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RESEARCH

Bornyl Acetate and Menthol Provide Neuroprotection Against Lipopolysaccharide-Induced Alzheimer's Disease-Like Condition in C57BL/6 Mice by Downregulating NARC-1 Lipid Antagonist

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Abstract

Alzheimer's disease (AD) is a progressive neurological illness that causes Aβ deposition and cognitive impairments. Anticholinesterase and anti-depressant drugs are used as medications; however, their side effects spotlight the need for alternate treatments. Bornyl acetate and menthol are monoterpenes with bioactive potential investigated against inflammation induced by lipopolysaccharide (LPS) in C57BL/6 mice. In our study, we analysed various behavioural changes along with memory activities as well as assessed neuronal damage, acetylcholinesterase activity, amyloid deposition, mitochondrial membrane integrity, calcium deposition and oxidation derivatives. In addition, we also examined gene and protein expression associated with lipid dysfunction in neuroinflammation. Our findings revealed that monoterpenes such as bornyl acetate and menthol potentially improved LPS-induced behaviour changes and cognitive activities. In addition, these compounds have the potential effects against amyloid plaque formation, calcium build-up, mitochondrial membrane damage and oxidative markers (malondialdehyde, protein carbonyls and advanced glycation end products) in the LPS-injected C57BL/6 mice. Treatment with bornyl acetate and menthol also inhibited neural apoptosis-regulated convertase (NARC-1)/proprotein convertase subtilisin/kexin type 9 (PCSK-9) by upregulating low-density lipoprotein receptor-related protein (LRP)-1 protein expression. Cholesterol oxidation genes, including 11β-hydroxysteroid dehydrogenase 1 & 2, as well as proinflammatory microglial, apoptotic and amyloidogenic protein and gene expression, were decreased respectively when treated with monoterpenes while promoting the upregulation of anti-inflammatory. Based on the results, we concluded that these compounds can potentially target and prevent neuroinflammation, including Alzheimer's disease.

Keywords Lipopolysaccharide · Lipid dysfunction · Cognitive impairment · Amyloid formation · Alzheimer's disease · Monoterpenes

Introduction

Alzheimer's disease (AD) is a widely discussed neurodegenerative disorder that results from the loss of neurons, which was caused by the chronic inflammatory process in the nervous system [1, 2]. Furthermore, recent data analysis, encompassing previous studies conducted across Europe, Asia and USA, has reported a consistency of over 57 million individuals suffering from dementia, with an alarming 35% of deaths attributed to Alzheimer's disease [3]. The prevalence of neurological disorders in Asian countries is consistently lower compared to Western countries. However, recent data indicate a simultaneous increase in incidence, which may continue at a faster rate in the future [4]. In India,

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the prevalence rate was reported to be 14.2%, with Southern Indian provinces accounting for 1.91% [5, 6].

Alzheimer's disease patients often exhibit an accumulation of amyloid beta (Aβ) peptides and neurofibrillary tangles, which tend to clump together, that contribute to the development of plaques in the brain. These processes are closely linked to the progression of disease as well as neurodegeneration [7]. The development of AD-related conditions is triggered by the harmful pathway of amyloidogenic processing, where beta-secretase activity, controlled by β site APP cleaving enzyme 1 (BACE1), causes the production of N and C terminal fragments Aβ 1–42 [8], which are highly prone to plaque formation and neurotoxic, causing brain damage [9]. The brains of Alzheimer's patients and transgenic animals showed significantly greater levels of Aβ 1–42 [10–12]. Moreover, AD patients exhibit a specific pattern of degeneration in cholinergic neurons of the brain. This results in a significant decrease in levels of choline acetyltransferase (ChAT) and acetylcholine (ACh), as observed by Dastmalchi et al. [13]. Hence, clinically AD is characterized by progressive dementia that can initially be asymptomatic and eventually results in cognitive decline, including symptoms such as memory loss, disorganized thinking, changes in personality and altered emotions and behaviour [14, 15]. Regarding the symptoms of AD, researchers have found that altering the function of nicotinic ACh receptors (nAChRs) and using acetylcholinesterase inhibitors (AChEIs) can be effective in mitigating the disease. Several cholinesterase inhibitors such as tacrine, galantamine, donepezil and rivastigmine as well as the glutamatergic modulator memantine have been developed as potential treatments for AD. In addition, certain antidepressants have also been explored as potential therapies. According to Weller & Budson [16], these medications are commonly prescribed to individuals with moderate to severe mental health issues to enhance their overall well-being and possibly prolong their lifespan. However, it is important to note that certain potent anticholinergic and antipsychotic drugs may also bring unwanted side effects such as increased heart rate, impaired vision and adverse effects on cognitive function and perception [17, 18]. As a result, continuous use of these medications may worsen the individual's condition and even lead to a fatal outcome [19].

To investigate the AD condition, several research models have been established. It was found that pathogens are known to be present in the brains of individuals with Alzheimer's disease along with specific components such as lipopolysaccharide (LPS), capsular proteins, fimbrillins and flagellin. These microorganisms can easily bypass the blood–brain barrier, ultimately contributing to the buildup of toxic amyloid beta fibrils. As a result, this leads to a gradual increase in inflammation and the death of crucial neuronal cells [20, 21]. In this study, LPS was used in animal models to induce neuroinflammatory conditions similar to those seen in AD. The presence of lipopolysaccharide (LPS) in the brain has been shown to have a profound impact on the hippocampal region. This is due to the activation of the acetylcholinesterase enzyme, which leads to decreased levels of acetylcholine and is linked to aggressive behaviour often seen in individuals with dementia [22]. Additionally, LPS has been linked to the loss of dopaminergic neurons and memory deficits, although the exact relationship is not yet fully understood [23]. Further studies have revealed that LPS can induce a pro-inflammatory state in microglia, which is associated with cognitive impairments such as memory loss and anxiety, commonly observed in individuals with Alzheimer's disease (AD) [24].

On the limitations of the treatment, two monoterpene derivatives have been employed against pathology in this study. Bornyl acetate is a bicyclic monoterpene derived from the precursor borneol extracted from various plants. Bornyl acetate has been reported for numerous biological mechanisms within cells and tissues. It was structurally similar to borneol that was established clinically, yet its pharmacological potential in various disease mechanisms is to be investigated in depth [25]. It is remarkable for their ability to reduce neutrophil and macrophage infiltration, ultimately decreasing the inflammatory response caused by LPS. Bornyl acetate exhibits inhibitory actions on pro-inflammatory cytokines, such as TNF-α, IL-1β and IL-6, as well as the activation of extracellular regulated protein kinases, c-Jun N-terminal kinase, and p38 mitogen-activated protein kinase, demonstrating promising anti-inflammatory capabilities [26]. Notably, in vitro studies have further supported these findings, with bornyl acetate extracted from *Cinnamomum osmophloem* inhibiting the production of prostaglandins and nitric oxide in lipopolysaccharide stimulation in 264.7 macrophages [27]. Recent studies have reported the potential of bornyl acetate as a powerful neuroprotective agent with the ability to combat oxidative stress within PC12 cells, showing its anti-cholinesterase properties [28, 29]. In mice with encephalomyelitis, bornyl acetate has established its capability to safeguard the brain's endothelial cells, maintain blood–brain barrier integrity and prevent demyelination of the spinal cord by suppressing key neuroinflammatory cytokines such as IL-1β, IL-6, COX-2, MCP-1 and MIP-1. These promising findings were also reflected in improved behavioural function and motor neuron activity in mice treated with bornyl acetate [30]. Additionally, inhalation of bornyl acetate has been found to have a relaxing effect on the autonomic nervous system, as reported in a clinical trial involving human volunteers [31].

Menthol is another cyclic terpene compound generally synthesized from essential oils of mint plants, citronella and eucalyptus. The pharmacological application of menthol involves its role as an antipruritic, antiseptic and cooling sensation compound. Moreover, menthol works as local anaesthetic agent by blocking calcium channels and activating the kappa-opioid receptor [32]. It also acts as an anti-cholinesterase and inhibitor of nicotine and gamma-aminobutyric acid (GABA)

receptors. This compound has a stimulating effect and reducing oxidative stress, effective in reducing lipid peroxidation and protecting dopaminergic neurons through its efficacy of inhibiting signalling pathways like AKT, JNK1/2, ERK1/2 and p65 phosphorylation [33, 34]. Menthol is also able to suppress the production of proinflammatory mediators, such as nitric oxide and prostaglandins in monocytes [35]. In vitro experiments revealed its ability to protect against damage caused by both cisplatin and amyloid, through the regulation of BCL-xl and JNK pathways in neuroblastoma cells [36]. Furthermore, topical application of menthol in rat models has proven effective in reducing neuropathic pain induced by spinal cord injury and ischemic stroke [37, 38]. Menthol has also been found to maintain a balance between pro-inflammatory and anti-inflammatory cytokines and possesses antioxidant properties that neutralize free radicals and prevent activation of various signalling pathways [39]. In addition to the limited study of bornyl acetate and menthol, a detailed investigation was performed on the pathological pathway of lipopolysaccharide-injected Alzheimer's disease progression.

Materials and Methods

Maintenance and Experimentation of Animals

Active C57BL/6 mice of age 13 to 15 weeks, weighed about 25 to 30 g purchased from Mass Biotech Pvt. Ltd, (CPCSEA approved animal suppliers), Padi, Chennai. Mice were reared in polypropylene cages allowing them to habituate the laboratory set up before the experiment and fed *ad libitum* (Hindustan Lever Limited). To ensure a regular day-night cycle, we provided artificial lighting for 12 h each day. Additionally, all experimentation strictly adhered to ethical norms as consented by the Government of India and our Institutional Animal Ethics Committee (Approval number: BRULAC/SDCH/SIMATS/IAEC/04–2022/094).

Preliminary assays

Preliminary assays were conducted in this study and the data were presented separately in the supplementary section. Further tests were conducted using the design described below, with six animals per group for the assays.

Experimental design

Group I	Control 0.9 % saline
Group II	Mice administered with LPS (5 mg/kg b.w.) intra-peritoneally
Group III	Mice co-administered with LPS (5 mg/kg b.w.) and bornyl acetate (100 mg/kg b.w.) intra-peritoneally
Group IV	Mice co-administered with LPS (5 mg/kg b.w.) and menthol (100 mg/kg b.w.) intra-peritoneally
Group V	Mice co-administered with LPS (5 mg/kg b.w.) and donepezil (5 mg/kg b.w.) intra-peritoneally

Detection of Amyloid Accumulation in Brain Tissue

To detect the presence of amyloid fibrils in the brain tissue, we utilized the method of Cheung et al. [47] and Zhang et al. [48]. Brain tissue sections were prepared and then treated with 0.7 % congo red solution for 5 to 10 min. After being washed with distilled water, the sections were carefully examined under a bright field microscope (Carl Zeiss Axiovision, Germany). In the same manner, 100 µl of congo red stain was applied to brain sections, which were then incubated for 10 min. Afterwards, the sections were observed under a microscope (Carl Zeiss Axiovision, Germany).

Detection of Mitochondria in Brain Tissue Using Janus Green B

The integrity of the mitochondria in formalin-fixed brain tissue sections was assessed using the method of Adamstone & Taylor [49]. The brain sections were processed, deparaffinized and then rehydrated with absolute alcohol. The sections were stained with 100 µl of 2 % janus green B for 20 min. After staining, the sections were washed with autoclaved double-distilled water to remove excess dye and then the sections were examined under a fluorescent microscope (Carl Zeiss Apotome 2.0, Germany).

Detection of Calcium Accumulation in Brain Tissue by Alizarin Red S

To determine the presence of calcium in brain tissue, we followed the method of Roos & Colleagues [50]. The formalin-fixed tissue sections were processed and deparaffinized followed by re-hydration. Then, 2 % solution of alizarin red S was overlaid on the slides and allowed to incubate for 5 min. The excess stain was then carefully removed with autoclaved double-distilled water. Finally, the calcium accumulation in the brain tissue sections was observed under a microscope (Carl Zeiss Axiovision, Germany).

Analysis of DNA Fragmentation

In this experiment, DNA fragmentation was detected through agarose gel electrophoresis, following the method of Kasibhatla et al. [51] with slight modifications. After the experimental period, the brain tissue from each group was lysed with buffer consisting of tris–HCl (pH 8.0), 100 mM NaCl, SDS, EDTA and 0.1 mg/ml proteinase K. The lysed brain tissues were incubated at 50 °C for 30 min and subsequently treated with a mixture of phenol: chloroform: isoamyl alcohol (in a 25:24:1 ratio) and centrifuged at 10,000 rpm for 10 min at 4 °C. The aqueous layer was collected and mixed with 50 µl of ice-cold ethanol and 50 µl of 7.5 M ammonium acetate centrifuged at 10,000 rpm for 10 min at 4 °C. After retrieving the pellet, it was combined with 1 ml of 70 % ethanol and spun in a centrifuge to collect the DNA. The DNA pellets were then carefully transferred into 20 µl of TE buffer and separated through 1 % agarose gel electrophoresis, the results were observed under the gel documentation system (Vilber, Germany).

Estimation of Oxidation by-Products

(i) Lipid Peroxidation

The levels of malondialdehyde (MDA), a by-product of lipid peroxidation were measured [52]. After the treatment period, brain tissues were disrupted and the resulting supernatant was collected by centrifugation at 5000 rpm for 10 min at 4 °C. Thereafter, 0.5 ml of the supernatants from each group was independently added with 2 ml of thiobarbituric acid and 1 ml of trichloroacetic acid. This mixture was then heated at 80 °C and further centrifuged at 5000 rpm for 10 min at 37 °C. The resulting supernatant was read at a wavelength of 532 nm using a UV spectrophotometer. The levels of MDA were calculated in terms of nanomoles per milligram of protein.

(ii) Carbonylation of Proteins

Protein carbonylation levels were evaluated by following the method of Youn et al. [53] with slight modifications. Brain tissues from various experimental groups were treated with RIPA buffer and then centrifuged at 5000 rpm for 10 min at 4 °C. The total protein concentration in each group was then determined using the Lowry et al. (1951) method. Following this, 1 mg/ml of protein from each group was incubated with 1 ml of 2,4-dinitrophenylhydrazine (DNPH) for 45 min. After this incubation, the bound carbonylated protein was extracted by precipitating it using 10 % TCA. The protein precipitates were washed with a 1:1 ratio ethanol and ethyl acetate mixture. Then the absorbance of the sample was read at 375 nm using a UV spectrophotometer, resulting in the calculated carbonyl protein content expressed in µM/mg protein.

(iii) Advanced Glycation of Proteins

The presence of advanced glycation end products (AGEs) in protein was assessed by following method of Varsha et al. [54]. The samples of brain tissues obtained from every experimental group were treated with 0.1 N NaOH and centrifuged at 10,000 rpm at 4 °C for 10 min. The resulting supernatant was analysed using a spectrofluorometer (Perkin Elmer, Germany) with an excitation of 370 nm and an emission of 440 nm. As a standard, 1 mg/ml of bovine serum albumin (BSA) dissolved in 0.1 N NaOH was used to calculate unknown values, and the data was presented in arbitrary units.

Gene Expression Analysis

The method of Zhang et al. [55] was utilized for the isolation of total RNA and subsequent gene expression analysis. After the experimental period, brain tissues were collected from the subjects and lysed using ice-cold trizol reagent at a ratio of 100 µl per 10 mg pellet, then mixed with an equal volume of ice-cold chloroform, vigorously vortexed and incubated at 37 °C for 10 min, centrifuged at 10,000 rpm for 20 min at 4 °C. The aqueous layer from each sample were added a 1:2.5 ratio of ice-cold isopropanol and centrifuged at 10,000 rpm for 20 min at 4 °C to obtain RNA pellet and then washed thrice with 1 ml of ice-cold 75 % ethanol, centrifuged at 10,000 rpm for 20 min at 4 °C. Finally, the pellet was resuspended in 20 µl of nuclease-free water, the quantity RNA was accurately measured utilizing a Nano Drop spectrophotometer (Thermo Fisher Scientific, USA) at 260/280 nm wavelength ratio. To continue the process, the RNA was transformed into cDNA using reverse transcription with the help of first-strand cDNA synthesis kit (Thermo Scientific). The specific primers along with 100 ng of cDNA were then amplified through PCR. The amplified genes were subsequently separated via agarose electrophoresis and visualized using a gel documentation system (Vilber, Germany). To determine the intensity of the amplified genes, ImageJ software was utilized and the results were expressed in arbitrary units.

Protein Analysis

In order to assess protein expression in brain tissue samples, the western blot analysis was performed [56]. After the experimental period, the brain tissues were sonicated

with RIPA buffer for 5 min, centrifuged at 10,000 rpm for 10 min at 4 °C. The protein samples were collected and their concentrations in each sample were estimated by the method of Lowry et al., 1951. One hundred micrograms of extracted protein samples were boiled with sample buffer and loaded into 12% SDS-PAGE wells containing tris–glycine-SDS. The gel was run at 50 V current for the stacking gel and 100 V for the resolving gel to separate the proteins. The resulting proteins were then transferred to a nitrocellulose membrane via a wet sandwich western blot set-up, using a constant current of 150 V maintained at 4 °C for 2 h. The membrane after blotting was blocked with 5% BSA-TBS buffer containing 0.05% tween 20, washed with 1 X TBS three times and incubated overnight at 4 °C with constant shaking. Primary antibody targeting (rabbit anti-amyloid 1–42, BACE1, p65/NF-κB, Caspase-3, LRP-1 and NARC-1/PCSK9) were incubated with membrane and washed three times with 1X TBS. After which goat polyclonal anti-rabbit horse radish peroxidase (HRP) conjugated secondary antibody was incubated for an hour, followed by thorough rinsing with 1X TBS. The membrane was developed using a chemiluminescent HRP substrate (Cyanogen) and the bands were captured using a chemic doc imaging system (SYNGENE, USA). The relative intensity of the protein band was analysed using Image J software and expressed in arbitrary units.

In addition, an enzyme-linked immunosorbent assay was performed to quantify the levels of Aβ 1–42, BACE1, p65/NF-κB, Caspase-3, LRP-1 and NARC-1/PCSK9 in mice brains. Brain tissues were dissected and sonicated in RIPA buffer [55]. The supernatants were collected. One hundred micrograms of the protein sample has been added to the capture antibody-coated wells that bind to the antigen contained in the sample. The sample coated wells were incubated at 4 °C overnight and then blocked with blocking buffer followed by the incubation of the biotinylated detection antibody for 24 h at 4 °C. Then HRP-conjugated avidin is added, which binds to the biotinylated detection antibody. Finally, the substrate is added, which catalyses the reaction and provides colour intensity that represents the quantity of antigen and the absorbance of both the supernatant and the standards was measured at 450 nm. The protein concentrations were determined using the standard curve and expressed in terms of pg/ml.

Statistical Analysis

The values obtained from three separate determinants were used to obtain the results. Comparison between experimental groups was conducted through the use of one-way ANOVA and paired sample "t" tests, facilitated by the use of GraphPad Prism software (version 8).

Results

Effect of Bornyl Acetate or Menthol on the Formation of Amyloid Plaque in Brain Tissue of Lipopolysaccharide-Injected Mice

As shown in Figs. 1 and 2, our result demonstrates that in the control animals, there were no signs of amyloid fibril aggregates (Fig. 1 i & ii A). However, in the brain sections of LPS-treated animals (group II), a significant accumulation of amyloid plaque was observed in the cortex and hippocampus regions, characterized by dark red as well as green fluorescence aggregates of congo red and thioflavin T stains binding to the amyloid fibrils (Figs. 1 and 2 i & ii B). In contrast, with higher concentrations of bornyl acetate or menthol (100 mg/kg b.w.) as well as donepezil 5 mg/kg b.w. (groups III–V), treated animals amyloid plaque aggregates were markedly reduced similar to the control group (Figs. 1 and 2 i & ii C–E).

Effect of Bornyl Acetate or Menthol on Mitochondrial Membrane Integrity in Brain of Lipopolysaccharide-Injected Mice

The integrity of active mitochondria in the cortex and hippocampus regions of mice brain tissues were stained using janus green b. In the control group, both the cortex and hippocampus showed a high intensity of green fluorescence in the mitochondria (Fig. 3 i & ii A; group I). However, in the LPS-injected group, there were only a few randomly arranged live mitochondria with less green fluorescence, indicating damage to the mitochondrial membrane (Fig. 3 i & ii B; group II). Intriguingly, when treated with a higher concentration of bornyl acetate or menthol (100 mg/kg b.w.), there was a significant increase in the number of mitochondria with green fluorescence, similar to the control group (Fig. 3 i & ii C, D; groups III, IV). Surprisingly, in the hippocampal region of donepezil-treated animal (5 mg/kg b.w.) there was no intact mitochondria in the hippocampal region (Fig. 3 ii E; group V).

Effect of Bornyl Acetate or Menthol on Calcium Accumulation in Brain of Lipopolysaccharide-Injected Mice

The sections of mice brain tissue analysed using alizarin red S, the control animals brain showed no pathological changes

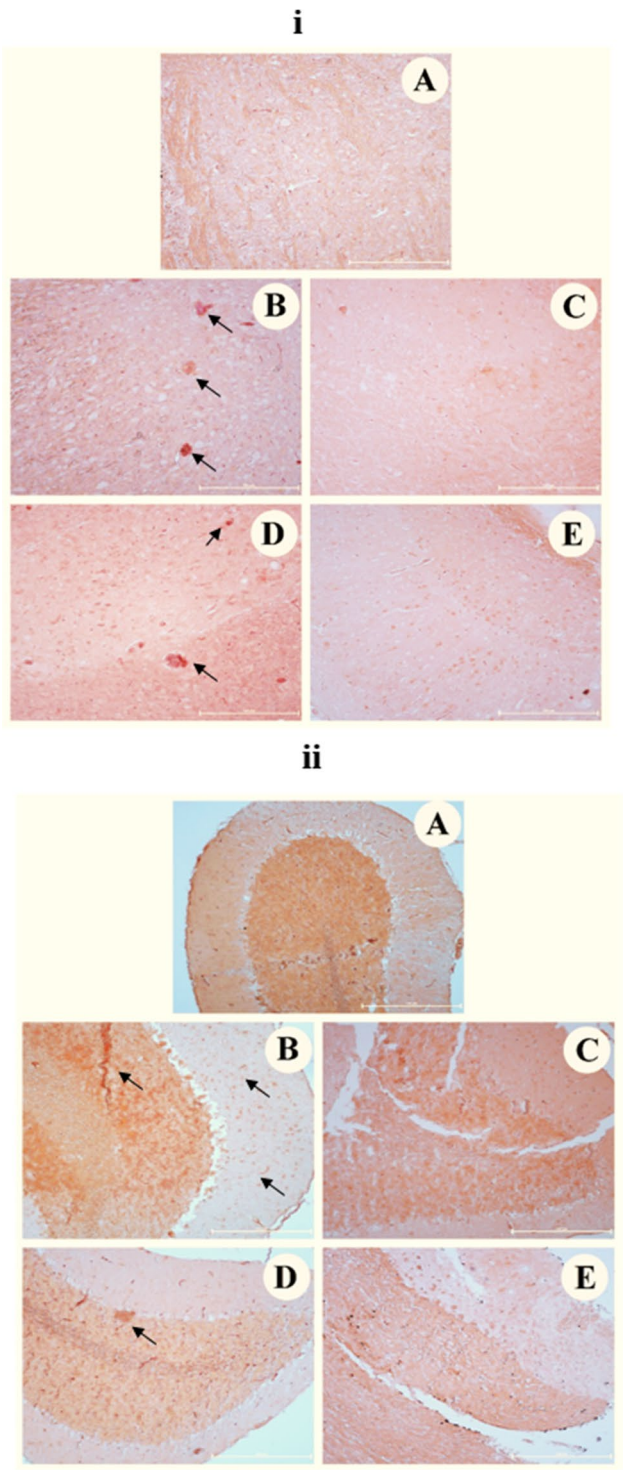


Fig. 1 Effect of bornyl acetate and menthol observed for amyloid fibril formation on the histological section (i) cortex and (ii) hippocampus region of brain tissues in lipopolysaccharide-injected mice determined using congo red. **A** Control animal, **B** LPS-injected animal, **C** bornyl acetate co-treated animal (100 mg/kg b.w.), **D** menthol co-treated animal (100 mg/kg b.w.), **E** donepezil co-treated animal (5 mg/kg b.w.). Note: arrows indicate the dark red-coloured amyloid deposits with $n = 6$

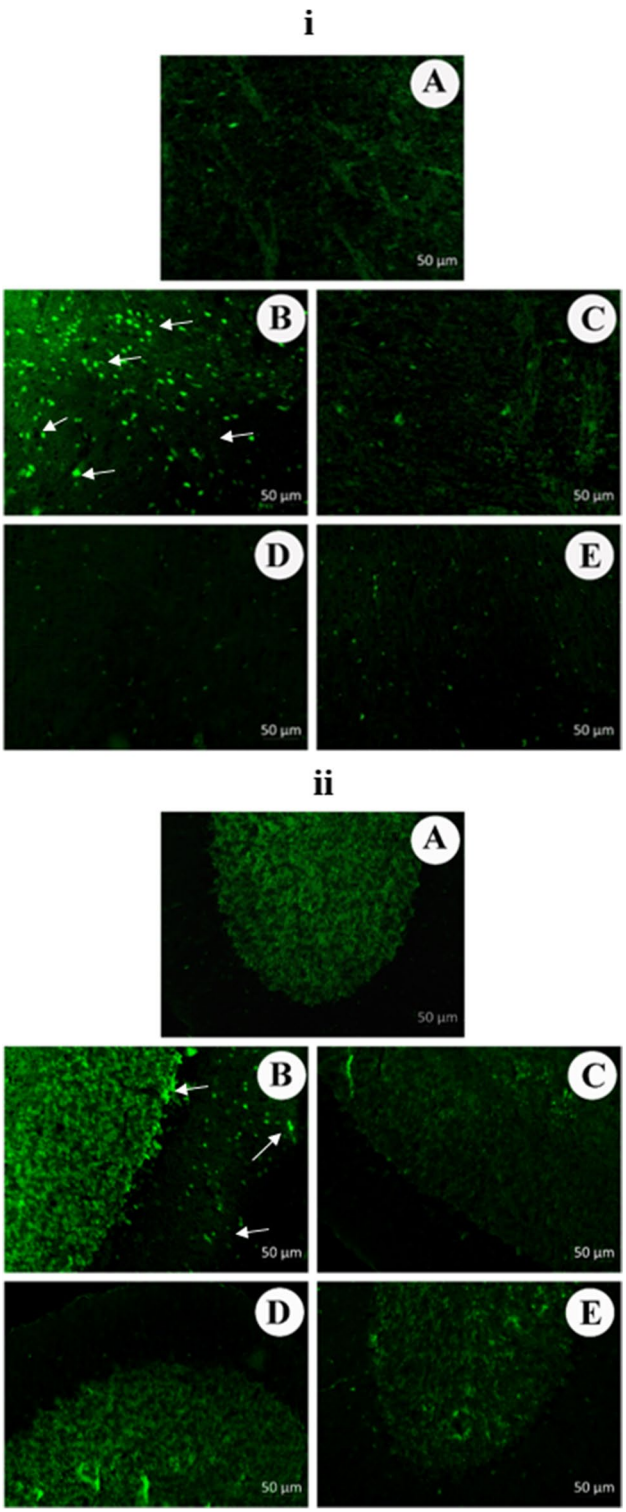


Fig. 2 Effect of bornyl acetate and menthol observed for amyloid fibril formation on the histological section (i) cortex and (ii) hippocampus region of brain tissues in lipopolysaccharide-injected mice determined using thioflavin t. **A** Control animal, **B** LPS-injected animal, **C** bornyl acetate co-treated animal (100 mg/kg b.w.), **D** menthol co-treated animal (100 mg/kg b.w.), **E** donepezil co-treated animal (5 mg/kg b.w.). Note: arrows indicate the green fluorescence emission in amyloid deposits with $n = 6$

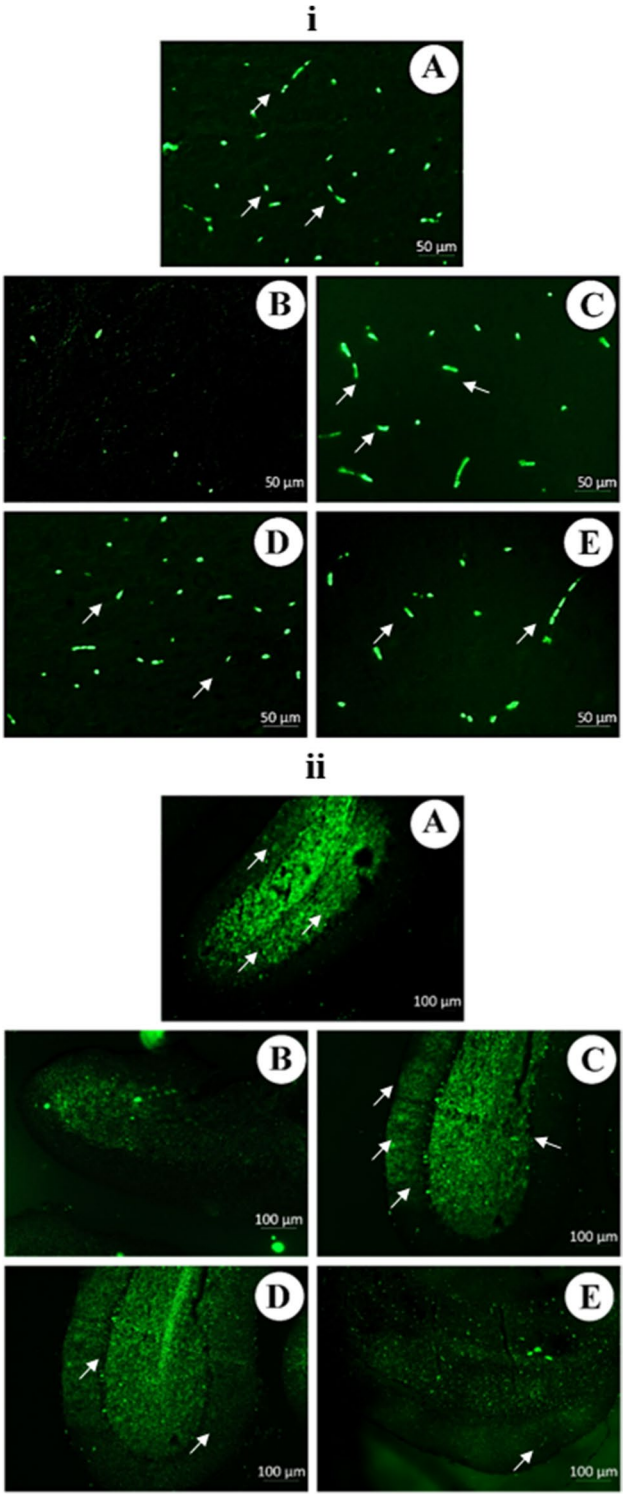


Fig. 3 Effect of bornyl acetate and menthol analysed for mitochondrial membrane integrity on the histological section (i) cortex and (ii) hippocampus region of brain tissues in lipopolysaccharide-injected mice determined using janus green B. **A** Control animal, **B** LPS-injected animal, **C** bornyl acetate co-treated animal (100 mg/kg b.w.), **D** menthol co-treated animal (100 mg/kg b.w.), **E** donepezil co-treated animal (5 mg/kg b.w.). Note: arrows indicate the high green fluorescence emission in live mitochondria with $n = 6$

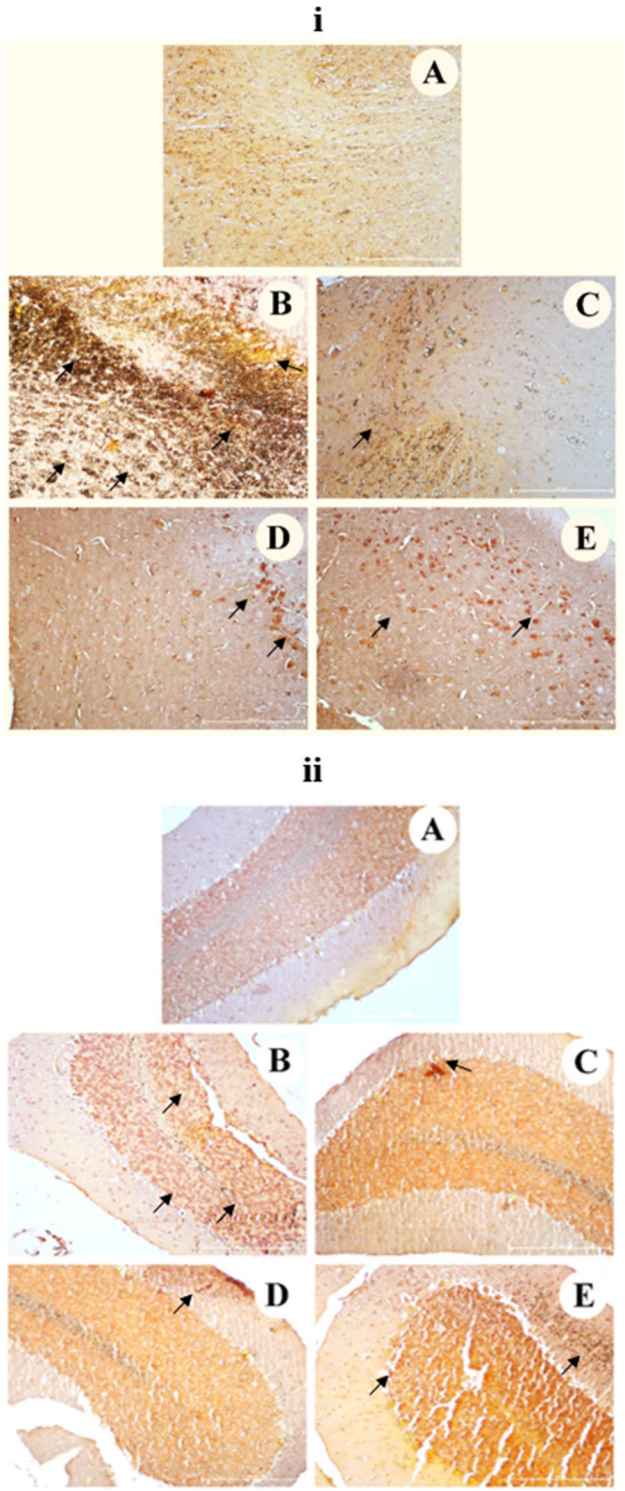


Fig. 4 Effect of bornyl acetate and menthol analysed for intracellular calcium accumulation on the histological section (i) cortex and (ii) hippocampus region of brain tissues in lipopolysaccharide-injected mice determined using alizarin red S. **A** Control animal, **B** LPS-injected animal, **C** bornyl acetate co-treated animal (100 mg/kg b.w.), **D** menthol co-treated animal (100 mg/kg b.w.), **E** donepezil co-treated animal (5 mg/kg b.w.). Note: arrows indicate the dark brown deposits of accumulated calcium with $n = 6$

or accumulation of calcium, whereas, LPS-administered animals showed an overabundance of active calcium ions in the cortex and hippocampus regions. These excess ions manifested as bright red deposits; a phenomenon absent in the brains of the control group (Fig. 4 i & ii B; group II). In contrast, treatment with a higher concentration of bornyl acetate or menthol (100 mg/kg b.w.) or donepezil (5 mg/kg b.w.) resulted in a reduction of red deposits (Figs. 4 i & ii C–E; groups III–V), indicating a decrease in calcium accumulation similar to that of the control animals (group I).

Effect of Bornyl Acetate or Menthol on Oxidative by-Products in Brain Homogenates of Lipopolysaccharide-Injected Mice

The levels of lipid peroxidation by-products malondialdehyde (MDA) were determined in the brain tissues. The control animals showed lower level of MDA. In contrary, animals administered with LPS (group II), demonstrate a significant increase in lipid peroxidation, compared to the control group (Table 1, group I). However, animals treated with higher doses of bornyl acetate or menthol (100 mg/kg b.w.) respectively, showed a decrease in lipid peroxidation when compared to the LPS-treated group (Table 1, groups III–V).

The levels of carbonylated and glycated proteins assessed in brain tissue homogenate revealed that the control animals (group I) showed the lowest levels of protein carbonylation and advanced glycation end products, while the animals treated with LPS (group II) showed a notable increase in these substances (Table 1). On the other hand, animals treated with higher doses of bornyl acetate or menthol (100 mg/kg b.w.) respectively exhibited a significant reduction in carbonylated and glycated proteins as compared to the LPS-injected animals (Table 1; groups III-V).

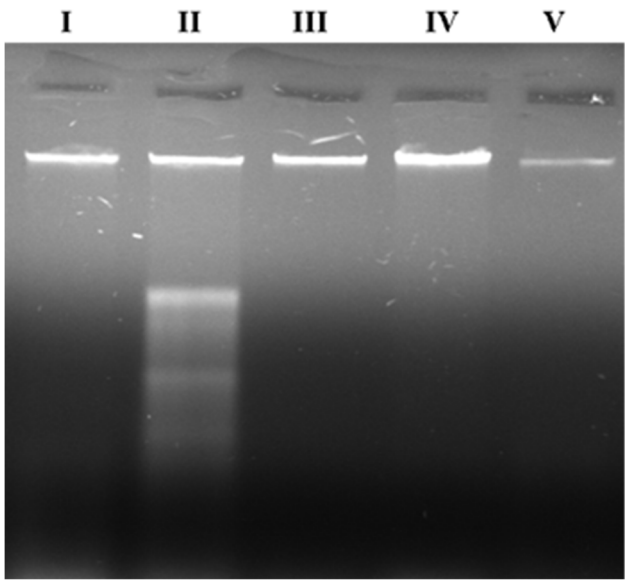


Fig. 5 Effect of bornyl acetate and menthol evaluated on total DNA fragmentation in lipopolysaccharide-injected mice brain tissue analysed using agarose gel electrophoresis. Lane I: Control animal, Lane II: LPS-injected animal, Lane III: bornyl acetate co-treated animal (100 mg/kg), Lane IV: menthol co-treated animal (100 mg/kg), Lane V: donepezil co-treated animal (5 mg/kg) with $n=6$

Effect of Bornyl Acetate or Menthol on DNA Fragmentation in Brain of Lipopolysaccharide-Injected Mice

The presence of DNA damage in brain tissue of all experimental animals was evaluated. Visual analysis of the agarose gel (Fig. 5) in the control brain tissue homogenates exhibited a single intact DNA with no sign of fragmentation (group I; Lane I). In contrast, the LPS-injected animals revealed distinct streak formations in the gel indicating multiple fractioning of DNA fragments (group II; Lane II). Interestingly,

Table 1 Effect of bornyl acetate or menthol on the levels of malondialdehyde, protein carbonyls and advanced glycation end products in lipopolysaccharide-induced mice

Experimental groups	Malondialdehyde (nmol/mg protein)	Protein carbonyl content (μM/mg protein)	Advanced glycation end product (Arbitrary units)
Group I	5.76 ± 0.05	1.86 ± 0.013	109 ± 1.41
Group II	13.26 ± 0.02**	2.77 ± 0.012**	149 ± 2.8*
Group III	6.26 ± 0.01**	1.75 ± 0.013**	117 ± 1.4**
Group IV	7.5 ± 0.02**	2.0 ± 0.013*	128 ± 1.4**
Group V	9.58 ± 0.01*	2.22 ± 0.012*	134 ± 0.7*

Each value represents mean ± SD of three determinations. The difference in the MDA, protein carbonyl, advanced glycated end products levels between groups I & II and groups II & III–V were statistically significant at * $p<0.05$ and ** $p<0.01$

co-treatment with either bornyl acetate or menthol (100 mg/kg b.w.) respectively was found to effectively prevent DNA damage, as evidenced by the gel image resembling that of the control animals (Fig. 5; groups III–V). This highlights the potential of these compounds in protecting against LPS-induced DNA damage in the brain tissues.

Effect of Bornyl Acetate or Menthol on Gene Expression in Brain of Lipopolysaccharide-Injected Mice

The investigation focused on examining gene expression levels related to surface markers, cytokines, amyloidogenic and inflammatory pathways, cholesterol regulatory genes and microglial markers that are linked to neuroinflammation and development of Alzheimer's disease.

(i) Surface marker

The mRNA levels of TLR 4 surface receptor increased by 1.1 fold in the animal brain stimulated by lipopolysaccharide (Fig. 6 i & ii; group II). Interestingly, mice co-treated with bornyl acetate or menthol significantly reduced expression by 1.1, 1.0 and 1.0 folds, respectively (Fig. 6 i & ii; groups III–V).

(ii) Amyloidogenic markers

The amyloidogenic markers revealed the significant upregulation of APP (1.9 folds), BACE1 (1.5 folds), misfolded protein marker GRP78 (1.3 folds) and ATF-6 (1.2 folds) in the lipopolysaccharide-injected animal (Fig. 6 i & ii; group II), whereas bornyl acetate or menthol co-treated animals downregulated the APP expression by 1.0, 1.3, 1.7 folds, BACE1 by 1.2, 1.1, 1.11 folds, GRP78 by 1.1, 1.3, 1.07 folds change and ATF-6 expression by 1.2, 1.9, 1.0 folds change, respectively, which was similar to that of control (Fig. 6 i & ii; groups III–V).

(iii) Pro-inflammatory cytokines

Upregulation of TLR4 surface marker and amyloidogenic gene expressions influenced the significant upregulation in pro-inflammatory genes IL-6, IL-1β, iNOS and MMP-9 (Fig. 6 i & ii; group II). Animals co-treated with bornyl acetate reduced IL-6 expression by 1.3, but remained unaltered in co-treatment with menthol and donepezil. Similarly, bornyl acetate or menthol or donepezil downregulated the expression of IL-1β by 1.6, 1.2, 1.1 folds, iNOS by 6, 2.4, 1.4 folds and MMP-9 by 1.9, 2.4, 2.9 folds respectively, which was almost identical to the control (Fig. 6 i & ii; groups III–V).

(iv) Pro-apoptotic markers

Cathepsin S, a pro-apoptotic gene, was elevated by 3.4 folds in lipopolysaccharide-injected animals (Fig. 6 i & ii; group II) compared to controls but was significantly reduced by 1.81, 1.4 and 2.1 folds in bornyl acetate or menthol co-treated animals (Fig. 6 i & ii; groups III–V). PARP1 was elevated 1.64 times when compared to controls; however, it was downregulated by 1.8, 1.2 and 3.4 folds in bornyl acetate or menthol co-treated mice, respectively. However, the conventional medicine donepezil was unsuccessful in modifying PARP-1. Caspase 3 expression was also increased by 4.6 folds in lipopolysaccharide-injected animals, which was significantly reduced by 3.5, 6.5 folds in bornyl acetate or menthol co-treated animals (Fig. 6 i & ii; groups III & IV) and donepezil group showed no significant changes (Fig. 6 i & ii; group V).

(v) Cholesterol efflux genes

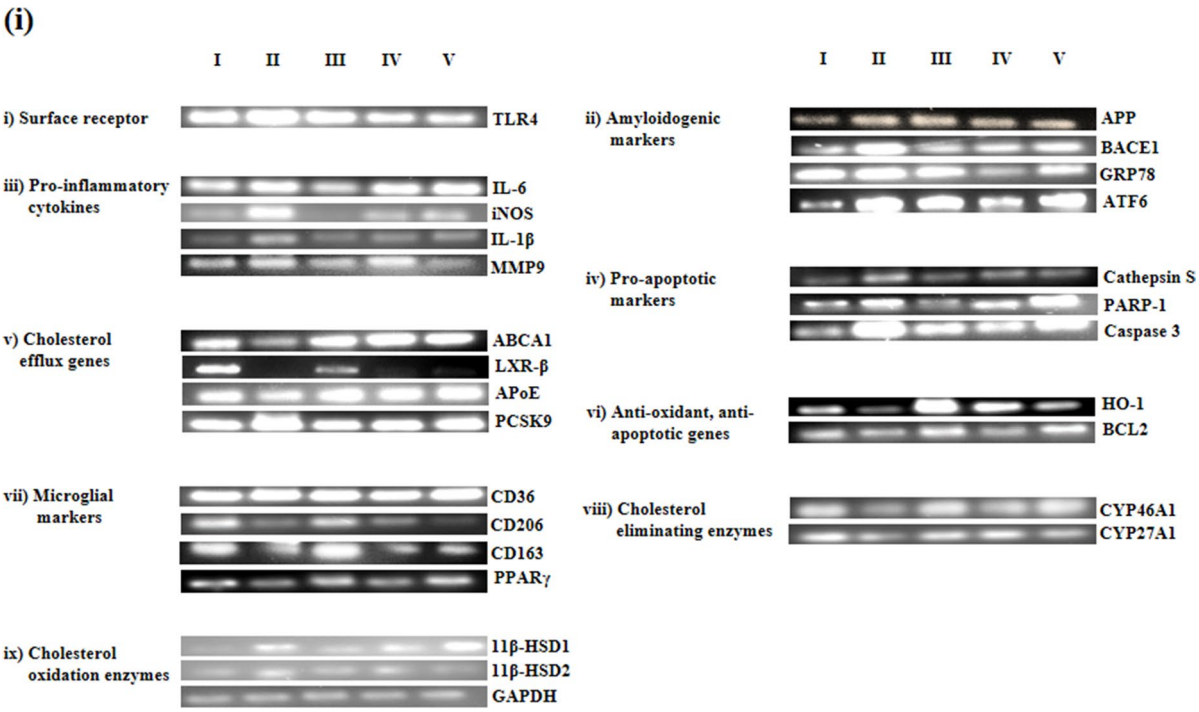
The levels of cholesterol efflux gene ABCA1, LXR-β and ApoE were significantly decreased 1.2, 1.2, 2.6, fold respectively, in animals treated with lipopolysaccharide, as compared to the control group (Fig. 6 i & ii; group II). Conversely, co-treatment with bornyl acetate or menthol resulted in an upregulation of ABCA1 (1.5, 1.91, 1.99 folds), LXR-β (1.5, 1.81, 1.91 folds) and ApoE (2.1, 1.8, 2.3 folds), which was similar to the levels observed in the control group. Interestingly, while lipopolysaccharide exposure led to a 1.2-fold increase of PCSK9/NARC-1, bornyl acetate or menthol normalized the expression similar to that of control (Fig. 6 i & ii; groups III–V).

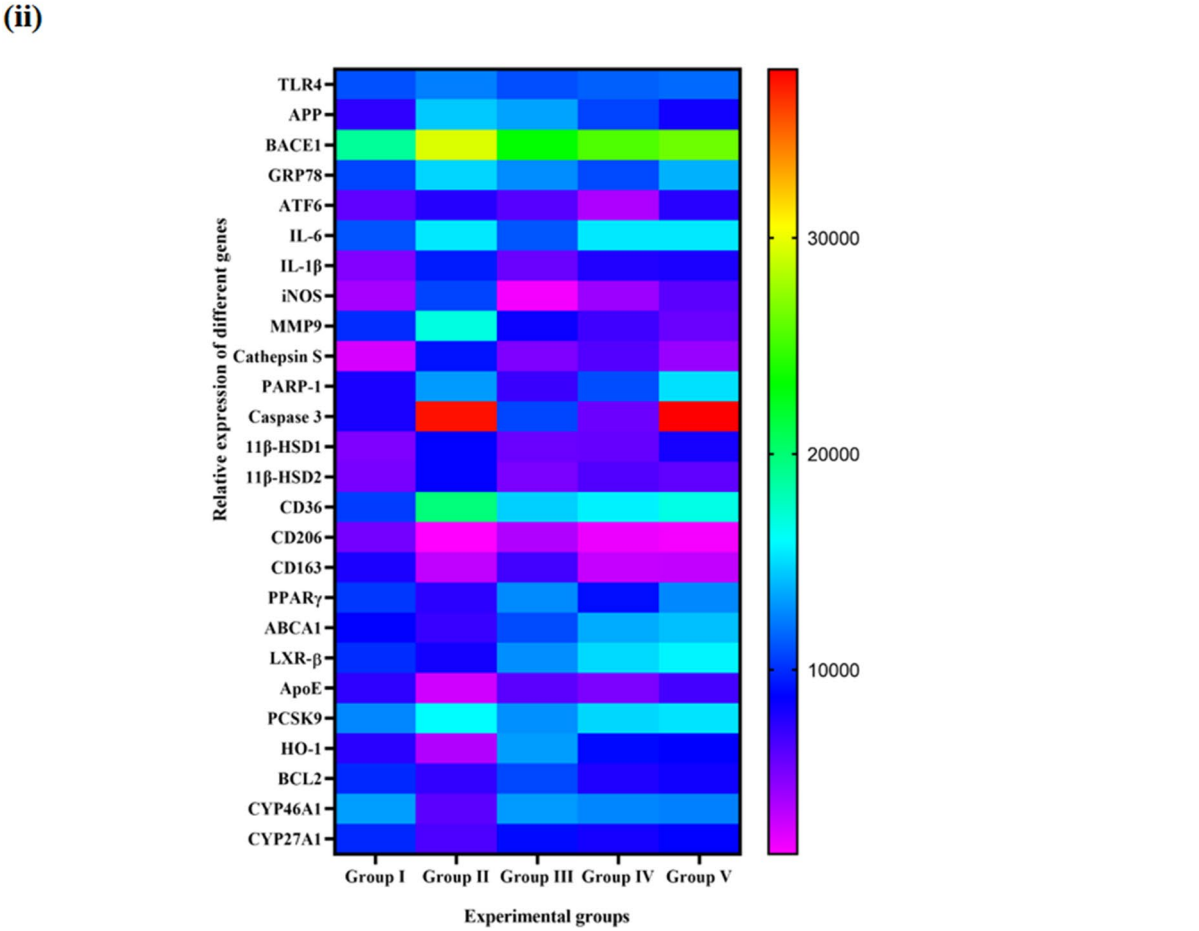
(vi) Anti-oxidant and anti-apoptotic genes

Additionally, the expression of the anti-oxidant gene HO-1 and anti-apoptotic gene BCL2 was found to be suppressed by 2 and 1.35 folds, respectively in lipopolysaccharide-injected animals when compared to that of the control group (Fig. 6 i and ii, group II). In contrast, administering either bornyl acetate or menthol resulted in an upregulation of 3.6 and 2.4-fold or 1.4 and 1.0-fold, respectively. These findings were similar to those observed in the control group (Fig. 6 i & ii; groups III-V).

(vii) Microglial markers

In the lipopolysaccharide-injected animals, we observed a significant increase 1.8 fold, in the mRNA level of the microglial marker CD36, which is associated with pro-inflammatory activity. However, when these animals were co-treated with bornyl acetate or menthol, they showed a significant decrease in CD36 expression, with reductions of 1.3,


(i)
I II III IV V
i) Surface receptor
TLR4
iii) Pro-inflammatory cytokines
IL-6
iNOS
IL-1β
MMP9
v) Cholesterol efflux genes
ABCA1
LXR-β
APoE
PCSK9
vii) Microglial markers
CD36
CD206
CD163
PPARγ
ix) Cholesterol oxidation enzymes
11β-HSD1
11β-HSD2
GAPDH
I II III IV V
ii) Amyloidogenic markers
APP
BACE1
GRP78
ATF6
iv) Pro-apoptotic markers
Cathepsin S
PARP-1
Caspase 3
vi) Anti-oxidant, anti-apoptotic genes
HO-1
BCL2
viii) Cholesterol eliminating enzymes
CYP46A1
CYP27A1


(ii)
TLR4
APP
BACE1
GRP78
ATF6
IL-6
IL-1β
iNOS
MMP9
Cathepsin S
PARP-1
Caspase 3
11β-HSD1
11β-HSD2
CD36
CD206
CD163
PPARγ
ABCA1
LXR-β
ApoE
PCSK9
HO-1
BCL2
CYP46A1
CYP27A1
Relative expression of different genes
30000
20000
10000
Group I Group II Group III Group IV Group V
Experimental groups

◂**Fig. 6** Effect of bornyl acetate and menthol analysed for the mRNA expression of various gene markers in lipopolysaccharide-injected mice brain tissue. Lane I: Control animal, Lane II: LPS-injected animal, Lane III: bornyl acetate co-treated animal (100 mg/kg), Lane IV: menthol co-treated animal (100 mg/kg), Lane V: donepezil co-treated animal (5 mg/kg). (ii) Relative density of mRNA level expression in lipopolysaccharide-injected animals co-treated with bornyl acetate and menthol. The difference in the mRNA levels between control and treated animals were statistically significant at ** $p < 0.001$ with $n = 6$

1.2 and 1.1 fold, respectively, in groups III–V. These results were similar to the levels seen in the control group (Fig. 6 i & ii; group II), indicating the potential anti-inflammatory effects of bornyl acetate and menthol.

In the experimental animals, levels of anti-inflammatory microglial markers CD206, CD163 and PPAR-γ were examined in brain samples. Lipopolysaccharide induction resulted in significant downregulation of these markers, with decreases of 3.7, 2.4 and 1.37 folds evident (Fig. 6 i & ii; group II). However, co-treatment with bornyl acetate or menthol or donepezil led to increased expression of CD206 (2.5-fold increase), as well as PPAR-γ (1.3- and 1.1-fold increases, respectively). Interestingly, only bornyl acetate co-treated animals showed upregulation of CD163, with 2.1 folds increase similar to the control. No significant modulation was observed in the other co-treatment groups (Fig. 6 i & ii; groups III–V).

(viii) Cholesterol-eliminating enzymes

The levels of cholesterol-eliminating enzymes, specifically CYP46A1 and CYP27A1, showed a significant decrease in the expression of these enzymes, with a 2.1 and 1.4-fold reduction in the brain tissue of lipopolysaccharide-injected animals (Fig. 6 i & ii; group II) compared to control animals. However, in animals treated with bornyl acetate or menthol, similar to control (Fig. 6 i & ii; groups III-V), the gene expression of CYP46A1 increased by 2.1, 2.0 and 2.0 folds, while CYP27A1 saw a 1.3, 1.2 and 1.3-fold upregulation.

(ix) Cholesterol oxidation enzymes

On the other hand, there was a significant increase of 1.6 times in the levels of cholesterol oxidation enzymes (11β-HSD 1 & 2) in lipopolysaccharide-injected animal brain compared to control (Fig. 6 i & ii; group II). However, a contradictory result was seen in animals co-treated with bornyl acetate or menthol, where the mRNA levels of these enzymes were reduced by 1.5, 1.4, 1.0 and 1.6, 1.3, 1.4 times respectively, similar to the levels observed in the control animals (Fig. 6 i & ii; groups III–V). These results reveal the beneficial effects of bornyl acetate and menthol in regulating the expression of these enzymes.

Effect of Bornyl Acetate or Menthol on Protein Expression in Brain of Lipopolysaccharide-Injected Mice

The levels of key proteins involved in transcription, apoptosis and lipid regulation were assessed using the western blot analysis. These proteins included p65/NF-κB, caspase 3, BACE1, Aβ42, LRP-1 and NARC-1. Results from the experiments on animals showed that lipopolysaccharide-induced the translocation of cytosolic p65/NF-κB to the nucleus (Fig. 7 i & ii; group II), resulting in a significant decrease in its expression compared to the control animals. However, the co-treatment of bornyl acetate or menthol led to an increase in cytosolic p65/NF-κB expression similar to that of the control group, indicating a reduction in nuclear translocation (Fig. 7 i & ii).

Upon analysing the protein expression of apoptotic activator caspase 3, BACE1 enzyme, Aβ42 and NARC-1 in the experimental animals, it was observed that lipopolysaccharide induction resulted in a significant increase in comparison to control animals (Fig. 7 i & ii; group II). Conversely, treatment with bornyl acetate or menthol demonstrated a noteworthy decrease in protein expressions, similar to control animals (Fig. 7 i & ii; groups III–V). Concerning this, the experimental animals were examined for the presence of LDL receptor like protein (LRP) 1 in their brains. Results showed a significant decrease in LRP1 protein expression in the brain homogenates of animals injected with lipopolysaccharide, compared to those in control conditions (Fig. 7 i & ii; group II). However, when bornyl acetate or menthol was co-administered, LRP1 expression was found to be similar to that of the control group (Fig. 7 i & ii; groups III–V). Similarly, the ELISA quantification revealed significantly elevated levels of caspase 3, BACE1 enzyme, Aβ42 and NARC-1 when compared to the control (Fig. 8); however, the mice brains treated with bornyl acetate or menthol showed significantly lower levels of these proteins, while a contrary result was observed in the levels of LRP-1.

Discussion

Alzheimer's disease is an irresistible condition that has been linked to cognitive impairments, neuroinflammation and the build-up of amyloid peptides in the brain. Despite efforts to find effective treatments, a major obstacle remains: maintaining proper brain lipid balance [57–59]. However, promising research has shown that compounds like bornyl acetate and menthol have powerful effects against neurodegeneration that might be potentially used in lipopolysaccharide-induced Alzheimer's disease, targeting the root cause of altered brain lipid homeostasis [27, 29–31, 36–38].

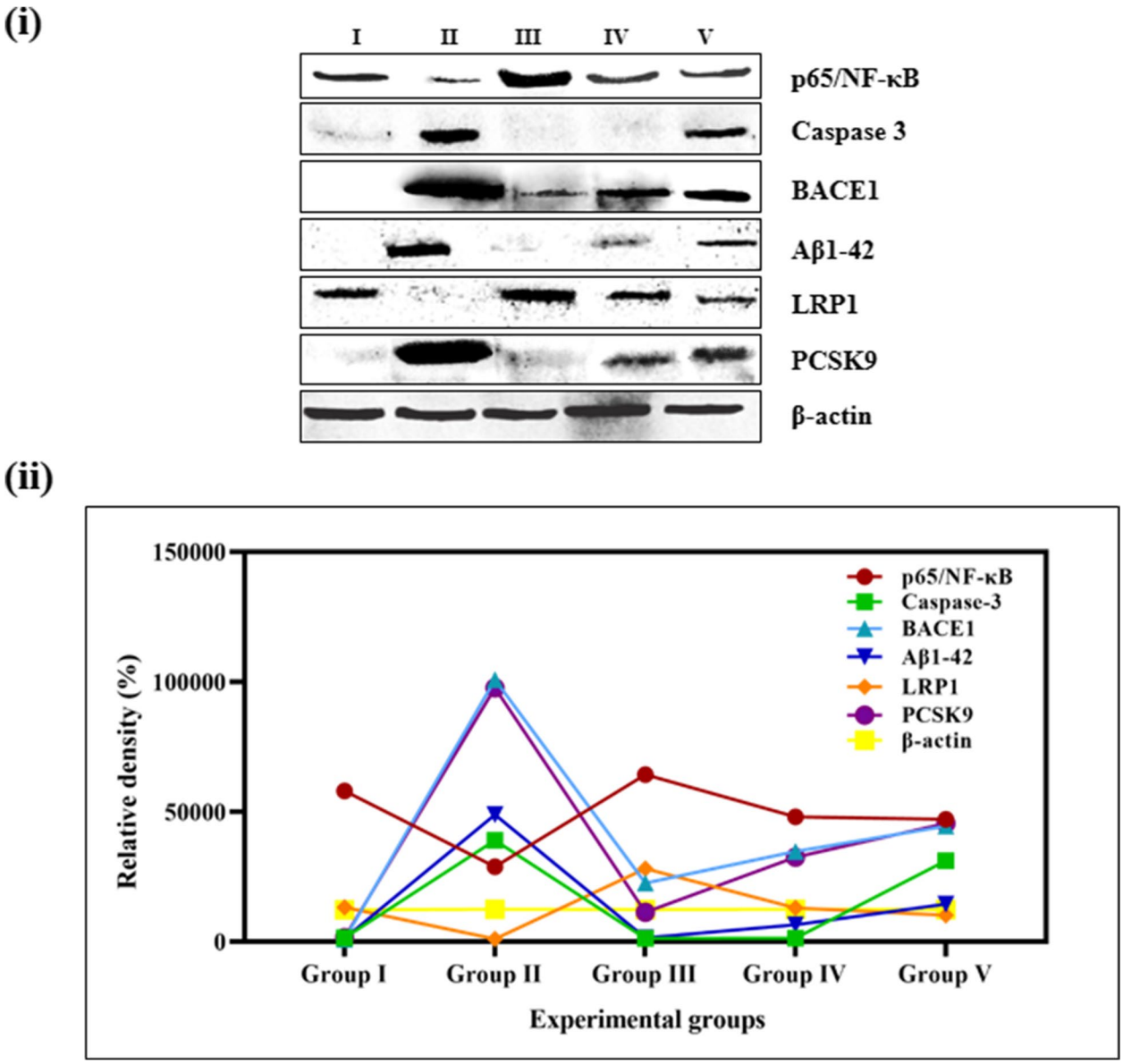


Fig. 7 Effect of bornyl acetate and menthol investigated on the protein expression of p65/NF-κB (65 kDa), caspase 3 (32 kDa), BACE1(49 kDa), Aβ(1–42) (140 kDa), LRP-1 (600 kDa) and PCSK-9/NARC-1 (62 kDa) in lipopolysaccharide-injected mice brain tissue. Lane I: Control animal, Lane II: LPS-injected animal, Lane III: bornyl acetate co-treated animal (100 mg/kg), Lane IV: menthol co-treated animal (100 mg/kg), Lane V: donepezil co-treated animal (5 mg/kg). (ii) Relative density of protein expression of amyloidogenic markers in animals co-treated with bornyl acetate and menthol. The difference in the protein levels between control and treated animals was significant at ** $p < 0.001$ with $n = 6$

In our preliminary investigation (presented as supplementary data), we put forth the primary emphasis on the behaviour of the animals found striking alterations such as dementia, anxiety and habituation due to the damaged neural cells and elevated acetylcholinesterase enzyme during exposure to LPS. Researchers have previously examined the effects of LPS-induced inflammation and have also documented brain tissue damage, manifested by distorted and diminished neurons in the cortex and altered cell arrangement in the hippocampal region, which receives cholinergic input associated with learning and memory, as well as a decline in behavioural and cognitive capabilities. Notably, our research revealed that the administration of bornyl acetate or menthol resulted in a reduction of neuronal damage and acetylcholinesterase enzyme with significant enhancements in behaviour, memory and neuromuscular function, as well as a decrease in aggressive tendencies. These results align with the previous research that also found similar protective effects for limonene, carvacrol, rosiridin and linalool [20, 60–64].

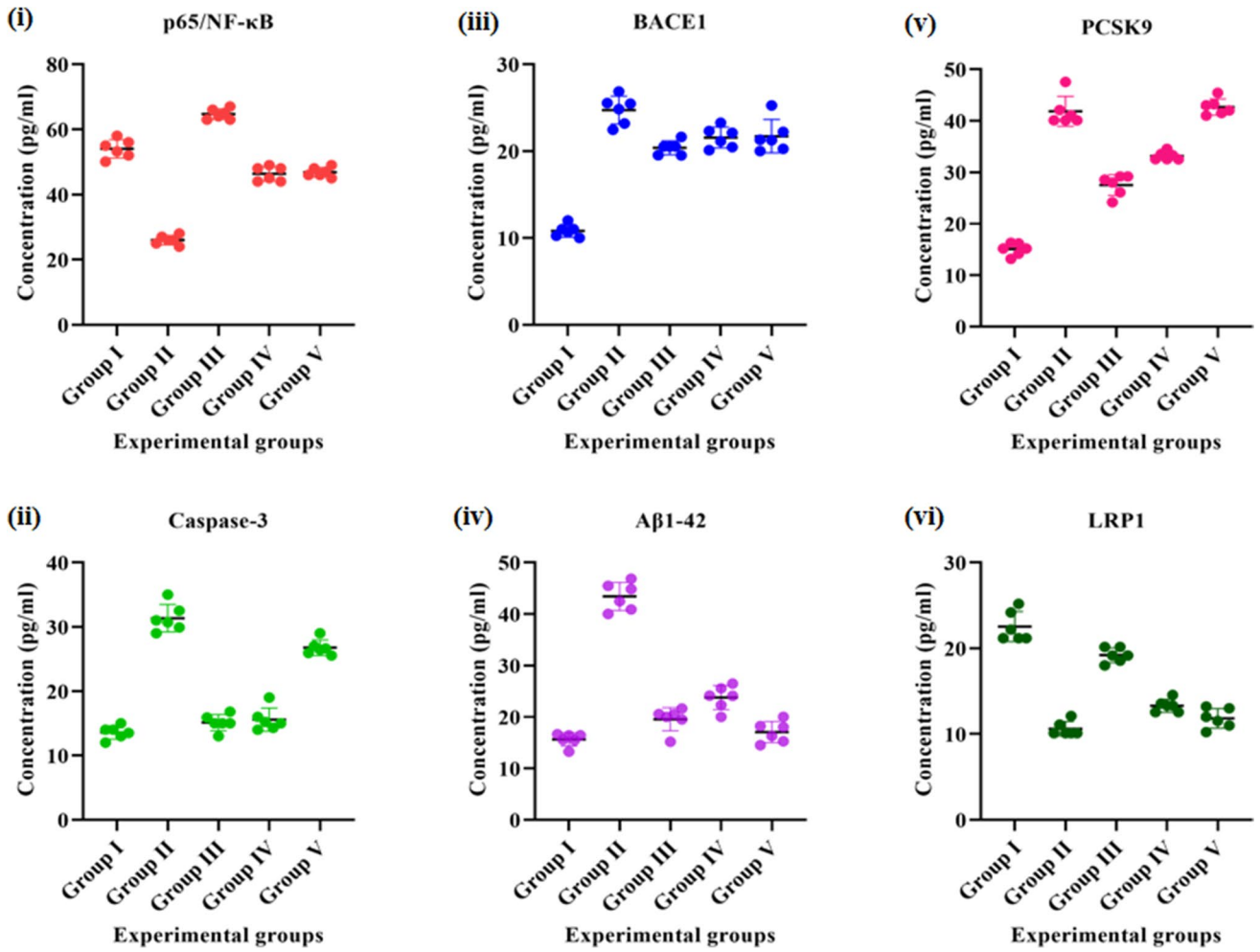


Fig. 8 Effect of bornyl acetate and menthol on the protein levels of **i** p65/NF-κB, **ii** caspase 3, **iii** BACE1, **iv** Aβ(1–42), **v** LRP-1 and **vi** PCSK-9/NARC-1 quantified using ELISA in lipopolysaccharide-injected mice brain tissue. The difference in the protein levels between control and treated animals was significant at ** $p < 0.005$ with $n = 6$

Discussing cognitive impairment and cholinergic neuronal loss, amyloid hypothesis is the most dominant pathology listed for the development of Alzheimer's disease. The production of amyloid β 42 peptides leads to a buildup of amyloid plaques, contributing to the neurodegeneration in Alzheimer's patients [65]. In a healthy brain, the non-canonical pathway is usually activated, with alpha and gamma-secretases cleaving to form soluble amyloid alpha peptides that can easily be cleared. However, when the NF-κB pathway is activated under pathological conditions, the canonical pathway triggers BACE-1, leading to the production of neurotoxic amyloid beta peptides that clump together and accumulate within the brain [66, 67].

The other pathological process involved in neurodegeneration is disturbed lipid balance, which leads to an increase in reactive oxygen species, causing lipid peroxidation and the release of harmful compounds like oxysterols, MDA, HNE, acrolein, eicosanoids and ceramide. This process can also weaken the body's natural defence against oxidative stress, increasing the risk of developing neurodegenerative diseases [68]. Luckily, antioxidants can counteract this harmful free radical generation by scavenging them. However, in cases of neuroinflammation, the antioxidant levels may decrease, leaving the body vulnerable to oxidative stress and resulting in the oxidation of proteins and lipids [69]. In our study, we observed a similar condition with higher levels of lipid peroxidation and protein oxidation markers, specifically protein carbonyls, in response to LPS induction. However, our findings elucidated the ameliorative effects of bornyl acetate and menthol to reverse these changes, similar to previous demonstrations [27, 70, 71]. Geraniol was previously demonstrated reducing free radicals and oxidative markers such as malondialdehyde, hydroperoxides and protein carbonyls in rat models [72].

DNA damage has been connected to the emergence of neurological diseases. A greater proportion of Alzheimer's disease patients with chromosomal or cytogenetic abnormalities have been found to have DNA damage as a result of oxidative stress, one of the body's first insults. [73] have also shown that the injection of LPS can duplicate Alzheimer's conditions, which results in DNA damage and apoptosis. This conclusion is further supported by our research, which showed that the brains of mice treated with LPS had fragmented DNA. However, we were able to preserve the integrity of the DNA and avoid damage by administering menthol and bornyl acetate. Similarly, previous research highlights the efficacy of *Acacia hydaspica*, pomegranate extract and polyphenols in reducing DNA damage [74–76].

A stressed endoplasmic reticulum can elevate calcium, which can enter the mitochondria through mitochondrial communication. This influx of calcium can alter the mitochondria-associated membrane, leading to dysfunctional mitochondria and the production of oxidative stress and free radicals, reported by Wang et al. [77]. Calcium deposition has been observed in various structures of the brain. Prolonged deposition of calcium in the mitochondria can result in structural damage and harm to neuronal cell bodies, as reported by Oliveira et al. [78]. Our investigation revealed an undesirable calcium accumulation in the mitochondria with LPS administration, which was similarly documented by Seabra et al. [79]. Saponin, considerably reduced the influx of calcium into the brain that was caused by LPS [80] similar to bornyl acetate and menthol, which is another favourable effect that our research demonstrated [81, 82].

Various investigations have revealed that oxidative stress plays a significant role in the development of Alzheimer's disease. Amyloid beta triggers the production of harmful free radicals, having a detrimental impact on mitochondrial functioning, inflammation, proteolysis and nucleic acid cleavage. Ultimately, this chain of events can result in cell death, as observed by Kulkarni et al. [83] in their examination of mouse brains treated with LPS. Similarly, we noted mitochondrial destabilization with excessive generation of free radicals upon LPS administration. According to Xi et al. [84], genistein effectively eliminates reactive oxygen species and activates a redox-sensitive pathway to maintain antioxidant levels in mitochondria. Similar findings were observed in bornyl acetate and menthol treatment; their antioxidant nature was noted by Schmidt et al. [85] and Perez-Rose et al. [86]. Further, these compounds also boosted the activity of mitochondria and their potent antioxidant properties effectively scavenged free radicals.

While considering the consequences of disease pathologies, it is vital to acknowledge the pivotal role of complex genes and protein mediators. Our research focused on the signalling mediators at the molecular level, investigating how bornyl acetate and menthol impact on mRNA and protein expressions. Numerous reports have suggested that AD is influenced by the administration of LPS activates TLR-4 receptors, leading to the production of proinflammatory cytokines and stimulation of NF-κB, which promotes the formation of Aβ42 peptide fibrils [87, 88]. In mice, LPS administration has been found to increase the activity of beta-secretase and the production of Aβ1–42, the carboxy-terminal fragments (c-99) [20, 89, 90] which is supported by our findings. Necrodane and crocin inhibited amyloid deposition. Similarly, vinillic acid provided neuroprotection against Alzheimer-like condition triggered by LPS, reducing NF-kB, nitric oxide synthase 2 (NOS-2) and proinflammatory cytokines, cognitive impairment and synaptic dysfunction [91–93]. We propose that bornyl acetate and menthol are anti-amyloidogenic and regulate the NF-kB proinflammatory pathway by their antioxidant ability [33, 94].

Inflammatory markers, surface receptors and transcription factors, specifically involved in the activation of AD, were examined. A previous study conducted by Topchiy et al. [95] revealed that the administration of lipopolysaccharide led to an increase in proinflammatory cytokines (IFN-γ, IL-1β, TNF-α, iNOS, IL-6 and NF-κB) and a decrease in levels of low-density lipoprotein receptors (LDLR) in the bloodstream [96]. This ultimately decreases the expression of genes such as sirtuin1, Nrf2, SOD and HO-1 [97], leading to a systemic inflammatory response. This inflammation, caused by the introduction of LPS into the circulatory system, can result in sepsis and peripheral inflammation. In mice, this can lead to a neuroinflammatory state, activating microglia and astrocytes that produce pro-inflammatory cytokines, ultimately resulting in neuronal death and the development of various neurodegenerative diseases [98]. Our results support the previous evidence, suggesting that LPS-induced inflammation disrupts the lipid metabolism of brain by increasing proinflammatory activity. However, our research also discovered that bornyl acetate and menthol can counteract this effect by reducing oxidative stress and sustaining normal cholesterol metabolism, thus suppressing inflammatory signals. Like our study, the compounds like parthenolide and cantalasaponin-1 have been reported earlier, which downregulate the proinflammatory cytokines in brain inflammation [99, 100].

Our results reveal the important role of cholesterol in brain health and the potential for LXR agonists to regulate its levels. The presence of oxysterols, a by-product of brain cholesterol auto-oxidation, can lead to changes in intracellular cholesterol and the processing of amyloid precursor protein into the Aβ42 peptide, a hallmark of Alzheimer's disease. However, the LXR agonist can inhibit this process by promoting lipid efflux and enhancing the clearance of Aβ through the activation of ABCA1 and ApoE genes [101]. Our results also highlight the effect of LPS administration in mice, on the decreased expression of ABCA1, LXR-β

and ApoE cholesterol efflux genes, with significant upregulation seen in bornyl acetate and menthol co-treatment. In addition, previous research observed that decrease in the expression of LDLR, a receptor responsible for the transport of high cholesterol to the brain, in the LPS-induced sepsis model. Furthermore, our findings indicate that PCSK9 downregulates LDLR in the brain following LPS administration. However, treatment with bornyl acetate and menthol showed an increase in LDLR levels, potentially aiding in LPS clearance and preventing neurodegeneration caused by lipid dysfunction.

Microglia, the necessary macrophages present in the central nervous system (CNS), have crucial roles in the uptake and degradation of Aβ. They use macropinocytosis and LDL-receptor-related proteins (LRPs) to absorb soluble Aβ, whereas insoluble Aβ activates innate immune cell surface receptors and promote phagocytosis. Alzheimer's disease progression decreases microglial function of Aβ clearance. Recent research shows that activating TLRs with CD14 can improve microglia's ability to remove fibrillar Aβ [102]. However, prolonged exposure to LPS may lead to the continual presence of proinflammatory cytokines, hindering microglial phagocytic abilities and promoting the production of fibrillar Aβ [103]. In comparison to our results, ginsenoside has been reported to alleviate neuroinflammation LRP1 [104]. LXRβ agonist treatment in the NPC1 mouse model eliminated the cholesterol by upregulating sterol transporters ABCA1 and ABCG1, while suppressing neuroinflammatory activation receptors CD14, MAC1, CD11c and iNOS [105]. In addition, 2'-hydroxycinnamaldehyde upregulated LRP1, which counteracted microglial activation and inflammatory pathways ERK, JNK, p38 MAPK and NF-κB [106].

LPS-induced changes in the mouse brain mediated by CD36 upregulation and increased level of Aβ42 in our study, as CD36 facilitates the binding of microglia and macrophages to harmful substances such as β-amyloid fibrils and oxidized low-density lipoprotein (ox-LDL). In addition, CD36 also collaborates with the secretion of hydrogen peroxide (H_2O_2) to promote adhesion to Aβ fibrils found in senile plaques. This interaction activates a signalling process that leads to the release of harmful substances like reactive oxygen species (ROS). Furthermore, CD36 may also contribute to the breakdown of the blood–brain barrier and increased permeability of cerebral microvessels by interacting with fibrillar Aβ in the subendothelial space [107]. However, bornyl acetate and menthol were found to reduce the CD36 microglial receptors. Quoting our results, PP2 and piceatannol compounds were also involved in inhibiting CD36 microglial activation, abrogating neurotoxic peptides [108].

Microglial cells can become proinflammatory (M1) when exposed to certain stimuli such as LPS and proinflammatory interferons and are also capable of switching to a more beneficial anti-inflammatory state (M2) by various receptors, including PPAR-γ, CD163 and 206 to efficiently clear debris and protect the nervous system. These receptors influence microglia and can limit the development of neuroinflammatory diseases were downregulated by LPS administration as highlighted by a recent study by Monoranu et al. [109, 110]. In contrast, bornyl acetate and menthol increased the receptors of CD163, PPAR-γ revealing the expression of anti-inflammatory microglia that modulated the neuroinflammation in LPS-induced AD mice model, similar to previous literature [111]. This scores a significant role as these anti-inflammatory phenotype receptors, further emphasizing the potential of these compounds in controlling inflammation.

Furthermore, the impact of LPS stimulation on brain lipid metabolism was assessed by examining the mRNA levels of enzymes involved in cholesterol elimination and oxidation. 24-hydroxylase enzyme, CYP46A1, maintains brain cholesterol homeostasis which converts the common derivative, 24-s hydroxycholesterol, into a form that can be removed from the brain [112]. This essential process is disrupted during inflammation, as the dominating oxysterol, 24-hydroxycholesterol, is metabolized in the liver. However, abnormal metabolism can lead to the enzyme CYP27A1 taking over and reducing CYP46A1's effectiveness [113]. The activity of CYP46A1 is further impacted by LPS administration, as inflammation triggers alterations in the TLR4/MyD88/NF-κB signalling pathway [114]. In our study, we observed a notable decrease in CYP46A1 expression when examining the brains of mice injected with LPS. Interestingly, we also found that the compounds bornyl acetate and menthol were able to boost CYP46A1 expression and prevent the harmful effects of cholesterol oxidation by regulating the enzymes CYP27A1, 11β-HSD 1 & 2. These enzymes are responsible for the production of toxic oxysterols that can accumulate in the brain and contribute to Alzheimer's disease pathology. This highlights the potential of bornyl acetate and menthol in maintaining healthy brain cholesterol levels through lipid transport and inhibition of oxysterol formation.

Many of the world's population currently rely on traditional medicines, often incorporating plant materials into extracts and food. These natural remedies have demonstrated impressive health benefits, specifically monoterpenes, which have been found to possess robust biological properties [115].

Conclusion

In conclusion, our recent research shows two natural compounds, bornyl acetate and menthol, have shown remarkable efficacy in shielding against neurological damage. In mice injected with lipopolysaccharides, these compounds

effectively counteracted neuroinflammation and inhibited amyloid plaque formation. These results from our experiments provide basic evidence for the potential of these compounds as a targeted treatment for the aforementioned neuroinflammatory pathway. Detailed future investigations concentrating on multiple pathways of Alzheimer's disease, such as the efficacy of monoterpenes in comparison to AD medications in well-established AD models, will provide more insight and supporting data for these substances as possible treatments. More pre-clinical research is needed to completely investigate the efficacy of bornyl acetate and menthol as potent drugs and recommend them as conventional AD therapies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s12035-025-05333-2.

Author Contribution Mahalakshmi Krishnan conceptualized the hypothesis, compiled the protocols and performed all methodologies and accumulated the data, Sangeetha Ravi, Livya Catherene Martin and Manikandan Kumaresan, assisted in data curation and Dr. Beulaja Manikandan and Dr. Thiagarajan Raman helped in literature writing and revising and correcting the manuscript. Dr. Manikandan Ramar provided resources, supervision and validation for the research work. The manuscript has been read and approved for submission by all the authors.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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