Scopoletin Ameliorates Lipopolysaccharide Induced Neuroinflammation, Oxidative Stress and Cognitive Dysfunction in Mice: A Mechanistic Study

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Authors' contributions

Article Information

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ABSTRACT

Background: Alzheimer's disease (AD), a progressive neuropsychiatric disorder, is characterized by neuroinflammation, β-amyloid plaques and neurofibrillary tangles. It has been demonstrated that systemic inflammation and neuroinflammation caused by lipopolysaccharide (LPS) injections in rodents leads to elevation in Aβ levels and neuronal cell death, finally resulting in cognitive impairment.
Methods: Animals received the experimental compound – Scopoletin (2.5, 5 or 10 mg/kg, i.p) for 31 days. Initially for 14 days, they received only the experimental drug; thereafter for the next 7 days they received LPS (0.25 mg/kg i.p) along with the experimental molecules.
Treatment with the experimental molecules was continued for the next 10 days and behavioural tests were performed. Animals were sacrificed on day 32 and biochemical tests were performed on brain tissue homogenate.

Results: Scopoletin administration improved memory and cognition as indicated by reduction of escape latency in Morris water maze test and increased number of entries in Y maze test. Malondialdehyde was significantly increased after LPS administration and reduced glutathione, catalyse and superoxide dismutase were significantly reduced in mice brains. Treatment with scopoletin significantly reduced oxidative stress. Inflammatory markers. β-secretase and apoptotic markers were also markedly reduced.

Conclusion: At medium and high dose, Scopoletin showed promise response as a prophylactic entity in the LPS induced neuroinflamation and cognitive dysfunction model in mice.

1. INTRODUCTION

Alzheimer's disease (AD), a progressive neurodegenerative disease associated with hallmarks such as accumulation of β-amyloid plaques, deposition of neurofibrillary tangles composed of hyperposphorylated, and neuroinflammation was described by Alios Alzheimer more than 100 years ago. The autopsy performed by Alios Alzheimer, in 1906, on the brain of late Auguste Deter, a female who presented with abnormal behaviours such as short-term amnestic disorder, disorientation and dysphrasia, and whose symptoms had been studied for many years by Alzheimer, revealed significant cerebral atrophy, abnormality in the regions of the brain that were considered responsible for judgement, language and memory; intraneuronal plaques and tangles in nerve fibres were detected [1-3]. The number of cases of AD globally are estimated to increase to 106.8 million by 2050 [4]. According to a systematic analysis conducted by Global Burden of Diseases, Injuries, and Risk Factors Study (GBD) Dementia Collaborators [5], the global burden of dementia was 20.2 million in 1990 and it rose to 43.8 million by 2016; women are affected to a greater extent compared to men. In 2016, dementia was the fifth leading cause of death, claiming 2.4 million lives worldwide [5]. In 2020, the estimated economic burden of AD was $305 billion and it is predicted to rise to more than $1 trillion by 2050 [6]. The interventions that are in clinical practice today, only provide symptomatic relief by counterbalancing the neurotransmitter disturbances; three of these molecules are Acetylcholineterase inhibitors and have been approved for the indication mild to moderate AD, whereas, memantine is an NMDA receptor noncompetitive antagonist that is indicated for moderate to severe AD [7,8]. Extensive research is being carried out to identify disease-modifying drugs that will be able to halt the progression of the disease by impeding an essential characteristic process in the pathophysiology leading to clinical symptoms [9,10].

Compiling evidence suggested that immune responses play a major role in the pathogenesis of AD, and that AD is not a disease that is only restricted to the neuronal system. Understanding the interactions of these two key systems may help us in preventing or delaying the progression of the disease. In AD, neuro inflammation is not merely a beholder that is triggered by deposition of plaques or neurofibrillary tangles, but actually contributes equally, if not more, to the pathogenesis of AD; TREM2 and CD33, genes for immune receptors, have recently been implicated in the development of AD [11-14]. Recent reports suggest that lipopolysaccharide (LPS), a gram-negative endotoxin has been detected in AD affected brain tissues [15,16]. LPS level in blood of AD patients has been found to be 3-fold the levels in control subjects [17]. Additionally, LPS has been found to be co-localized with amyloid plaques, peri-vascular amyloid, neurons, and oligodendrocytes in AD brains [18]. Zhao et al have shown that LPS injection stimulates microglia via the NF-κB signaling pathway leading to increase in brain Aβ levels and neuronal cell death [19].

Scopoletin (SCP) has been shown to possess anti-amnesic activity comparable to that of donepezil in scopolamine induced amnesia model in mice [20]. It has also been proven to restore the disruption of memory induced by administration of the anticholinergic agent scopolamine, when administered i.c.v., suggesting a potent ameliorating action on the impaired cholinergic transmission [21]. However, its potential activity in AD has not been evaluated. A drug that enhances cholinergic neurotransmission and exerts antioxidative and anti-inflammatory actions [22] would be highly desirable as a therapeutic for neurodegenerative diseases such as Alzheimer's dementia (AD).

The aim of this study was to evaluate the prophylactic effect of Scopoletin, 6-methoxy-7-hydroxycoumarin, in LPS induced neuroinflammation and cognitive impairment in mice. The doses selected for evaluation i.e. 2.5, 5, or 10 mg/kg were based on experimental results obtained by Malik et al[20] and Chang et al [23].

2. MATERIALS AND METHODS

2.1 Animals

Swiss albino mice weighing 25-35 g were procured from the National Institute of Biosciences, Pune. Mice were housed in polypropylene cages in a temperature and humidity-controlled environment under a 12 hours light-dark cycle. Food and water were provided *ad libitum*. Experimentation was started after a 7-days acclimatisation period. Behavioural experiments were carried out between 10 a.m. and 4 p.m.

2.2 Experimental Design and Drug Administration

A total of 30 mice were divided into 5 groups (each with n = 6). They received saline or SCP (2.5, 5 or 10 mg/kg i.p) totally for 31 days. Initially for 14 days, they received the experimental drug alone; thereafter for the next 7 days, they received LPS (0.25 mg/kg i.p) along with the SCP. Treatment with the SCP was continued for the next 10 days and behavioural tests were performed. The experimental scheme has been described in Fig. 1.

2.3 Behavioural Evaluation

2.3.1 Morris water maze (MWM) test

The morris water maze test, which is a commonly used test to evaluate AD-like dysfunction was used to study hippocampal spatial memory impairment [24,25]. This test is designed in such a way that the mice swim in a circular pool containing an opaque fluid until they find the hidden platform, which is the only safe place in the pool. The apparatus consisted of a circular pool (122 cm in diameter, 50 cm in height, and filled to a depth of 30 cm with opaque water [prepared by dissolving milk powder in water]) and a hidden platform (10 ×10 cm^2) was placed in the pool such that its surface was 1 cm below the surface of opaque water. Bright coloured clues were placed around the pool and the placing was such that they would be visible to the mouse from the pool. This was done to provide spatial clues to the mice, so that the mice will be able to locate the platform easily when the mice would be exposed to the same challenge the next time. The experimental setup was maintained exactly identical during the training and evaluation challenges; the environmental conditions and spatial clues were uniformly maintained. Two perpendicular threads were secured firmly on the circumference of the pool in order to divide the pool into 4 equal quadrants, such that the threads would form a '+' and the pool would be divided; this was done so that the animal could be placed in a random quadrant during training and evaluation challenges. The hidden platform was placed in the third quadrant throughout the training and evaluation process. During the training phase, mice were placed randomly in one of the other three quadrants with their face towards the wall of the circular pool. The mice were to swim through the pool and identify the safe place i.e. the hidden platform. The maximum time allowed for finding the hidden platform was 120s. If the mouse failed to find the platform, he was gently guided towards the platform. The mouse was allowed to stay on the platform for 30s in order to explore the spatial clues. Four consecutive training sessions were carried out at an interval of 30 min between training sessions for four days. Escape latency (time taken to locate the hidden platform) was recorded subsequently.
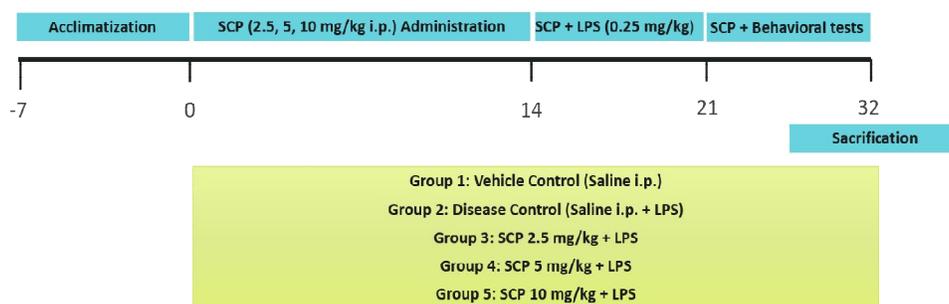

Fig. 1. Experimental groups and study design

2.3.2 Y maze task [26]

The apparatus consisted of three identical arms of equal size-the first arm, the familiar arm and the novel arm. The animal was always placed in the first arm which was always open. The familiar arm was also always open for the animal to explore. The novel arm was blocked during the training trail but was open during the evaluation phase. The training trial was carried out and then after an hour, the evaluation trial was carried out. In the training trial, the novel arm was blocked, and the animal was allowed to explore the start arm and the familiar arm for a period of 10 min. After an hour, the novel arm was unblocked, and the animal was placed in the start arm. The animal was free to explore all the three arms for 5 min. The number of entries in the novel arm was recorded. A novel arm 'entry' was counted when all four limbs of the animal were in the novel arm.

2.4 Biochemical Evaluation

2.4.1 Brain tissue processing for biochemical evaluation

Mice were euthanized by CO_2 overdose on the 32nd day and perfused with ice-cold 0.1 M phosphate buffer (pH 7.4). The brains were isolated and weighed, and 10% tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 4000 rpm for 10 min and the supernatant was stored at -80°C until further use.

2.4.2 Determination of lipid peroxidation

Free radical attacks on polyunsaturated fatty acids leads to formation of reactive electrophilic aldehydes such as malondialdehyde (MDA). This process of lipid peroxidation leads to neuronal damage. The content of MDA in the supernatant was assayed in the form of Thiobarbituric acid reactive substance (TBARS) by the method described by Niehaus and Samuelsson, 1968. Reaction of thiobarbituric acid (TBA) with MDA produces a red complex which has a peak absorbance of 535 nm. 0.1 ml of the supernatant was treated with 2 ml of TBA-TCA-HCl (1:1:1) reagent (0.37% thiobarbituric acid, 0.25 N HCl and 15% TCA) and heated on a boiling water bath for 15 min. It was cooled and centrifuged at 4000 rpm for 10 min at room temperature and the absorbance of the clear supernatant was measured at 535 nm using a spectrophotometer

[27]. The MDA content was determined by extrapolating from the standard curve.

2.4.3 Determination of Reduced glutathione (GSH) concentration

GSH is a potent antioxidant that scavenges free radicals and protects the cells from their damaging effects. The method described by Moron et al. 1979 was followed [28]. 0.4 ml of the supernatant was precipitated with an equal quantity of 20% TCA. This mixture was then centrifuged at 10,000 rpm at 4ºC for 20 min. From the resultant supernatant 0.25 ml was mixed with 2 ml of 0.6 mM DTNB reagent and the final volume was made up to 3ml with 0.2 M phosphate buffer. The absorbance of the yellow product that was formed in-situ was measured at 412 nm in a spectrophotometer.

2.4.4 Determination of Catalase (CAT) activity

According to the method described by Aebi, 1984 [29], decomposition of H_2O_2 was followed by measuring the change in absorbance at 240 nm. In this assay, 0.1 ml of the supernatant was mixed with 0.8 ml of phosphate buffer (50 mM, pH 7.0) and 0.1 ml of 0.02% Triton X-100. This mixture was incubated for 10 min at room temperature. This mixture was added to 2.0 ml of 0.03M H_2O_2 and the change in absorbance was monitored every minute for five minutes at 240 nm. The activity was expressed as micromole H_2O_2 decomposed/min/mg protein.

2.4.5 Determination of Superoxide dismutase (SOD) activity

SOD activity was measured based on the method described by Marklund and Marklund, 1974 [30]. The principle of this method was based on the ability of SOD to prevent the auto-oxidation of pyrogallol. In this assay, 10 µl of tissue homogenate, 180 µl of phosphate buffer, and 10 µl of pyrogallol were added to each well of a 96-well microplate. The absorbance was read at 325 nm for 5 min. For control solution, the tissue homogenate was replaced with distilled water. One-unit activity of SOD activity was defined as 50% inhibition of autoxidation of pyrogallol.

2.4.6 Determination of Acetylcholine esterase (AChE) inhibitory activity

The assay method described by Ellman et al., 1961 was followed [31]. AChE hydrolyses

acetylthiocholine iodide to form choline iodide. Choline iodide reacts with DTNB and produces TNB (yellow colored compound). Thus, the quantity of choline iodide produced is an indicator of AChE activity. Briefly, 40 µl of supernatant was mixed with 2.6 ml of 0.1 M phosphate buffer and 100 µl of DTNB n (39.6 mg DTNB and 15 mg of NaHCO3 dissolved in 10 mL of 0.1 M phosphate buffer). To this reaction mixture, 20 µl of 75 Mm acetylthiocholine iodide was added and the change in absorbance was monitored per minute over a period of 5 minutes. Acetylcholinesterase activity was calculated using formula [32];

$$R = 5.74 \times 10^{-4} \times A / CO$$

Where,

R-Rate in moles of substrate hydrolyzed/min/g of brain tissue
A-Change in absorbance/min.
CO-Original concentration of the tissue (mg/ml).

2.4.7 Determination of inflammatory cytokines (TNF-alpha and IL-6) by ELISA

Content of TNF-alpha in the brain tissue supernatants was determined by using commercially available ELISA kit (Krishgen biosystems). Results were expressed as pg/mg protein.

2.4.8 Determination of Caspase-3 by ELISA

Caspase-3 activation has been demonstrated as an early neurodegenerative event in the progression of AD; Caspase-3 is the primary executioner of apoptosis. Numerous studies have presented elevated level of caspase-3 in AD. Caspase-3 level in the brain homogenate were estimated using commercially available ELISA kit (Bioassay Technology Laboratory)

2.4.9 Determination of NF-κB by ELISA

To determine the mechanistic pathway by which LPS induces pathological changes and to study the mechanism of action of the experimental molecules, NF-kB levels in the brain homogenate were estimated using commercially available ELISA kit (Bioassay Technology Laboratory)

2.4.10 Determination of BACE-1 (β-secretase) by ELISA

BACE-1 activity was evaluated using commercially available ELISA kit (Bioassay Technology Laboratory)

2.4.11 Total protein

Protein estimation was carried out by using Bovine serum albumin (BSA) as the standard and
extrapolating the values from the standard curve.

2.4.12 Statistical analysis

The data obtained was analyzed statistically using analysis of variance (ANOVA) followed by Tukey's Multiple Comparison Test. P<0.05 is considered as significant. The analysis was performed using Graph Pad Prism 5.0. U.S.A.

3. RESULTS AND DISCUSSION

3.1 Effect of SCP on Escape Latency in Morris Water Maze Test

MWM task is a standard behavioral tool designed to access spatial learning and memory. Over the course of training, animals learned to find the hidden platform; animals in the disease control group took significantly longer to find the hidden platform (escape latency) than animals in the vehicle control group. Administration of SCP showed dose-dependent effect on escape latency and animals could find the platform in a shorter span of time (Fig. 2).

3.2 Effect of SCP on Number of Entries in Novel Arm in Y Maze Test

The number of entries in the novel arm significantly reduced in the disease control group; this effect was significantly (p<0.001) reversed by administration of high dose of SCP (Fig. 3).

3.3 Effect of SCP on Lipid Peroxidation

Administration of LPS significantly increased (p<0.001) MDA level, a lipid peroxidation biomarker, in the brain of mice. Treatment with SCP reduced MDA level in a dose dependant manner (Fig. 4).

3.4 Effect of SCP on GSH Content

GSH, a major free radical scavenger in the brain, was reduced significantly after LPS administration. SCP significantly improved (p<0.01) GSH level in the brain at the highest dose administered (Fig. 5).
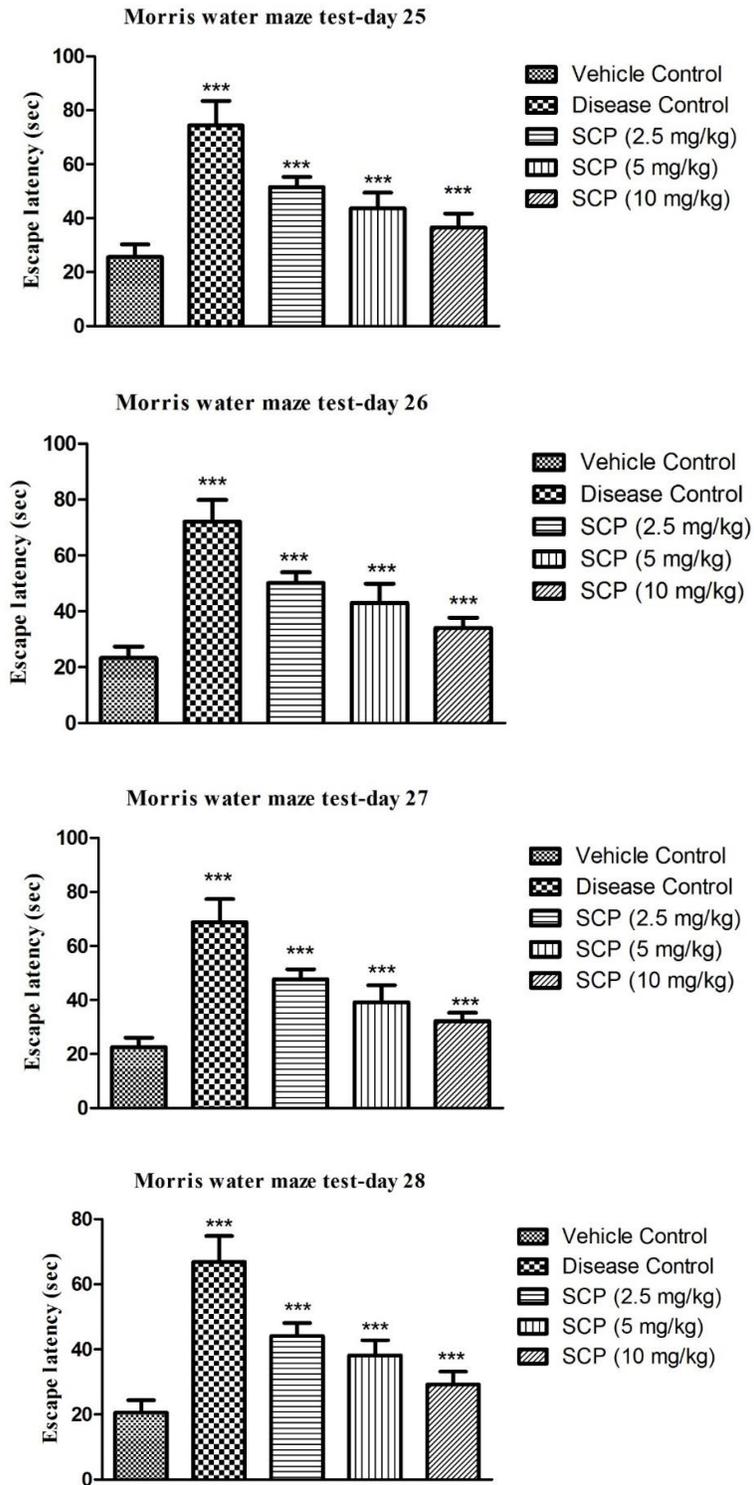
Fig. 2. Effect of SCP on escape latency in MWM test on day 25, 26, 27, and 28
*Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p< 0.001*

Fig. 3. Effect of SCP on number of entries in novel arm in Y test
*Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p< 0.001, ns=not significant*
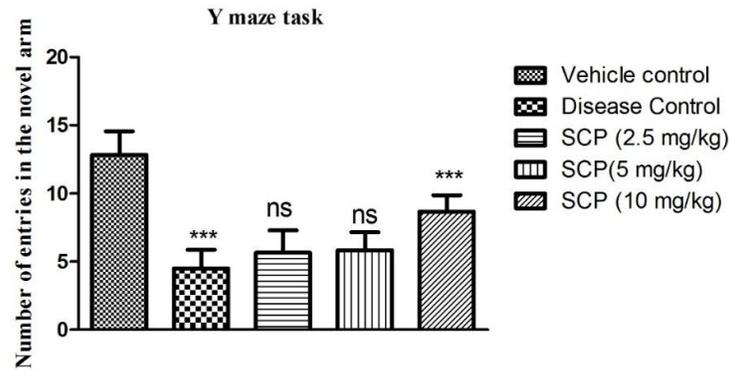

Fig. 4. Effect of SCP on lipid peroxidation
*Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p< 0.001, ns=not significant*
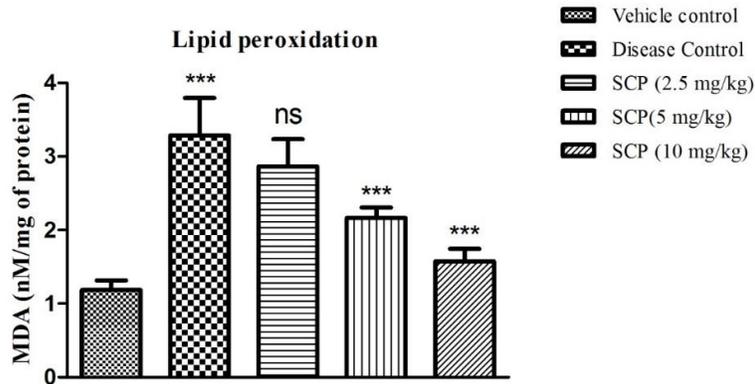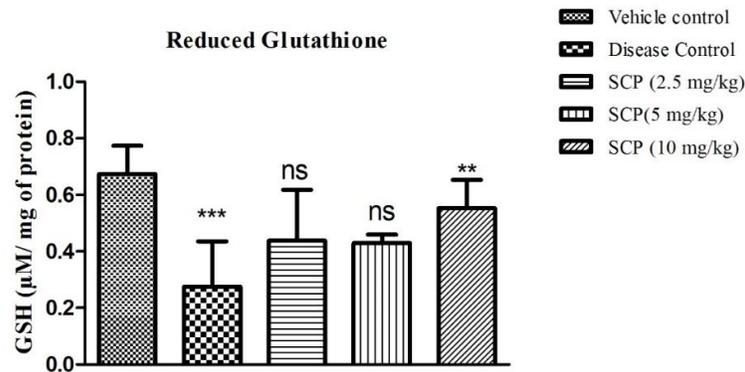

Fig. 5. Effect of SCP on GSH
*Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p< 0.001, **p< 0.01, ns=not significant*

3.5 Effect of SCP on CAT Activity

Catalase, an enzyme involved in preventing cellular oxidant damage by metabolizing hydrogen peroxide to water and oxygen, was significantly reduced after LPS administration. There was a significant ($***p<0.001$, $**p<0.01$) dose-dependent increase in catalase activity following the administration of SCP (Fig. 6).

3.6 Effect of SCP on SOD Activity

The activity of SOD, the first detoxification enzyme and most powerful antioxidant in the cell, was significantly reduced in the brain of mice post the administration of LPS. All the three doses of SCP significantly ($p<0.001$) improved the SOD activity all the tested dose levels (Fig. 7).
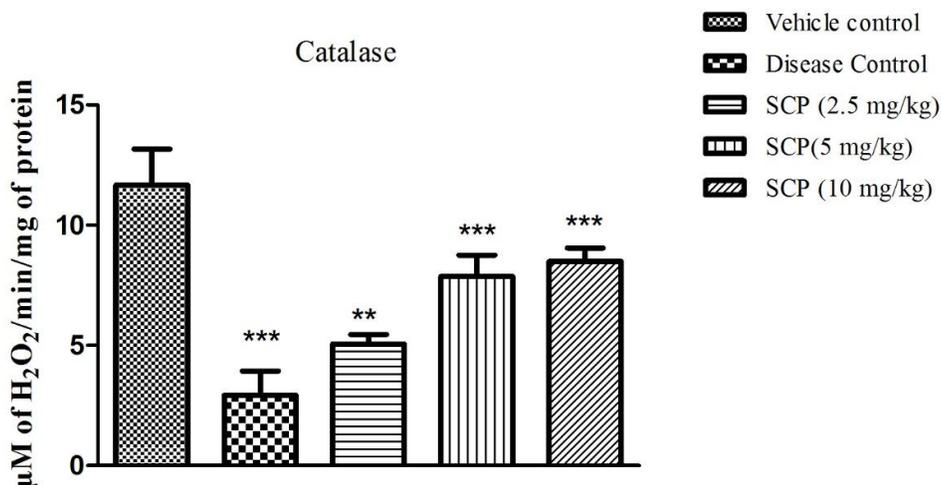

Fig. 6. Effect of SCP on CAT activity
*Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p< 0.001, **p< 0.01*
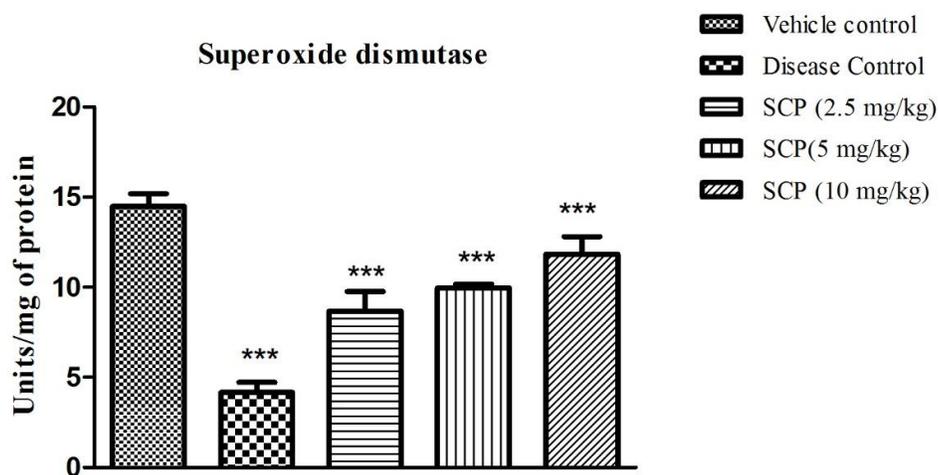

Fig. 7. Effect of SCP on SOD activity
*Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p<0.001*

3.7 Effect of SCP on AChE Activity

Acetylcholinesterase, the enzyme responsible for the breakdown of acetylcholine, was significantly inhibited ($p<0.001$) by SCP at all the administered doses (Fig. 8).

3.8 Effect of SCP on TNF-α and IL-6 level

TNF-α, the multifunctional cytokine, was significantly elevated after LPS administration; this elevation was dose dependently alleviated (*** $p<0.001$) by administration of SCP at medium and high dose. Brain level of the proinflammatory cytokine IL-6 was significantly increased after LPS administration. SCP administration significantly reduced (*** $p<0.001$, *$p<0.05$) IL-6 level at all studied dose levels (Fig. 9).

3.9 Effect of SCP on Caspase-3 Level

Caspase-3, a critical executioner of apoptosis, was significantly elevated in the disease control group. SCP administration caused a significant (*** $p<0.001$, *$p<0.05$) reduction in the apoptotic marker level (Fig. 10).

3.10 Effect of CSP on NF-κB Level

Brain level of NF-κB, a mediator of proinflammatory gene induction, was significantly increased after LPS injection. SCP administration reduced NF-κB level in a doses dependant manner at all three dose levels (Fig. 11).

3.11 Effect of CSP on BACE-1 Activity

BACE-1, the enzyme involved in abnormal APP processing, was significantly increased after administration of LPS. High-dose SCP significantly ($p<0.001$) inhibited BACE-1 (Fig. 12).

The pathophysiology of AD has been shown to encompass immunological responses, and brains of AD patients have shown chronic inflammation. It has been hypothesised that contribution of inflammation in AD pathophysiology starts decades before the clinical onset and cognitive decline. Also, there is a strong association between Aβ accumulation, tau hyperphosphorylation and neuroinflammation [33-41]. In this study, we examined the pharmacological effect of SCP at three dose levels in LPS induced neuroinflammation and cognitive impairment in mice. Administration of LPS resulted in increase in escape latency in MWM task, whereas it lead to a significant reduction in number of entries in novel arm in Y maze test. MWM task is a standard behavioral tool designed to access spatial learning and memory. Over the course of training, animals learned to find the hidden platform; animals in the disease control group took significantly longer to find the hidden platform (escape latency) than animals in the vehicle control group. This finding was consistent with the previously reported effect of LPS on learning and memory [42]. Administration of SCP showed dose-dependent effect on escape latency and animals could find the platform in a shorter span of time. The Y maze test novel arm preference test is a widely-accepted test to study hippocampus-dependant spatial memory; mice with special deficits tend to enter the novel arm less frequently in comparison to normal mice. We observed that mice receiving the highest dose of SCP entered the novel arm more frequently that the mice in the disease control group, thus indicating that SCP has a positive effect on spatial memory [43-45]. The brain is extremely sensitive to oxidative stress since it utilizes 20-30% of the inspired oxygen. Previous studies have demonstrated that i.p. injection of LPS caused significant changes in oxidative stress markers in the brain of mice [46]. Moreover, the brain contains high levels of redox transition metals and polyunsaturated fatty acids (PUFA). Lipid peroxidation, an early event in the progression of AD, is a major source of free-radical mediated injury that leads to neuronal membrane damage and yields a number of secondary products that were also in turn responsible for extensive cellular damage [47]. Free radical attack to PUFAs leads to the formation of highly reactive electrophilic aldehydes such as malondialdehyde (MDA) which is used as a marker of oxidative stress [48]. SCP administration (medium and high dose) significantly lowered brain MDA level. Diminished GSH levels make cells more vulnerable to oxidative stress. Post-mortem studies of AD brains have shown diminished GSH levels and increased glutathione disulphide (GSSG) content in patients with mild cognitive impairment, as mild/moderate and severe AD patients compared to controls. Similarly in proton magnetic resonance spectroscopy studies, reduction in in vivo GSH levels have been observed in AD patients; the reduction has mainly been observed in frontal cortex and hippocampus i.e. regions of the brain that show pathological changes in AD.
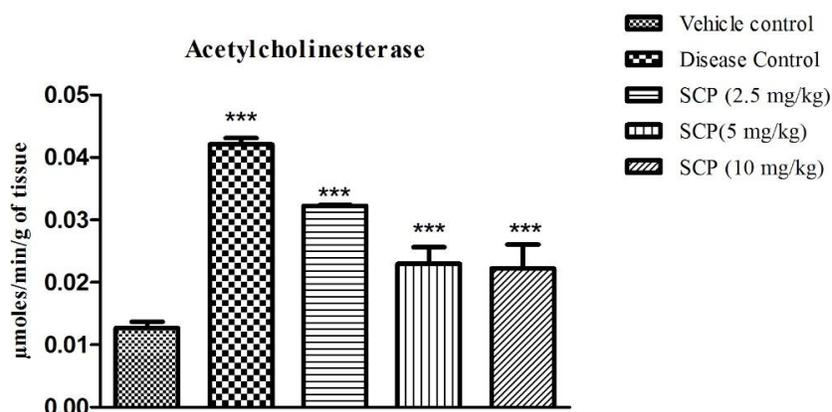
Fig. 8. Effect of SCP on AChE activity
*Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p< 0.001*
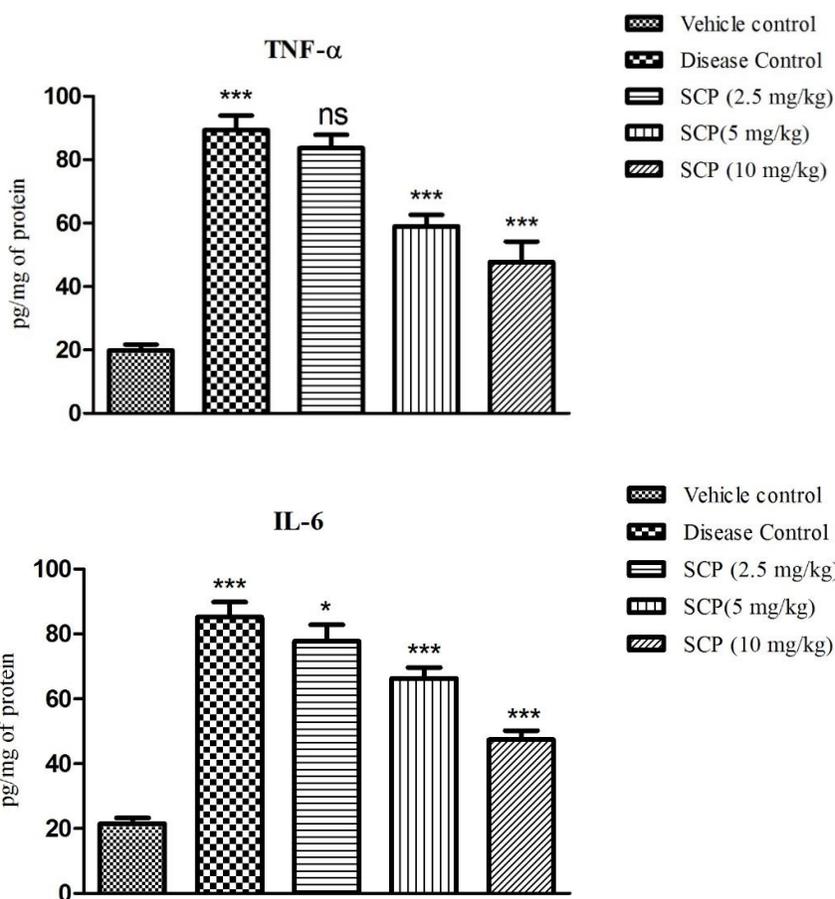

Fig. 9. Effect of SCP on proinflammatory cytokines TNF-α and IL-6 level
*Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p< 0.001, *p< 0.05, ns=not significant*
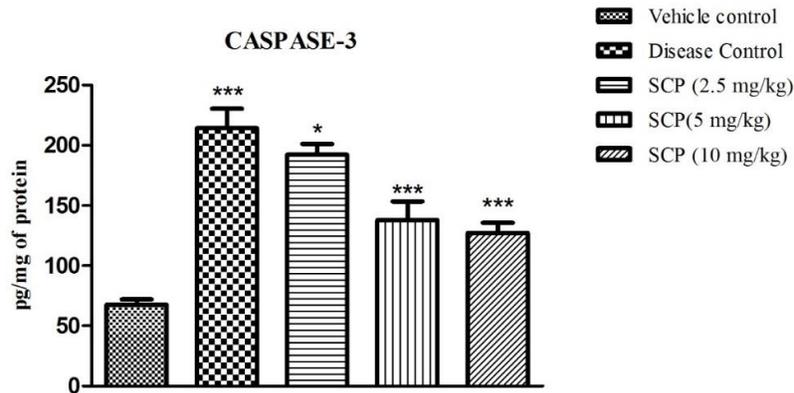
Fig. 10. Effect of SCP on Caspase-3 level
Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p< 0.001, *p< 0.05

Fig. 11. Effect of SCP on NF-κB level
Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p<0.001, **p<0.01

Fig. 12. Effect of SCP on BACE-1 activity
Results are represented as Mean± S.D (n = 6). Disease control group has been compared with the vehicle control group; all treatment groups have been compared with disease control. *** p< 0.001, ns=not significant
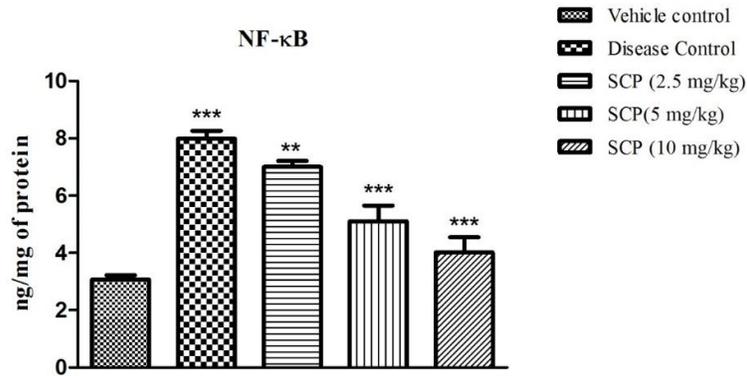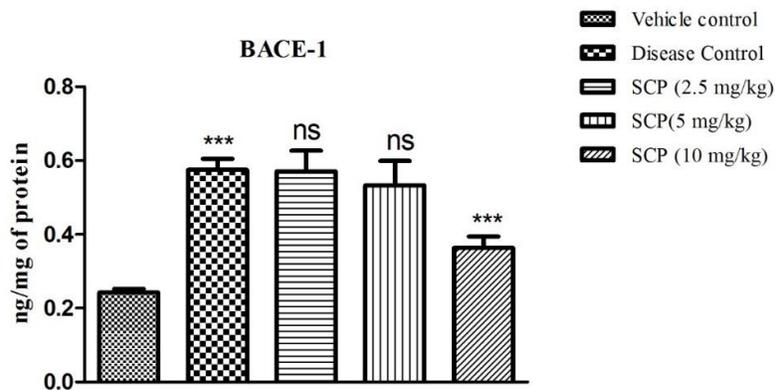
LPS administration diminished GSH level in disease control mice brains. This effect was significantly reversed in mice receiving high dose of SCP [49-51]. Similarly, the activity of SOD, the first detoxification enzyme and most powerful antioxidant in the cell, was significantly reduced in the brain of mice post the administration of LPS; SOD levels were significantly rejuvenated after administration of each of the SCP doses. Clinically, it has been observed that in post-mortem AD brains, catalase level is significantly reduced [52]. Administration of LPS significantly reduced catalse level, and this effect was reversed by prophylactic administration of SCP at all three doses. The pathogenesis of AD has been associated with reduced level of the brain neurotransmitter acetylcholine. Subsequently, acetylcholinesterase inhibitors (AChEIs) were introduced for the symptomatic treatment of AD and are the mainstay of symptomatic AD management. The current opinion is that the efficacy of AChE inhibitors is due to the improvement of acetylcholine-medicated neuron to neuron transmission. However, AChE inhibitors also protect cells from free radical toxicity and β-amyloid-induced injury and increased production of antioxidants. It has also been shown that AChE inhibitors directly inhibit the release of cytokines from microglia and monocytes. These observations indicate a role of acetylcholine in suppression of cytokine release through a 'cholinergic anti-inflammatory pathway' [53]. Significant ACHE inhibitory activity of SCP was observed *in vivo*. The exact pathophysiology of AD is still unknown; however, mounting evidence suggests the role of inflammation in neurodegenerative diseases, specially AD [54-56]. It has been suggested that accumulation of Aβ leads to the activation of caspases and signal-dependent transcription factors (NF-kB and AP-1) which cause regulation of inflammation amplifiers (e.g., IL-1β, TNF-α, IL-6); these proinflammatory cytokines may directly act on neurons to cause apoptosis [57,58] or activate astrocytes, and these factors released from astrocytes possess the capacity to further activate microglia [59]. Studies have demonstrated that TNF-α triggers cytotoxic cascades and apoptotic cell death; Aβ causes neuronal cell death via TNF-α-mediated signalling. Moreover, it also effects learning and memory processes by disrupting synaptic plasticity and has detrimental effects on synaptic transmission and plasticity [60,61]. There was a significant reduction in expression of proinflammatory cytokines in SCP-administered mice brain as compared to disease control.

Caspase-3 is a principal cell death protease involved in neuronal apoptosis during physiological development and under pathological conditions. It has been shown that wild-type Amyloid precursor protein (APP) is an intrinsic activator of caspase-3-mediated death machinery in postmitotic neurons [62]. SCP administration diminished caspase-3 level at all three test doses. APP, presenilin (a component of γ secretase) and BACE1 (β-secretase) have NF-kB sites in their promoters, and thus their expression is governed by NF-kB; Sastre et al have demonstrated that proinflammatory cytokines upregulate the expression of these factors in neurons [63]. Inflammatory mediators acting on neurons increase production of Aβ, further activating microglia-mediated inflammation. Thus, neurons and glia communication may amplify the production of hallmark AD pathological factors. Guzman-Martinez et al have hypothesized that during the course of AD, damage signals activate glial cells leading to NF-κB activation and synthesis and release of proinflammatory cytokines that affect neuronal receptors with an overexpression of protein kinases [64]. Furthermore, NF-kB, a pleiotropic transcription factor, regulates several other genes that are involved in inflammatory responses and stimulates numerous cellular signalling pathways, which leads to the increased production of inflammatory cytokines. The LPS-induced NF-kB activation in glia has been reported to upregulate the expression of inflammatory mediators [65,66]. SCP administration significantly lowered brain NF-κB level. BACE1, the β-secretase enzyme, cleaves the APP and releases a C99 fragment which gives rise to various species of Aβ peptide upon further processing by γ-secretase. BACE inhibition will result in an upstream interference with the amyloid cascade. Several studies report that BACE1 inhibitors hold great potential as a target strategy to reduce Aβ brain concentrations, thus preventing the progression of AD [67,68]. High dose of SCP demonstrated significant BACE-1 inhibitory effect.

4. CONCLUSION

In summary, intraperitoneal injection of LPS lead to cognitive and memory impairment in behavioral tests, and an increase in pro-inflammatory cytokines (TNF-α and IL-6) via the activation of NF-κB signaling pathway, oxidative stress (increase in MDA; reduction in SOD, GSH, and CAT), upregulation of AChE, cell death (caspase-3), and BACE-1 activity. Our findings

revealed that SCP attenuated oxidative stress, inflammation and degeneration in a dose-dependent manner. Further, it is important to study the activity of SCP in other chemically induced animal models. Catalase, an enzyme involved in preventing cellular oxidant damage by metabolizing hydrogen peroxide to water and oxygen, was significantly reduced after LPS administration.

5. FUNDING

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by University Grants Commission.

CONSENT

It's not applicable.

ETHICAL APPROVAL

Approval for animal experimentation was obtained from Institutional Animal Ethics Committee of T. N. Medical College and BYL Nair Charitable Hospital, Mumbai (Protocol No. IAEC/4A dated 29th February 2016) and all the experimentation was carried out in accordance with the Committee for Control and Supervision of Experimentation on Animals (CPCSEA) guidelines.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. DeTure MA, Dickson DW. The neuropathological diagnosis of Alzheimer's disease. Mol Neurodegeneration. 2019;14:32 (2019). DOI:10.1186/s13024-019-0333-5

2. Apostolova LG. Alzheimer Disease. Continuum (Minneap Minn). 2016;22(2 Dementia):419-434. DOI:10.1212/CON.0000000000000307

3. Yang HD, Kim DH, Lee SB, Young LD. History of Alzheimer's Disease. Dement Neurocogn Disord. 2016;15(4):115-121. DOI:10.12779/dnd.2016.15.4.115

4. Bird TD, Miller BL. Alzheimer's disease and primary dementias. In: Harrison's Principles of Internal Medicine, 17th ed. New York: McGraw–Hill. 2008;2393–2406.

5. GBD. Dementia Collaborators. Global, regional and national burden of Alzheimer's disease and other dementias, 1990–2016: A systematic analysis for the Global Burden of Disease Study 2016. The Lancet Neurology. 2019;18(1):88-106. DOI:https://doi.org/10.1016/S1474-4422(18)30403-4.

6. Deb A, Thornton JD, Sambamoorthi U, Innes K. Direct and indirect cost of managing Alzheimer's disease and related dementias in the United States. Expert Rev Pharmacoecon Outcomes Res. 2017;17(2):189-202. DOI:10.1080/14737167.2017.1313118 PMID: 28351177; PMCID: PMC5494694.

7. Birks J. Cholinesterase inhibitors for Alzheimer's disease. Cochrane Database Syst Rev. 2006;(1):CD005593. DOI: 10.1002/14651858.CD005593 PMID: 16437532.

8. McShane R, Areosa Sastre A, Minakaran N. Memantine for dementia. Cochrane Database Syst Rev. 2006;(2):CD003154. DOI: 10.1002/14651858.CD003154.pub5

9. Galimberti D, Scarpini E. Disease-modifying treatments for Alzheimer's disease. Ther Adv Neurol Disord. 2011;4(4):203-216. Galimberti D, Scarpini E. Disease-modifying treatments for Alzheimer's disease. Ther Adv Neurol Disord. 2011;4(4):203-216. DOI:10.1177/1756285611404470

10. Golde TE. The Abeta hypothesis: Leading us to rationally-designed therapeutic strategies for the treatment or prevention of Alzheimer disease. Brain Pathol. 2005;15(1):84-7. DOI:10.1111/j.1750-3639.2005.tb00104.x PMID: 15779241.

11. Zhang B, Gaiteri C, Bodea LG, Wang Z, McElwee J, Podtelezhnikov AA et al. Integrated systems approach identifies

genetic nodes and networks in late-onset Alzheimer's disease. Cell. 2013;153:707–20.
DOI:10.1016/j.cell.2013.03.030.
PMID: 23622250;
PMCID: PMC3677161.

12. Guerreiro R, Wojtas A, Bras J, Carrasquillo M, Rogaeva E, Majounie E et al. TREM2 variants in Alzheimer's disease. N Engl J Med. 2013;368:117–27.
DOI:10.1056/NEJMoa1211851.
Epub 2012 Nov 14.
PMID: 23150934;
PMCID: PMC3631573.

13. Bradshaw EM, Chibnik LB, Keenan BT et al. CD33 Alzheimer's disease locus: Altered monocyte function and amyloid biology. Nat Neurosci. 2013;16:848–50.

14. Griciuc A, Serrano-Pozo A, Parrado AR, Ottoboni L, Raj T, Tang A et al. Alzheimer's disease risk gene CD33 inhibits microglial uptake of amyloid beta. Neuron. 2013;78:631–43.
DOI:10.1038/nn.3435.
Epub 2013 May 23.
PMID: 23708142;
PMCID: PMC3703870.

15. Bhattacharjee S, Lukiw WJ. Alzheimer's disease and the microbiome. Front. Cell. Neurosci. 2013;7:153.
DOI: 10.3389/fncel.2013.00153

16. Foster JA, Lyte M, Meyer E, Cryan JF. Gut Microbiota and Brain Function: An evolving field in neuroscience. Int J Neuropsychopharmacol.
2016;19(5):pyv114.
DOI:10.1093/ijnp/pyv114.
PMID: 26438800;
PMCID: PMC4886662.

17. Zhang R, Miller RG, Gascon R, Champion S, Katz J, Lancero M et al. Circulating endotoxin and systemic immune activation in sporadic amyotrophic lateral sclerosis (sALS). J Neuroimmunol. 2009;206(1-2):121-124.
DOI:10.1016/j.jneuroim.2008.09.017

18. Zhan X, Stamova B, Jin LW, DeCarli C, Phinney B, Sharp FR. Gram-negative bacterial molecules associate with Alzheimer disease pathology. Neurology. 2016;87(22):2324-2332.
DOI:10.1212/WNL.0000000000003391.
Epub 2016 Oct 26.
PMID: 27784770.
PMCID: PMC5135029.

19. Zhao J, Bi W, Xiao S, Lan X, Cheng X, Zhang J. Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. Sci Rep. 2019;9:5790.
DOI:10.1038/s41598-019-42286-8.

20. Malik J, Karan M, Vasisht K. Attenuating effect of bioactive coumarins from Convolvulus pluricaulis on scopolamine-induced amnesia in mice. Nat Prod Res. 2016;30(5):578-82.
DOI: 10.1080/14786419.2015.1025398.
Epub 2015 Apr 1.
PMID: 25828605.

21. Hornick A, Lieb A, Vo NP, Rollinger JM, Stuppner H, Prast H. The coumarin scopoletin potentiates acetylcholine release from synaptosomes, amplifies hippocampal long-term potentiation and ameliorates anticholinergic- and age-impaired memory. Neuroscience. 2011;197(1):280-92.
DOI:10.1016/j.neuroscience.2011.09.006.
Epub 2011 Sep 10.
PMID: 21945033;
PMCID: PMC3212650.

22. Mogana R, Teng-Jin K, Wiart C. "Anti-Inflammatory, Anticholinesterase and Antioxidant Potential of Scopoletin Isolated from Canarium patentinervium Miq. (Burseraceae Kunth)", Evidence-Based Complementary and Alternative Medicine. Article ID 734824. 2013;7.
DOI:https://doi.org/10.1155/2013/734824

23. Chang TN, Deng JS, Chang YC, Lee CY, Jung-Chun L, Lee MM et al. Ameliorative effects of scopoletin from crossostephium chinensis against inflammation pain and its mechanisms in mice. Evid Based Complement Alternat Med. 2012;2012:595603.
DOI:10.1155/2012/595603.
Epub 2012 Sep 6.
PMID: 22991572.
PMCID: PMC3443580.

24. West MJ. Regionally specific loss of neurons in the aging human hippocampus. Neurobiol Aging. 1993;14(4):287-93.
DOI:10.1016/0197-4580(93)90113-p.
PMID: 8367010.

25. Morris R. Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods. 1984;11(1):47-60.
DOI: 10.1016/0165-0270(84)90007-4.
PMID: 6471907.

26. Sopian NF, Ajat M, Shafie NI, Noor MH, Ebrahimi M, Rajion MA, Meng GY, Ahmad H. Does short-term dietary omega-3 fatty acid supplementation influence brain

hippocampus gene expression of zinc transporter-3? Int J Mol Sci. 2015;16(7):15800-10.
DOI: 10.3390/ijms160715800.
PMID: 26184176.
PMCID: PMC4519926.

27. Niehaus WG Jr, Samuelsson B. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem. 1968;6(1):126-30.
DOI:10.1111/j.1432-1033.1968.tb00428.x
PMID: 4387188.

28. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta. 1979;582(1):67-78.
DOI: 0.1016/0304-4165(79)90289-7
PMID: 760819.

29. Aebi H. Catalase *in vitro*. Methods Enzymol. 1984;105:121-6.
DOI:10.1016/s0076-6879(84)05016-
PMID: 6727660.

30. Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur J Biochem. 1974;47(3):469-74.
DOI:10.1111/j.1432-1033.1974.tb03714.x
PMID: 4215654.

31. Ellman GL, Courtney KD, Andres V Jr, Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol. 1961;7:88-95.
DOI:10.1016/0006-2952(61)90145-
PMID: 13726518.

32. Raju TR, Kutty BM, Sathyaprabha TN, Rao BSS. Assay of acetylcholinesterase activity in the brain. Brain Behav. 2004;42-144.

33. Heneka MT, Carson MJ, Khoury J El, Landreth GE, Brosseron F,BFeinstein DL, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol. 2015;14:388-405.
DOI: 10.1016/S1474-4422(15)70016-5

34. Akiyama H, Barger S, Barnum S, Bradt B, Bauer J, Cole GM, et al. Inflammation and Alzheimer's disease. Neurobiol Aging. 2000;21:383-421.
DOI:10.1016/S0197-4580(00)00124-X

35. Rogers J, Webster S, Lue LF, Brachova L, Civin WH, Emmerling M, et al. Inflammation and Alzheimer's disease pathogenesis. Neurobiol Aging. 1996;17:681686.

DOI:10.1016/0197-4580(96)00115-7

36. McGeer PL, McGeer EG. The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. Brain Res Brain Res Rev.1995;21:195-218.
DOI:10.1016/0165-0173(95)00011-9

37. Brosseron F, Krauthausen M, Kummer M, Heneka MT. Body fluid cytokine levels in mild cognitive impairment and Alzheimer's disease: A comparative overview. Mol Neurobiol. 2014;50:534-44.
DOI:10.1007/s12035-014-8657-1

38. Nordengen K, Kirsebom B-E, Henjum K, Selnes P, Gísladóttir B, Wettergreen M, et al. Glial activation and inflammation along the Alzheimer's disease continuum. J Neuroinflammation. 2019;16:46
DOI:10.1186/s12974-019-1399-2

39. Fan Z, Brooks DJ, Okello A, Edison P. An early and late peak in microglial activation in Alzheimer's disease trajectory. Brain. 2017;140:aww349.
DOI:10.1093/brain/aww349

40. Edison P, Brooks DJ. Role of neuroinflammation in the trajectory of Alzheimer's disease and in vivo quantification using PET. J Alzheimer's Dis. 2018;64:S339-51.
DOI:10.3233/JAD-179929

41. Parbo P, Ismail R, Hansen K V, Amidi A, Mårup FH, Gottrup H, et al. Brain inflammation accompanies amyloid in the majority of mild cognitive impairment cases due to Alzheimer's disease. Brain. 2017;140:2002-2011.
DOI:10.1093/brain/awx120

42. Xu Y, Zhang ZQ, Zhao Y, Dong XW, Wang XY, Zhang LQ. Enhanced effect of guizhi plus Gegen Decoction on learning and memory disorder in LPS induced neuroinflammatory mice. Zhongguo Zhong Xi Yi Jie He Za Zhi. Chinese.2014;34(2):179-84.
PMID: 24672942.

43. Akwa Y, Ladurelle N, Covey DF, Baulieu EE. The synthetic enantiomer of pregnenolone sulfate is very active on memory in rats and mice, even more so than its physiological neurosteroid counterpart: Distinct mechanisms? Proceedings of the National Academy of Sciences of the United States of America. 2001;98:14033-14037.

44. Sanderson DJ, Bannerman DM. The role of habituation in hippocampus-dependent spatial working memory tasks: Evidence

from GluA1 AMPA receptor subunit knockout mice. Hippocampus. 2012;22(5):981-94.
DOI: 10.1002/hipo.20896
Epub 2010 Dec 1.
PMID: 21125585.
PMCID: PMC3490380.

45. Kraeuter AK , Guest PC, Sarnyai Z. The Y-Maze for Assessment of Spatial Working and Reference Memory in Mice Methods Mol. Biol. 2019;1916:105-111.

46. Zhang XY, Cao JB, Zhang LM, Li YF, Mi WD. Deferoxamine attenuates lipopolysaccharide-induced neuroinflammation and memory impairment in mice. J Neuroinflammation. 2015;12:20.
DOI:10.1186/s12974-015-0238-3
PMID: 25644393.
PMCID: PMC4323121.

47. Markesbery WR, Kryscio RJ, Lovell MA, Morrow JD. Lipid peroxidation is an early event in the brain in amnestic mild cognitive impairment. Ann Neurol. 2005;58(5):730-5.
DOI:10.1002/ana.20629
PMID: 16240347.

48. Esterbauer H, Schaur RJ, Zollner H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radic Biol Med. 1991;11(1):81-128.
DOI:10.1016/0891-5849(91)90192-6
PMID: 1937131.

49. Ansari MA, Scheff SW. Oxidative stress in the progression of Alzheimer disease in the frontal cortex. J Neuropathol Exp Neurol. 2010;69(2):155-167.
DOI:10.1097/NEN.0b013e3181cb5af4

50. Karelson E, Bogdanovic N, Garlind A, Winblad B, Zilmer K, Kullisaar T, et al. The cerebrocortical areas in normal brain aging and in Alzheimer's disease: noticeable differences in the lipid peroxidation level and in antioxidant defense. Neurochem Res. 2001;26(4):353-61.
DOI:10.1023/a:1010942929678
PMID: 11495345.

51. Mandal PK, Saharan S, Tripathi M, Murari G. Brain glutathione levels - a novel biomarker for mild cognitive impairment and Alzheimer's disease. Biol Psychiatry. 2015;78(10):702-10. Erratum in: Biol Psychiatry. 2016 Jan 1;79(1):71.
DOI:10.1016/j.biopsych.2015.04.005.
Epub 2015 Apr 14.
PMID: 26003861.

52. Gsell W, Conrad R, Hickethier M, Sofic E, Frölich L, Wichart I, Jellinger K, Moll G, Ransmayr G, Beckmann H, et al. Decreased catalase activity but unchanged superoxide dismutase activity in brains of patients with dementia of Alzheimer type. J Neurochem. 1995;64(3):1216-23.
DOI:10.1046/j.1471-4159.1995.64031216.x.
PMID: 7861154.

53. Tabet N. Acetylcholinesterase inhibitors for Alzheimer's disease: anti-inflammatories in acetylcholine clothing! Age Ageing. 2006;35(4):336-8.
DOI:10.1093/ageing/afl027
PMID: 16788077.

54. Akiyama H. Inflammatory response in Alzheimer's disease. Tohoku J Exp Med. 1994;174(3):295-303.
DOI: 10.1620/tjem.174.295
PMID: 7539162.

55. Cartier L, Hartley O, Dubois-Dauphin M, Krause KH. Chemokine receptors in the central nervous system: role in brain inflammation and neurodegenerative diseases. Brain Res Brain Res Rev. 2005;48(1):16-42.
DOI:10.1016/j.brainresrev.2004.07.021
PMID: 15708626.

56. Wyss-Coray T, Loike JD, Brionne TC, Lu E, Anankov R, Yan F, Silverstein SC, Husemann J. Adult mouse astrocytes degrade amyloid-beta in vitro and in situ. Nat Med. 2003;9(4):453-7.
DOI:10.1038/nm838
Epub 2003 Mar 3.
PMID: 12612547.

57. McCoy MK, Tansey MG. TNF signaling inhibition in the CNS: Implications for normal brain function and neurodegenerative disease. J Neuroinflammation. 2008;5:45.
DOI:10.1186/1742-2094-5-45
PMID:18925972.
PMCID: PMC2577641.

58. Simi A, Tsakiri N, Wang P, Rothwell NJ. Interleukin-1 and inflammatory neurodegeneration. Biochem Soc Trans. 2007;35(Pt 5):1122-6.
DOI:10.1042/BST0351122
PMID: 17956293.

59. Saijo K, Winner B, Carson CT, Collier JG, Boyer L, Rosenfeld MG, Gage FH, Glass CK. A Nurr1/CoREST pathway in microglia and astrocytes protects dopaminergic neurons from inflammation-induced death. Cell. 20093;137(1):47-59.

DOI:10.1016/j.cell.2009.01.03
PMID:19345186.
PMCID: PMC2754279.

60. Cheng X, Shen Y, Li R. Targeting TNF: A therapeutic strategy for Alzheimer's disease. Drug Discov Today. 2014;19(11):1822-1827.
DOI:10.1016/j.drudis.2014.06.02
Epub 2014 Jul 4.
PMID: 24998784.

61. Li DH, Havell EA, Brown CL, Cullen JM. Woodchuck lymphotoxin-alpha - beta and tumor necrosis factor genes: Structure, characterization and biological activity. Gene. 2000;242:295-305.

62. Uetsuki T, Takemoto K, Nishimura I, Okamoto M, Niinobe M, Momoi T, Miura M, Yoshikawa K. Activation of neuronal caspase-3 by intracellular accumulation of wild-type Alzheimer amyloid precursor protein. J Neurosci. 1999;19(16):6955-64.
DOI:10.1523/JNEUROSCI.19-16-06955.1999
PMID:10436052.
PMCID:PMC6782871.

63. Sastre M, Walter J, Gentleman SM. Interactions between APP secretases and inflammatory mediators. J Neuroinflammation. 2008;5:25.
DOI:10.1186/1742-2094-5-25
PMID:18564425
PMCID:PMC2442055.

64. Guzman-Martinez L, Maccioni RB, Andrade V, Navarrete LP, Pastor MG, Ramos-Escobar N. Neuroinflammation as a Common Feature of Neurodegenerative Disorders. Front Pharmacol. 2019;10: 1008.
DOI:10.3389/fphar.2019.01008.

65. Kim BW, Koppula S, Hong SS, Jeon SB, Kwon JH, Hwang BY, Park EJ, Choi DK. Regulation of microglia activity by glaucocalyxin-A: Attenuation of lipopolysaccharide-stimulated neuroinflammation through NF-κB and p38 MAPK signaling pathways. PLoS One. 2013;8(2):e55792.
DOI:10.1371/journal.pone.0055792
Epub 2013 Feb 5.
PMID: 23393601.
PMCID: PMC3564949.

66. Negri A, Naponelli V, Rizzi F, Bettuzzi S. Molecular Targets of Epigallocatechin-Gallate (EGCG): A special focus on signal transduction and cancer. Nutrients. 2018; 10(12):1936. DOI:10.3390/nu10121936
PMID: 30563268.
PMCID: PMC6315581.

67. Citron M. Beta-secretase as a target for the treatment of Alzheimer's disease. J Neurosci Res. 2002;70(3):373-9.
DOI:10.1002/jnr.10393.
PMID: 12391600.

68. De Strooper B, Vassar R, Golde T. The secretases: Enzymes with therapeutic potential in Alzheimer disease. Nat Rev Neurol. 2010;6(2):99-107.
DOI:10.1038/nrneurol.2009.218
PMID: 20139999
PMCID:PMC2879045.

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