for comparison among groups. *Post hoc* multiple comparison tests of significant differences among groups were determined. Pair-fed comparisons between the groups were made by Duncan's multiple range test. Value of $P \leq 0.05$ was considered to be statistically significant.

RESULTS

Study materials

The UC was obtained by the solvent extraction of dried turmeric rhizomes and was found to contain 95.06% of total curcuminoids with a relative distribution of curcumin (77.3%), DMC (14.6%), and BDMC (3.16%). The CGM, on the other hand, had only 39.1% total curcuminoids; but with the same ratio of curcuminoids as observed in UC. Both UC and CGM were dispersed in water by homogenization and supplemented to rats by oral gavage.

Elevated Plus Maze

In the EPM test, a significant reduction in both the number of entries and percentage time spent in open arm ($P \leq 0.001$) was observed with LPS-treated rats (Group II) as compared to that of vehicle control Group I [Figure 1] (from 3.99 ± 0.14 to 0.82 ± 0.03 and from 13.61% to 4.5%, respectively). However, administration of CGM (Group III) resulted in 80% increase in the number of entries (from 0.82 ± 0.03 to 4.10 ± 0.15) and 62.71% increase in the time spent (from 4.5% to 12.07%) which were highly significant ($P \leq 0.001$) when compared to that of Group II (LPS). Although UC-treated Group IV also showed an increase in open arm entries (from 0.82 ± 0.03 to 2.87 ± 0.10) and no significant change in the percentage time spent (4.97% from 4.5%).

The observations on closed arm indicated that the number of entries were significantly ($P \leq 0.001$) increased after LPS injection which has been reduced significantly (from 13.12 ± 0.48 to 7.38 ± 0.27) on CGM treatment in Group III [Figure 1]. The number of entries in Group IV was 10.96 ± 0.40.

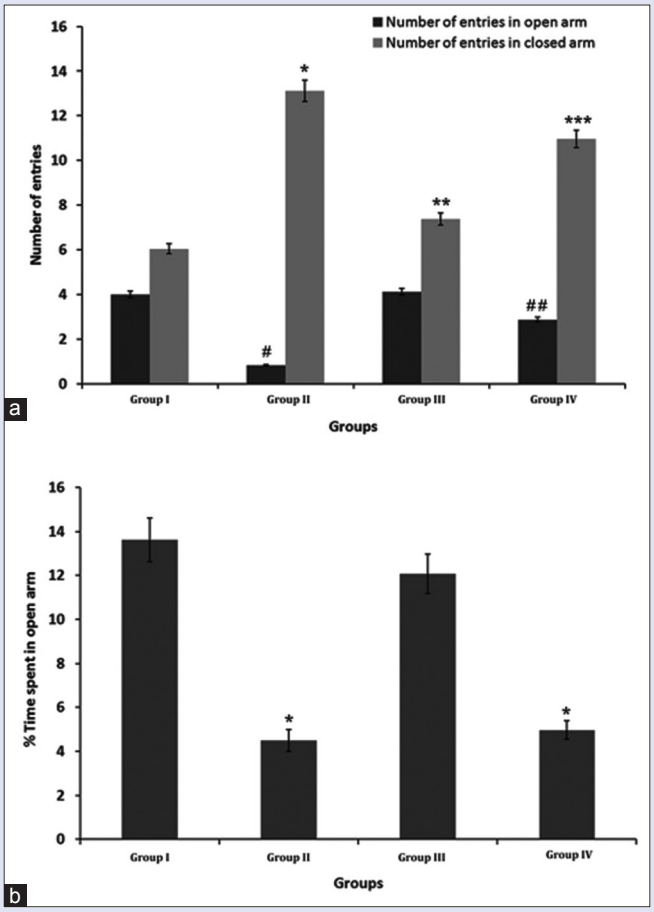

Figure 1: Elevated plus maze. (a) Number of entries in open arm and closed arm. (b) Percentage of time spent in open arm. Group I-Vehicle control, Group II-lipopolysaccharide treated, Group III-curcumin-galactomannan complex + lipopolysaccharide, Group IV–unformulated curcumin + lipopolysaccharide. Values are expressed as mean ± standard error of the mean The values which significantly differ at $P \leq 0.001$ are marked with * or #

Radial arm maze

Significant changes in memory errors were also observed on LPS-treatment and further treatment with both UC and CGM [Figure 2]. On LPS treatment, the values significantly increased ($P \leq 0.001$) from RME 4.51 ± 0.16, working memory error (WME) 2.05 ± 0.08 and time taken 225.50 ± 8.40 in Group I (vehicle control) to 6.25 ± 0.23, 6.97 ± 0.25 and 328.00 ± 12.22, respectively. However, supplementation of CGM significantly ($P \leq 0.001$) reduced both RME and WME [Figure 2a-c]. Although (UC, Group IV) also produced a reduction in RME, WME and in the time taken to complete one cycle in RAM, the percentage difference in reference and WMEs were 33.39% and 39.63%, respectively, as compared to CGM treated Group III.

Y-maze

The results of Y-Maze experiments showed a significant reduction [$P \leq 0.001$; Figure 3] in the percentage of spontaneous alteration behavior among LPS-treated Group II on the 7th and on 14th day of experiment, as compared to that of vehicle control Group I (72.77 in Group I vs 5.12 in Group II; 77.90 in Group I vs. 4.1 in Group II). Supplementation with CGM (Group III) significantly improved behavior (90.57%; from 5.12 in Group II to 54.32 in Group III) in comparison with LPS-Group II. Co-administration with UC in Group IV

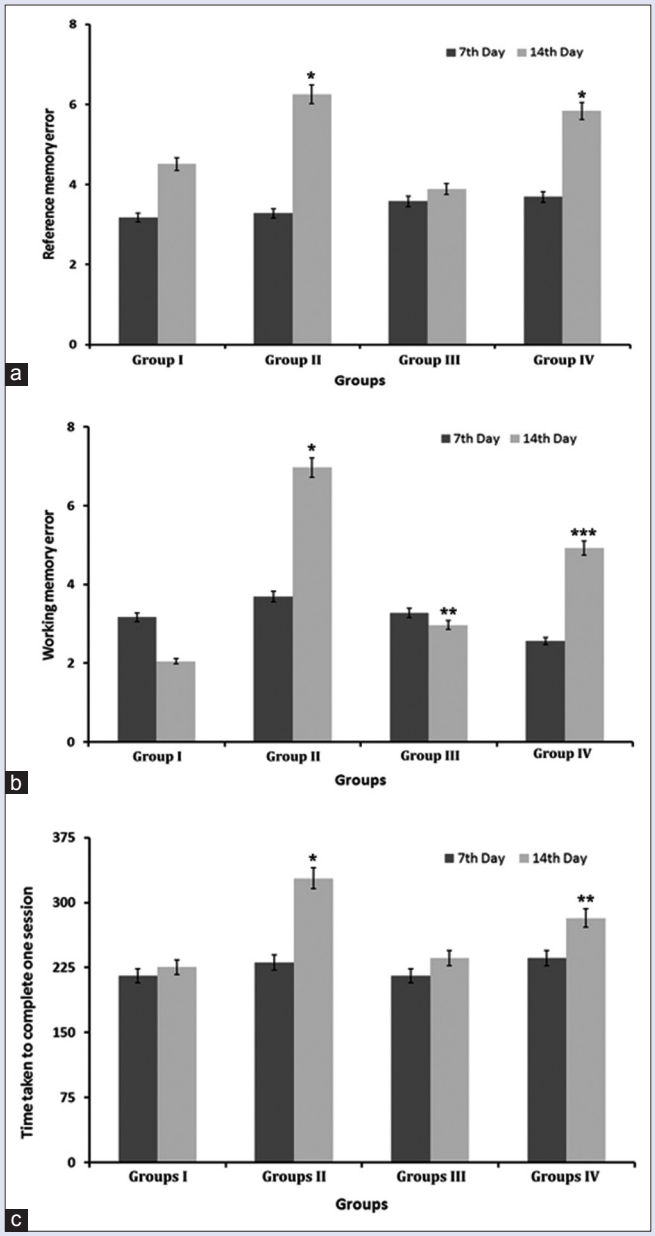

Figure 2: Radial arm maze. (a) Reference memory error, (b) working memory error, (c) time taken to complete one session. Group I-Vehicle control, Group II-lipopolysaccharide treated, Group III- curcumin-galactomannan complex + lipopolysaccharide, Group IV–unformulated curcumin + lipopolysaccharide. Values are expressed as mean ± standard error of the mean the values which significantly differ at $P \leq 0.001$ are marked with *

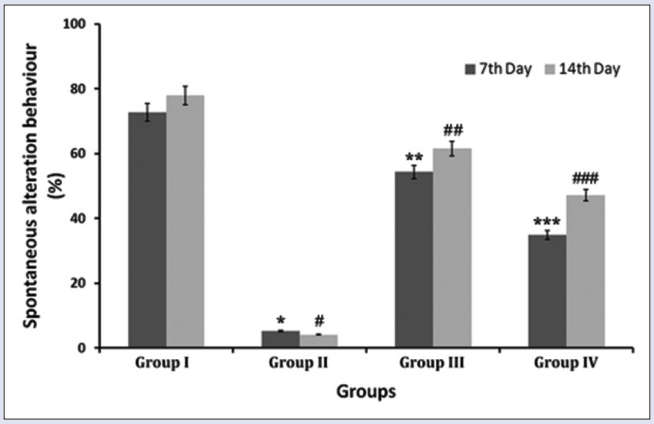

Figure 3: Y-maze test. Group I-Vehicle control, Group II-lipopolysaccharide treated, Group III-curcumin-galactomannan complex + lipopolysaccharide, Group IV–unformulated curcumin + lipopolysaccharide. Values are expressed as mean ± standard error of the mean The values which significantly differ at $P \leq 0.001$ are marked with * or #

also showed statistically significant ($P \leq 0.001$; 75.3%) improvement in behavior when compared to Group II (LPS). On the 14th day, Y-maze experiment results again demonstrated a significant improvement (93. 30%) in Group III (CGM-treated), as compared to Group II– LPS treatment ($P \leq 0.001$; Group II-4.1 ± 0.15; Group III-61.50 ± 2.29). UC supplementation (Group IV) also showed a reduction, but 23.3% less by that of Group III (CGM treatment).

Neurotransmitter levels

The activities of AchE and levels of glutamate [Figure 4a and b] were significantly increased in LPS treated group (from 3.63 ± 0.14 and 1.23 ± 0.05 to 6.47 ± 0.24 and 3.18 ± 0.12; 43.89% and 61.19%, respectively), which was significantly ($P \leq 0.001$) reduced on CGM administration in Group III (from 6.46 ± 0.24 and 3.17 ± 0.11 to 5.48 ± 0.20 and 2.35 ± 0.09). Group IV also showed a significant reduction (5.71 ± 0.21 and 2.67 ± 0.10, respectively) but was less when compared to CGM-treated Group III. The levels of dopamine and serotonin [Figure 4c and d] showed a significant reduction in LPS treated Group II in comparison with vehicle control, Group I (from 0.22 ± 0.01 and 0.56 ± 0.02 to 0.04 ± 0.00 and 0.07 ± 0.00, respectively). On CGM treatment (Group III), these levels were found to be significantly increased ($P < 0.001$). Although UC treatment (Group IV) also increased dopamine and serotonin levels significantly ($P < 0.01$) as compared to LPS-treated animals, the relative enhancement was only 10% in Group IV as compared to 50% hike in CGM treated Group III.

Nuclear factor-κB Expression

Being central to the inflammatory cascades, nuclear factor-κB (NFκB) expressions in the brain tissues of rats belonging to each group were investigated to have a general idea on the inflammatory levels. LPS-treatment (Group II) was found to cause a significant inflammation ($P \leq 0.001$) as clear from the upregulation of NFκB as compared to control Group I. Further treatment with both UC and CGM was found to downregulate the expressions significantly [Figure 5]. However, down-regulation by CGM was more prominent and it does not show significant difference with the normal control, Group I ($P > 0.05$).

Histopathology

Histopathology analysis results are shown in Figure 6. Normal control, Group I, showed normal brain structure with no signs of inflammation or tissue damage. In LPS-treated Group II, the presence of plenty of large pleomorphic astrocytes with signs of necrosis, edema, and brain tissue damage were observed. In the CGM treated animals (Group III), normal astrocytes and the tissues with minimal edema and necrosis were evident. Although Group IV (UC-treated) also showed reduced levels of edema, enlarged astrocytes were visible.

DISCUSSION

Neuroinflammation is a characteristic feature of neurodegenerative disorders. In this study, we used the LPS-induced neuroinflammation

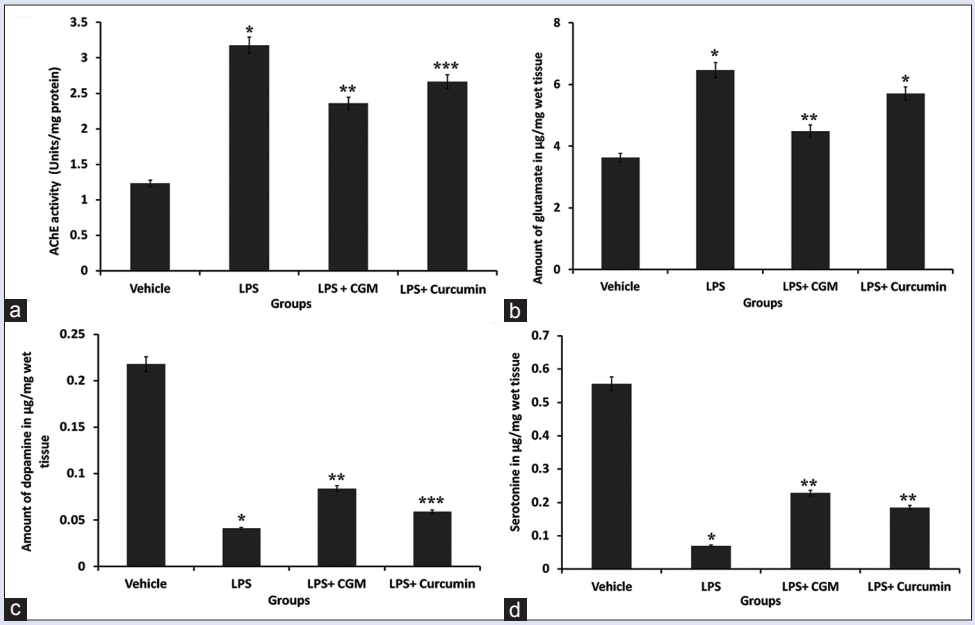

Figure 4: Neurotransmitters and activity of acetylcholine esterase. (a) Activities of acetylcholine esterase (b) amount of glutamate (c) amount of dopamine (d) amount of serotonin, Group I-Vehicle control, Group II- lipopolysaccharide treated, Group III-curcumin-galactomannan complex + lipopolysaccharide, Group IV–unformulated curcumin + lipopolysaccharide. Values are expressed as mean ± standard error of the mean the values which significantly differ at $P \leq 0.001$ are marked with *

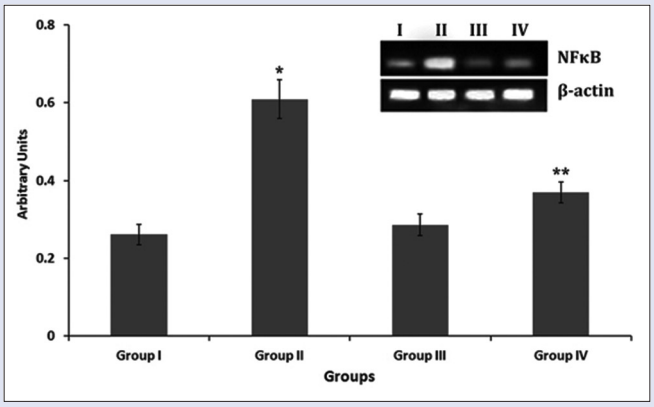

Figure 5: Expression of nuclear factor-κB. Expressions of nuclear factor-κB was analyzed in the cytoplasmic fraction of brain by agarose gel electrophoresis, and the intensities of the bands were normalized with that of the intensities of β-actin bands expressed in the samples. Intensities of the bands were quantified using Bio-rad gel doc and plotted. The results presented are average of quadruplicate experiments, ± standard error of the mean statistically significant at $P \leq 0.05$. The values which significantly differ at $P \leq 0.05$ are marked with *

model of rats since the molecular mechanisms of LPS-induced neurotoxicity are very much similar to that of neurodegenerative disorders, including AD.[19] To better understand the role of curcumin and the effect of its bioavailability and brain tissue distribution, the present study employed UC and CGM – a formulation that has already been shown to possess improved BBB-permeability and enhanced bioavailability.[18] The behavioral changes, modulation of neurotransmitters, expression of NFκB and histopathology of the brain tissues were analyzed to evaluate the relative efficacy of CGM in alleviating LPS-induced neurotoxicity.

Behavioral studies are of great importance in the systematic analysis of cognitive changes associated with neuroinflammation and neurodegeneration. In the present contribution, EPM, RAM, and Y-Maze were employed to analyze various aspects of cognitive functions. EPM is one of the most frequently used behavioral neuropsychopharmacology tools in animal models for screening drugs with potential anxiolytic effects.[32] Here, the decrease or increase in the number of entries and the time spent in open-arms were regarded as indicators of its anxiogenic or anxiolytic effects, respectively.[33] LPS-injected groups in the present study showed a significant decrease in both the number of entries and time spent at open arms. However, the number of entries and time spent in closed arm were significantly increased as compared to the normal control group of animals indicating enhanced anxiety and decreased cognition on LPS administration [Figure 2]. On treatment with curcumin and CGM, there was a significant reduction in anxiety as compared to LPS-treated groups. Further comparison between curcumin and CGM treated groups revealed a significant improvement in CGM groups and its behavior was almost similar to untreated normal control group of animals. Since LPS was established and validated as a model for anxiety-like behavior in animals,[33] the present study suggests the enhanced anxiolytic effect of CGM as compared to curcumin.

RAM is a validated test for neuronal damage and memory impairments in animals.[34] LPS control animals were already shown to exhibit a significant increase in both reference memory error and WME as compared to normal control group of animals indicating a significant loss in memory. The time taken to complete one session in RAM was also found to be high in LPS-rats as compared to a control group of animals, which was in agreement with early reports.[34] However, the reference and memory errors were found to be significantly decreased on treatment with both UC and on CGM treatments with a significant reduction in the time taken to complete one session in RAM.

Y-maze is yet another validated test method widely employed for investigating the learning and memory functions associated with various drugs.[24] In the present study, curcumin and CGM administration was found to increase the learning efficiency and memory of rats significantly

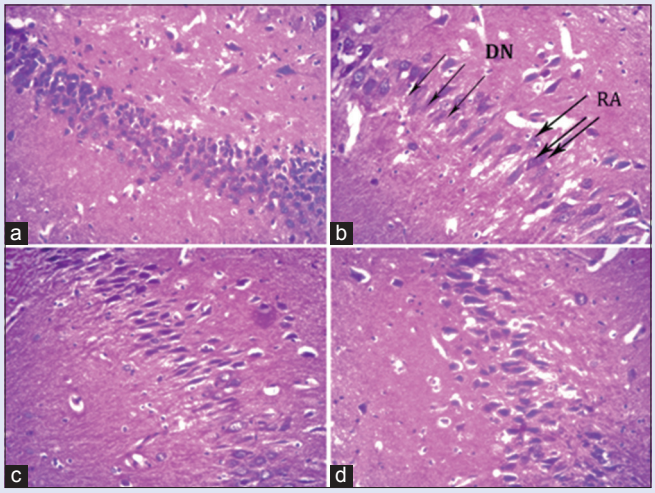

Figure 6: Histopathology of the brain. Microphotographs of histopathology using hematoxylin and eosin stain. (a) Vehicle control, (b) Lipopolysaccharide treated, (c) Curcumin-galactomannan complex + lipopolysaccharide, (d) unformulated curcumin + lipopolysaccharide

when compared to the LPS-rats. The improvement observed for CGM group was almost similar to the normal control group of rats indicating its effectiveness to protect LPS-induced neuronal damage. Thus, it was observed from EPM, RAM and Y-maze tests that both UC and CGM has neuroprotective effects against LPS in rats and the efficacy of CGM was significantly high as compared to UC.

It is known that the intricate and concerted activity of neurotransmitters, their receptors and degrading enzymes form the basis for neuronal communication system; the basis of a healthy cognitive function.[35] ACh, an important chemical messenger responsible for memory, has been shown to have its levels regulated by the hydrolytic enzyme acetylcholinesterase (AChE) in both the periphery and brain tissues.[36] Hence, AChE activity in the brain has been served as a reliable marker of cholinergic activity and progression of AD.[36] When treated with LPS, AChE activity in the rat brain was significantly increased, indicating a decrease in cholinergic activity. However, a significant improvement was observed with the supplementation of both curcumin (14%) and CGM (25%) treatment. Serotonin is a monoamine and inhibitory neurotransmitter to regulate appetite, sleep, memory and learning, temperature, mood, behavior, muscle contraction, heart, and hormone levels.[37] It has been shown that the stimulation of serotonergic neurotransmission disrupts behavioral performance, while inhibition enhances behavioral performances.[35] When treated with curcumin and CGM, brain levels of serotonin was found to be significantly decreased with a relatively higher effect for CGM, indicating significant inhibition ($P < 0.001$) in LPS-induced stimulation of serotonergic neurotransmission by CGM.

LPS-mediated inflammation enhances the production of nitric oxide from glial cells and death of mesencephalic dopaminergic neurons.[38] It has also been reported[38] that LPS can reduce dopamine and its metabolites in the striatum. However, treatment with curcumin and CGM could enhance dopamine levels in the brain, more significantly with CGM as compared to the UC group. Glutamate, a powerful excitatory neurotransmitter, is responsible for signaling between the nerve cells and has an important role in learning and memory.[35] Abnormally high glutamate levels might cause overexcitation of the receiving nerve cell, eventually culminating in cell death or nerve cells damage.[39] The present study showed a significant increase in brain glutamate levels among LPS treated animals and was found to be reduced significantly on CGM and curcumin administration, indicating their neuroprotective effects.

Even though inflammation is regulated by numerous molecules and factors, NFκB is the central regulator of inflammation which further elicits the various inflammatory pathways and cytokines.[40] Hence, we analyzed NFκB expressions in each group of animal brain tissues to learn the inflammation. The results of the NFκB expression study revealed significant upregulation by LPS which was further down regulated in CGM treated group. Although UC treatment was also downregulating NFκB, it was not as statistically significant as CGM. Further histopathology analysis of brain also revealed significant inflammation on LPS-treatment, as evident from the presence of large pleomorphic astrocyte, necrosis, edema, and the brain tissue damage as compared to the normal rat brain. On treatment with CGM, a significant reduction in inflammation was evident from the presence of normal astrocytes and the tissues with minimal edema and necrosis. However, UC treatment showed enlarged astrocytes though there was a reduction in edema.

Earlier studies with liquid chromatography-coupled triple, quadruple tandem mass spectrometry have established the improved BBB-permeability and tissue distribution of free (unconjugated) curcuminoids (curcumin, DMC, and BDMC) following the oral administration of CGM.[18] Although a number of enhanced bioavailable formulations, have been reported by measuring the plasma levels of total curcumin metabolites, recent studies have demonstrated the significance of free curcuminoids bioavailability over curcumin metabolites.[11,14-16] The significance of free curcuminoids in brain health is due to the BBB-permeability, antiamyloidic effect, better antiinflammatory and antioxidant effects over curcuminglucuronides.[11,15,17] Moreover, BDMC has been shown to possess better neuroprotective effects as compared to DMC and curcumin.[41] However, formulations of natural curcuminoids with oral bioavailability for BDMC and DMC are limited, especially due to the relatively low abundance of BDMC (<3% w/w) and DMC (<16% w/w) in commercially available natural curcuminoids with 95% purity. Thus, the enhanced brain health functions of CGM as demonstrated by the behavioral studies, neurotransmitter levels, NFkB expression, and histopathology can be attributed to the better brain bioavailability and pharmacokinetics of free curcuminoids as compared to UC.

CONCLUSION

Bacterial LPS-induced neuroinflammation and memory impairment in rats were considered as a validated model for the *in vivo* evaluation of the neuroprotective efficacy of any treatment regime. In the present study, CGM, a non-nano natural formulation of curcumin with enhanced bioavailability and improved BBB-permeability, has been investigated on LPS-induced neurotoxic rats. It was observed that CGM produced a significant effect as compared to UC in ameliorating the neuroinflammation and further changes associated with cognitive functions and neurotransmitter levels, indicating its potential in neurodegenerative disorders.

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Nil.

Conflicts of interest

The authors disclose the following conflict of interest."CurQfen™" is the registered trademark of M/s Akay Flavours and Aromatics Pvt. Ltd for CGM. SI, KR, and AS belongs to the university who have no conflict of interest.

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