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Protective effects of Embelin in Benzo[α]pyrene induced cognitive and memory impairment in experimental model of mice

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disease that affects the neurons in the hippocampus, resulting in cognitive and memory impairment. The most prominent clinical characteristics of AD are the production of amyloid-beta (Aβ) plaques, neurofibrillary tangles, and neuroinflammation in neurons. It has been proven that embelin (Emb) possesses antioxidant, anti-inflammatory, and neuroprotective properties. Therefore, we assessed the therapeutic potential of Emb in Benzo [α]pyrene (BaP)-induced cognitive impairment in experimental mice. BaP (5 mg/kg, i. p) was given to mice daily for 28 days, and Emb (2.5, 5, and 10 mg/kg, i. p) was given from 14 to 28 days of a protocol. In addition, locomotor activity was evaluated using open-field and spatial working, and non-spatial memory was evaluated using novel object recognition tasks (NORT), Morris water maze (MWM), and Y- maze. At the end of the study, the animal tissue homogenate was used to check biochemicals, neuroinflammation, and neurotransmitter changes. BaP-treated mice showed a significant decline in locomotor activity, learning and memory deficits and augmented oxidative stress (lipid peroxidation, nitrite, and GSH). Further, BaP promoted the release of inflammatory tissue markers, decreased acetylcholine, dopamine, GABA, serotonin, and norepinephrine, and increased glutamate concentration. However, treatment with Emb at dose-dependently prevented biochemical changes, improved antioxidant levels, reduced neuroinflammation, restored neurotransmitter concentration, and inhibited the NF-κB pathway. The current study's finding suggested that Emb improved cognitive functions through antioxidant, anti-inflammatory, and neuroprotective mechanisms and inhibition of acetylcholinesterase (AChE) enzyme activities and Aβ-42 accumulation.

1. Introduction

Alzheimer's disease (AD) was first mentioned in 1906 by Alois Alzheimer and was renamed by Emil Kraepelin many years later (Li et al., 2019). AD is a progressive age-related neurodegenerative disorder characterized by persistent memory loss and cognitive deficit. The pathogenesis of AD is multifactorial and caused by accumulation of β-amyloid peptide (Aβ), abnormal phosphorylation of the tau protein, oxidative stress, mitochondrial dysfunction, and degeneration of cholinergic neurons (Li et al., 2019). Extracellular deposition of amyloid β in the brain leads to activation of the microglia, which promotes of release various proinflammatory cytokines, including tumour necrosis factor-α (TNF-α), interleukin (IL)-1β and IL-6. Excessive oxidative stress neuroinflammation, leads to energy failure synaptic dysfunction and causes degeneration of the cholinergic neurons (Spuch et al., 2012). In addition, the accumulation of amyloid β triggers mitochondrial dysfunction through a number of pathways, such as impairment of oxidative phosphorylation, the elevation of reactive oxygen species production, alteration of mitochondrial dynamics, and interaction with mitochondrial proteins (Rojas-Gutierrez et al., 2017).

Oxidative stress has also been reported to play a role in the pathogenesis of AD (Fan et al., 2018). It has been recommended that cellular responses to increased stress cause aberrant activation of stress response molecules such as the nuclear factor-kappa b (NF-κB). It is a major cellular stress response protein activated by a number of stimuli, including UV light, cigarette smoke, viral and bacterial products, free radicals, carcinogens, neurotrophin, oxidative stress, and overload of endoplasmic reticulum protein (Oeckinghaus and Ghosh, 2009) and

Abbreviations: AD, Alzheimer's disease; Emb, Emb; SP, senile plaques; NFT, neurofibrillary tangles; NF, κB- nuclear factor-kappa b; IL-1, Interleukin-1; TNF, Tumour necrosis factor; LTD, long-term depression; LTP, long-term potentiation; BaP, Benzo(α)pyrene; IAEC, Institutional Animal Ethics Committee; INSA, Indian National Science Academy.

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environmental pollutants. The NF-κB transcription factor family in mammals consists of five proteins, p65 (RelA), RelB, c-Rel, p105/p50 (NF-κB1), and p100/52 (NF-κB2), that associate with each other to form distinct transcriptionally active homo- and heterodimeric complexes (Wideman et al., 2018). The misproportion of NF-κB dimer formation between c-Rel and RelA might result in the pathological process in specific neurons. In addition, various other mechanisms cause neurodegeneration using NF-κB, such as microglial activation, Aβ accumulation, cholinergic, and mitochondrial dysfunction (Goel and Singh, 2020). NF-κβ transcription factor is a known risk factor in AD-associated neurodegeneration by facilitates β-site AβPP cleaving enzyme 1 or BACE1 gene expression and AβPP processing and increases BACE1 expression in the brain (Jones and Kounatidis, 2017). Numerous studies have supported that Aβ, or a secreted form of AβPP, also provokes an up-regulation of NF-κβ expression, which leads to the expression of a large variety of proinflammatory molecules such as cytokines. These proinflammatory molecules contribute to the AD prognosis (Jha et al., 2019; Jones and Kounatidis, 2017).

It is noticeable that neurotransmitters like acetylcholine, dopamine, serotonin, norepinephrine, GABA, and glutamate play a significant role in the brain's memory storage (Abraham et al., 2019). Synaptic plasticity, including long-term depression (LTD) and long-term potentiation (LTP) are essential learning and memory phenomena influenced by neurotransmitter alteration (Xu et al., 2012). Indeed, there has been a decreased cholinergic and monoaminergic transmission (Sun et al., 2009) and a disparity of glutaminergic and GABAergic signalling in AD (Liu et al., 2019).

Some well-recognized co-factors in neurodegenerative disorders are oxidative stress, mitochondrial dysfunction, and microglial and astrocyte activation. Further, exposure to harmful chemicals and environmental pollution, like AD. Benzo [*a*]pyrene (BaP) is a polycyclic aromatic hydrocarbon resulting from incomplete combustion of organic matter at temperatures between 300 °C and 600 °C. The ubiquitous compounds can be found in coal tar, tobacco smoke, and many foods, especially grilled meats. BaP and its metabolites can pass the blood-brain barrier (BBB) and deposit in the brain due to their high lipophilicity, potentially inducing neurotoxicity (Calderon-Garciduenas et al., 2004). New research suggested that BaP exposure may have a role in the etiology of AD. Epidemiological studies have found that BaP exposure is linked to learning and memory problems in healthy adults and coke oven workers, which neurotransmitter changes may cause (Niu et al., 2010). Animal studies have additionally shown that BaP exposure causes some AD-like behavior/pathological changes, such as short-term memory deficits in C57BL/6 J mice, changes in Aβ-related mRNA levels, Aβ42 accumulation and neurodegeneration in adult Zebrafish, and tau hyperphosphorylation in SD rats (Liu et al., 2020). It's important to investigate whether BaP exposure to healthy adults and coke oven workers in the industry may linked to learning and memory problems. Therefore, the present study has been designed to investigate the effects of BaP-induced cognitive and memory impairment in mice through behavioral, biochemical, neuroinflammation, neurotransmitters acetylcholinesterase, beta amyloids, and NF-κB pathways analysis.

Long-term BaP exposure promotes neuroinflammation, cognition, and memory, followed by neuronal death (Cummings et al., 2019). Recent evidence shows that the NF-κB signalling pathway played an essential role in the neuroinflammation and neurodegeneration induced by benzo(α)pyrene.

Current clinical trials for AD include disease-modifying medications, symptomatic agents, and cognitive enhancers to treat neuropsychiatric and behavioral changes. Anti-amyloid and anti-tau agents are disease-modifying therapies that contain molecules, biological agents, or monoclonal antibodies (Khazdair et al., 2019). There are 121 agents currently undergoing clinical trials for the treatment of AD. Twenty-nine agents are undergoing Phase 3 trials, 65 are undergoing Phase 2 trials, and 27 are undergoing Phase 1 trials (Khazdair et al., 2019). Twelve agents are used for cognitive improvement, and twelve are used to treat neuropsychiatric and behavioral symptoms (Khazdair et al., 2019). Compared to the previous, there is an improvement in the number of disease-modifying agents targeting pathways other than amyloid or tau in the 2020 pipeline.

Several herbal compounds with neuroprotective effects have been widely studied in the last few decades to treat cognitive disorders, including neurodegenerative disorders such as AD and other memory-related diseases (Kundap et al., 2017). However, embelin (Emb), a natural, safe hydroxy benzoquinone, is considered one of the main constituents of plant Embelia ribes (family: Myrsinaceae) and has been reported to possess beneficial effects on a range of CNS disorders (Shaikh et al., 2016). An available study has shown that Emb has neuroprotective activities by inhibiting the activation of astrocytes and microglia and decreasing the generation of TNF-α, iNOS, and IL-1β (Carola et al., 2002). Emb has also been reported to possess cholinesterase inhibitory, anti-amyloidogenic, and neuroprotective effects *in vitro* and *silico* studies, as shown in Fig. 1a. Based on this evidence, the present study has been designed to investigate the neuroprotective potential of Emb as NF-κB inhibitor against Benzo(α) pyrene-induced cognitive and memory impairment in mice of behavioral, biochemical, neuroinflammation, neurotransmitters acetylcholinesterase, beta amyloids, and NF-κB pathways.

2. Materials and methods

2.1. Experimental animals

In the present study, experiments were carried out in male Swiss albino mice (30–35 g m) with 12 months and were obtained from the central animal house of ISF College of Pharmacy, Moga, Punjab (INDIA). The animals were divided into five groups, housed in a polyacrylic cage (25 × 19 × 13 cm), and maintained under standard husbandry conditions (room temperature 22 ± 2 °C and relative humidity of 55–60%). The animals were fed a commercial diet in dry pellets and water *ad libitium*. All the behavioral assessments were taken between 9:00–11:00 a.m. and 2:00–5:00 p.m. The experimental protocol was approved (ISFCP/CPCSEA/19/411) by the Institutional Animal Ethics Committee (IAEC), and experiments were carried out in accordance to Committee for the Purpose of Control and Supervision of Experimental on Animals (CPCSEA) guidelines for the use and care of experimental animals. All the experiments were performed for a given treatment using age-matched animals to avoid variability between experimental groups. The animal breeding and experimental facility are registered with the CPCSEA, Ministry of Environment and Forest and Climate Change, Government of India. Euthanasia was performed under sodium pentobarbital anaesthesia by decapitation, and efforts were made to minimize the pain and suffering of the animals.

2.2. Drugs and chemicals

BaP was purchased from Sigma–Aldrich (USA). Emb was purchased from INDOFINE Chemical Company, USA. BaP was prepared in olive oil as a concentration of 1 ml of olive oil per kg of mice, and Emb was always prepared afresh by suspending in 1 % Tween 80 (v/v) in a 0.9 % (w/v) saline at different doses (2.5, 5 and 10 mg/kg). The body weight of male Swiss albino mice was between 30 and 35 g m, and we calculated the dose of Emb based on the body weight of the mice. Interleukin-1 beta (IL-1β), interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α) kits were purchased from Krishgen Biosystem, India. All other chemicals used in the study are of analytical grade. The Solutions of the drugs and chemicals were freshly prepared before use.

2.3. Experimental procedure

Firstly, animals were divided into five groups, and seven mice were studied per group, whereas only six mice were in the control group. Mice

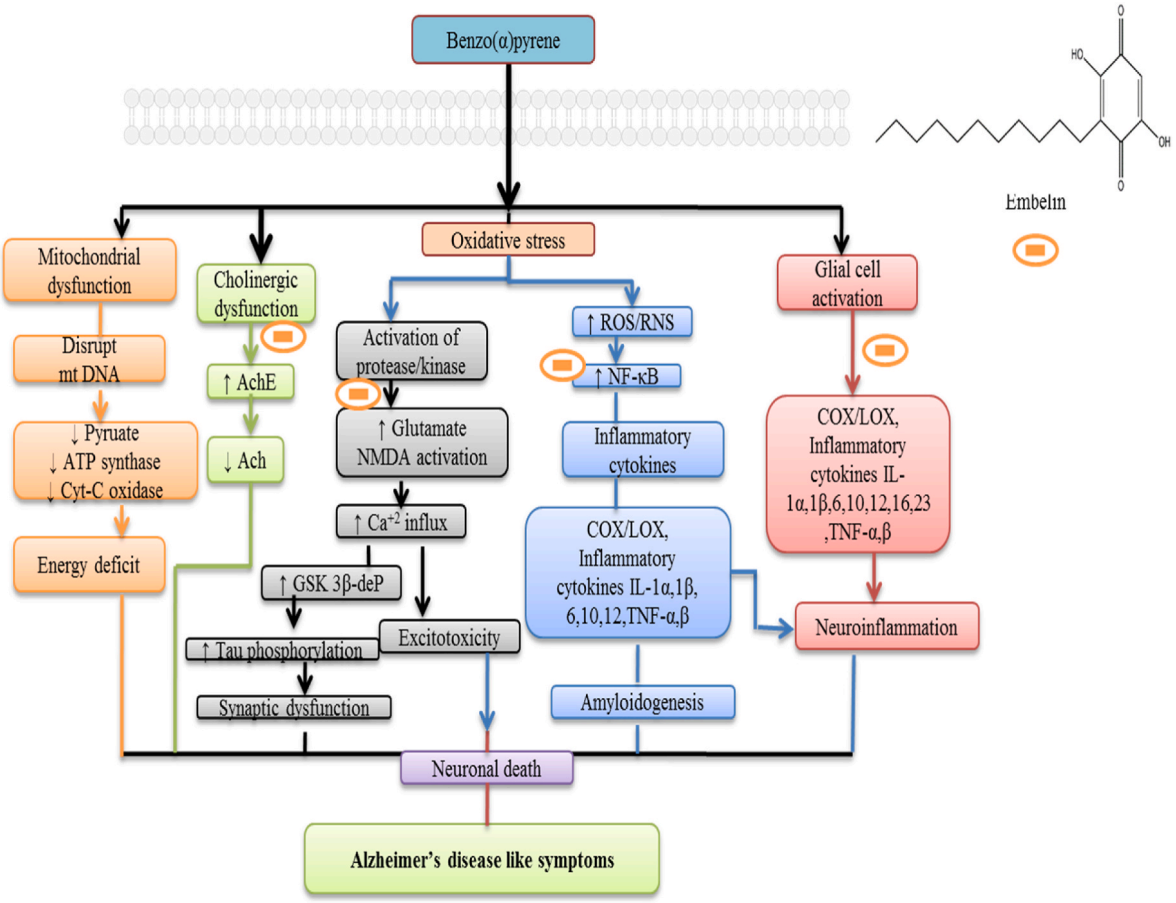

Fig. 1. Study rationale describing BaP induced neurodegeneration and Emb targets.

were placed in individual polyacrylic cages (25 × 19 × 13 cm), and in the control group, mice were injected with normal saline, and in the toxin group, mice were injected with BaP (5 mg/kg; i. p. For 28 days) whereas, in treatment groups, mice are injected with BaP as same as in toxin group along with the addition of Emb at different doses (2.5, 5, 10 mg/kg; i. p daily) from 14th to 28th days of a protocol which act as a treatment drug. Behavioral parameters are studied as per the given schedule on specified days in the work plan. On the 29th day, the animals are sacrificed with the means of euthanasia and then proceed for biochemical, neuroinflammatory, neurotransmitters, and other protein estimations (Fig. 1b).

3. Behavioral parameters

3.1. Measurement of body weight

Bodyweight was measured on days 1st, 7th, 14th, 21st' and 28th of the experiment schedule.

3.2. Open field task

To analyze the locomotor activity, open field task was performed. Each animal was tested for locomotor activity on days 1st, 6th, 13th, 20th and 27th of the experimental schedule. Rats were placed in the actophotometer chamber for 180 s for habituation and utilized to track ambulatory activity. The locomotor activity was measured using a digital actophotometer with infrared light-sensitive photocells, and the counts per minute were recorded for 5 min (Vorhees and Williams, 2006).

3.3. Morris water maze task

The Morris water maze test evaluated memory and cognitive impairment (Morris, 1984). It was constructed from a circular water tank filled with 40 cm of water (22 ± 3 °C). The tank had four equally spaced quadrants: east, west, north, and south. At a set point in one of the quadrants, an escape platform was fixed 2 cm below the surface of the water, and throughout the experiment, the platform stayed in the same quadrant. Animal received four trials for five days, and after 120 s, if the animal failed to locate a platform, it must be carefully selected and placed on the platform for 30 s. Starting latency was measured for each rat to assess their learning capacity. The location of this platform was kept constant throughout the entire experiment. Mice were allowed to swim freely in the pool for 120 s, without a platform during the training period. The four trial sessions per day were given for four days with 120 s of cut-off time, and the time gap between each trial was approximately 30 s during the training period. In this test, Escape latency was analyzed on the 22nd, 23rd, 24th, and 25th and recorded on the 26th day time spent in the target quadrant (TSTQ). (Zhang et al., 2012). The amount of time spent in the target quadrant after learning was reflected by the time spent there. The time spent on the target quadrant was measured for spatial memory check.

3.4. Novel object recognition test

The object recognition test (ORT) is a commonly used behavioral assay for investigating various aspects of learning and memory in mice. The test phase was divided into habituation, familiarization, and testing. The habituation phase lasted three days, with animals freely exploring the empty arena for 5 min every day. After the habituation phase,

animals were examined for the familiarization phase on the fourth day by placing two familiar identical items (rectangular wooden block) in the arena. All animals were allowed to explore for 5 min and given three exploration times for both items, which were counted as f1 and f2 in seconds. After 24 h of habituation, the test phase began by substituting one familiar object with a novel (triangular wooden block) object. Rats were allowed to investigate the arena's items for 5 min freely. For another 5 min, the animals were free to roam around the object that had been put in the arena. Time is allotted for the exploration of objects that fall under the categories of f1 and N1, respectively. The arena was routinely cleansed with 70% alcohol after each evaluation session. After experimentation, parameters like the novel object preference index was calculated to assess animals' recognition memory. Each animal was tested for memory impairment on days 1st and 28th of the experimental schedule. Memory impairment was analyzed using a novel object recognition test for 5 min, and values were expressed as recognition index in percentage (Zhang et al., 2016).

The object recognition index was calculated with the following formula:

Recognition index = (time spent in new object) / (time spent in the new object + time spent in the already known object).

3.5. Y-maze test

Spontaneous alternation behavior in Y-maze was used to assess short-term spatial memory. On days 1st, 7th, 14th, 21st, and 28th of the experimental schedule, mice were initially placed at the end of one arm and freely explored the three arms. The number of arm entries and the number of triads were recorded to calculate the percentage of an alternation. Over the course of multiple arm entries, the entry sequence (e.g., ABC, BCA, or CAB, where letters indicate the code of arms) was recorded manually over 5 min. An actual alternation was defined as entries into all three arms consecutively, i.e., ABC, CAB, or BCA but not BAB. An entry was defined as placing all four paws within the boundaries of the arm (Zou and Crews, 2010). The percentage of alternation (% alternation) was calculated as spontaneous alternation/(total number of arms entries-2) $\times$100.

4. Cellular and molecular markers

4.1. Dissection and homogenization for biochemical estimation

On day 29, the mice were sacrificed by cervical dislocation, and brain tissues were removed and preserved at −80 °C in an ultra-low temperature freezer. On an experimental day, brains were removed from the deep freezer, and the cortex and hippocampus were isolated, weighed, and then homogenized using phosphate buffer solution (0.1 M, pH 7.4) containing 1 mmol Ethylene Diamine-Tetra-Acetic acid (EDTA), 0.25 M sucrose, 10 mM potassium chloride (KCL), and 1 mM Phenyl Methyl Sulfonyl Fluoride (PMSF). After brain homogenization, the sample was centrifuged for 15 min at 10,000 g, supernatants were separated and used to estimate biochemical parameters, acetylcholinesterase (AChE), NF-κB protein, amyloid Beta $_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) level, oxidative stress, neuroinflammatory, and neurotransmitter estimations.

4.2. Determination of the expression levels of NF-κB protein

The NF-κB protein's expression level was determined using ELISA commercial kits (Krishgen diagnostics, India). This test was performed in brain homogenate as per the given standard procedure. The values are expressed as pg/ml protein (Singh and Kumar, 2016).

4.3. Preparation of brain homogenates for the measurements of neurotransmitters

The brains were removed from the deep freezer on the analysis day, and the hippocampus was separated, weighed, and homogenized with 0.2 M perchloric acid. After homogenization, the sample was centrifuged at 12,000 g for 5 min, and the supernatant was extracted using OPA/-ME and filtered through 0.22 mm nylon filters before being injected into the HPLC sample injector. The limitation brain homogenates to examine neurotransmitter levels is the difficulty to maintain the condition during protocol, which may lead to the degradation of neurotransmitters. Additionally, it's very difficult to maintain the temperature and pH conditions of derivatization chemicals.

4.4. Assessment of brain acetylcholine levels

Acetylcholine was measured by using a diagnostic kit (Krishgen diagnostics, India). All the reagents were prepared as per the standard procedure described in the kit. The optical density of the reaction mixture was determined at 540 nm in the microtiter plate.

4.5. Assessment of brain dopamine, serotonin, and norepinephrine levels

The level of dopamine, serotonin, and norepinephrine in the brain homogenate was determined according to the method of Singh and coworkers. The dopamine activity in mice brain homogenate is expressed as ng/mg tissue sample (Singh and Kumar, 2016; Donzanti and Yamamoto, 1988).

4.6. Assessment of brain GABA and glutamate levels

The estimations of GABA and glutamate were done by the method described by Donzanti and Yamamoto (Donzanti and Yamamoto, 1988) with slight modifications. The Waters standard system consists of a high-pressure isocratic pump, a 20 μl manual sample injector valve, and a C18 reverse phase column using an electrochemical detector. The mobile phase consisted of 100 mM disodium hydrogen phosphate anhydrous, 25 mm EDTA, and 22% methanol (pH- 6.5). The experimental electrochemical condition was +0.65 V, having sensitivity ranges from 5 to 50 nA. Separation was carried out at a 1.2 ml/min flow rate, and the column temperature was maintained at 40 °C. Samples (20 μl) were injected manually. Brain samples were homogenized in 0.2 mol/l perchloric acid on the day of the experiment. Then, the samples were centrifuged at 12,000 *g* for 15 min. The supernatant was derivatized using OPA/β-ME and then filtered through 0.22 mm nylon filters before injecting the HPLC sample injector. Data were recorded and analyzed with breeze software. The concentrations of amino acids were calculated from the standard curve using a standard with a concentration of 10–100 ng/ml.

5. Measurement of neuroinflammatory biomarkers in mice brain homogenate

5.1. Measurement of TNF- α, IL-6, and IL-1β levels

The levels of TNF- α, IL-6 and IL-1β were quantified by using mice TNF- α, IL-6, and IL-1β immunoassay kit (KRISHGEN BioSystem, USA). TNF- α, IL-6, and IL-1β activity in mice brain homogenates were expressed as pg/mg protein.

5.2. Estimation of p-Tau

The p-Tau level was determined using ELISA kits.

6. Estimation of biochemical parameters

6.1. Preparation of mice brain homogenate

On the 29th day of the protocol schedule, mice were sacrificed by decapitation, brains were removed, and homogenized with ten times (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000×*g* for 15 min, the supernatant was separated, and aliquots were used for biochemical estimation.

6.2. Protein estimation

The protein content was measured using a Coral protein estimation kit (Biuret method).

6.3. Assessment of acetylcholinesterase (AChE) levels

The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01 M sodium phosphate buffer (pH 8), 0.10 ml of acetylthiocholine iodide, and 0.10 ml of DTNB (Ellman reagent). The change in absorbance was measured immediately at 412 nm spectrophotometrically. The enzymatic activity in the supernatant was expressed as μmol/mg protein (Ayyappan et al., 2016).

6.4. Assessment of catalase levels

Catalase activity was measured according to the method described by Takahara, Hamilton, Nell, Ogubra, and Nishimura (1960). The 0.2 ml of tissue homogenate was mixed with 1.2 ml of phosphate buffer (0.05 M, pH 7.0), and the enzyme reaction was started by adding 1.0 ml of hydrogen peroxide (0.03 M). The decrease in absorbance was recorded at 240 nm for 3 min, and the enzyme blank was run simultaneously with 1.0 ml distilled water instead of hydrogen peroxide. The catalase activity was expressed as micromoles of hydrogen peroxide decomposed per minute per milligram protein (Green et al., 1982).

6.5. Estimation of reduced glutathione levels

Reduced glutathione in the brain was estimated according to the method described by Ellman, 1959). First, 1 ml supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4 °C for 1 h. Then, the samples were centrifuged at 1200×*g* for 15 min. To 1 ml of the supernatant, 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of 5,5′-dithiobis-(2-nitrobenzoic acid) (DTNB) were added. The yellow color that developed was measured immediately at 412 nm using a spectrophotometer. The glutathione concentration in the supernatant is expressed as μmol/mg protein (Ellman, 1959).

6.6. Assessment of nitrite levels

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO) is determined by a colorimetric assay using Greiss reagent (0.1% N-(1- naphthyl) ethylene diamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) as described by Green et al., (1982). Equal volumes of supernatant and Greiss reagent were mixed and incubated for 10 min at room temperature in the dark, and the absorbance was determined at 540 nm spectrophotometrically. The nitrite concentration in the supernatant is selected from the sodium nitrite standard curve and expressed as μmol/mg protein (Wills, 1966).

6.7. Estimation of malondialdehyde (MDA) levels

The quantitative measurement of the MDA end product of lipid peroxidation in brain homogenate was performed according to the method of Wills. The amount of MDA was measured after its reaction with thiobarbituric acid at 532 nm using a spectrophotometer. The concentration of MDA was expressed as μmol/mg protein (Galasko and Montine, 2010).

6.8. Estimation of amyloid Beta $_{1\text{-}42}$ ($A\beta_{1\text{-}42}$) level

The level of $A\beta_{1\text{-}42}$ was estimated by using the $A\beta_{1\text{-}42}$ ELISA kit ($A\beta_{1\text{-}42}$ ELISA kit protocol). It is a solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) using a microtitre plate reader read at 450 nm. Concentrations of $A\beta_{1\text{-}42}$ were calculated from a plotted standard curve (Jayasekara et al., 1992).

6.9. Statistical analysis

The results were expressed as mean ± Standard deviation (SD). Morris water maze and object recognition task were analyzed by repeated measure two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons and others behavior and molecular, biochemical, neuroinflammatory, and neurotransmitters results were analyzed using one-way analysis of variance followed by Tukey's post hoc test. Values with $P < 0.05$ were considered to be statistically significant.

7. Results

7.1. Effect of Emb on change in body weight in BaP-treated mice

BaP intraperitoneal injected group showed significant changes in body weight as compared to vehicle control. Treatment with Emb at doses 2.5, 5, and 10 mg/kg, significantly and dose-dependently attenuated BaP-induced decrease in body weight. Moreover, Emb (10 mg/kg) treated mice showed a significant increase in body weight than low doses of Emb (2.5, and 5 mg/kg). (Fig. 2).

7.2. Effect of Emb on spontaneous locomotor activity in BaP-treated mice

Mice injected with BaP showed significantly reduced spontaneous locomotor activity on days 1st, 7th, 14th, 21st, and 28th compared to the vehicle control group. Treatment with Emb at doses of 2.5, 5 and 10 mg/kg significantly and dose-dependently ameliorated locomotor activity in BaP-injected mice. Moreover, Emb (10 mg/kg) significantly improved

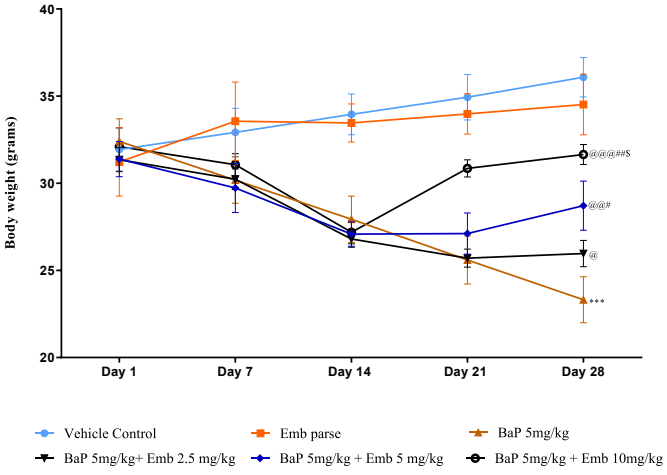

Fig. 2. Effect of Emb on change in body weight in BaP administered mice
Effect of Emb on change in body weight in BaP induced AD in mice. Values expressed as mean ± Standard deviation (SD), ***$p < 0.001$ versus vehicle control & Emb parse; $^{@}p < 0.05$, $^{@@}p < 0.01$ and $^{@@@}p < 0.001$ versus BaP 5 mg/kg; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus Emb 2.5 mg/kg; $^{\$}p < 0.05$ versus Emb 5 mg/kg [two-way Analysis of variance (ANOVA) followed by post hoc multiple comparison test Bonferroni].

spontaneous locomotor activity compared to Emb (2.5, and 5 mg/kg). (Fig. 3).

7.3. Effect of Emb on memory performance in novel object recognition task in BaP treated mice

On days 1st, 7th, 14th, 21st, and 28th, BaP injected group showed a significant decrease in memory performance compared to vehicle control on Morris water maze. Treatment with Emb at different doses 2.5, 5 and 10 mg/kg, significantly and dose-dependently showed improved memory performance in BaP-injected mice. Further, Emb (10 mg/kg) treated group showed significantly improved memory performance as compared to Emb (2.5, and 5 mg/kg) treated group (Fig. 4).

7.4. Effect of Emb on spatial learning and memory using Morris water maze task in BaP treated mice

Escape latency was checked on days 22nd, 23rd, 24th, and 25th and BaP-treated mice showed a gradual increase in escape latency on days 22nd, 23rd, 24th and 25th. Chronic treatment with Emb 2.5, 5, and 10 mg/kg showed a significant attenuation in escape latency than the BaP-injected group. Similarly, the effect of Emb 10 mg/kg significantly affected escape latency as compared to a low dose of Emb (Fig. 5).

On day 26th, time spent in the target quadrant (TSTQ) was estimated. BaP-injected mice show gradual decreases in TSTQ, but chronic treatment with Emb at doses 2.5, 5, and 10 mg/kg significantly increases TSTQ compared to BaP-injected groups. Long-term administration of Emb 10 mg/kg was found to be more effective in restoring TSTQ in BaP-injected mice (Fig. 6a).

Additionally, the average swim speed was performed in Morris water maze task in BaP administered mice and the data has shown no significant change in swim speed in BaP group as compared to vehicle control and Emb parse groups. Similarly, none of the Emb treatment doses (2.5, 5,10 mg/kg) caused any significant alteration in the swim speed of all mice which underwent BaP treatment (Fig. 6b).

7.5. Effect of Emb on learning and memory using Y-maze test in BaP-treated mice

Memory analysis was performed on days 1st, 7th, 14th, 21st and 28th. BaP-injected mice showed significant decreases in memory

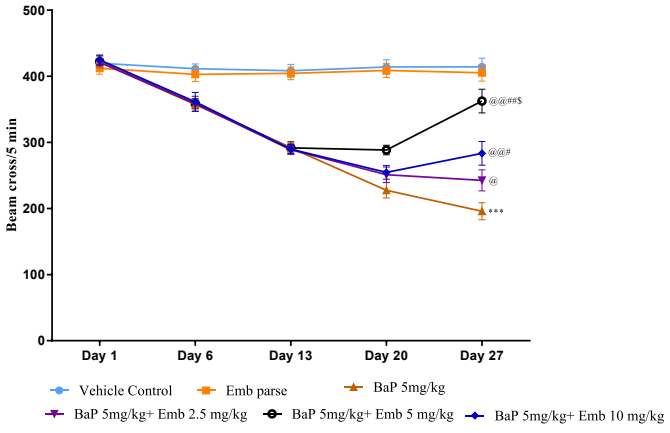

Fig. 3. Effect of Emb on spontaneous locomotor activity in BaP administered mice

Effect of Emb on spontaneous locomotor activity in BaP induced AD in mice. Values expressed as mean ± Standard deviation (SD), ***p < 0.001 versus vehicle control & Emb parse; @ p < 0.05, @@p < 0.01 and @@@ p < 0.001 versus BaP 5 mg/kg; # p < 0.05 and ## p < 0.01 versus Emb 2.5 mg/kg; $ p < 0.05 versus Emb 5 mg/kg [two-way Analysis of variance (ANOVA) followed by post hoc multiple comparison test Bonferroni].

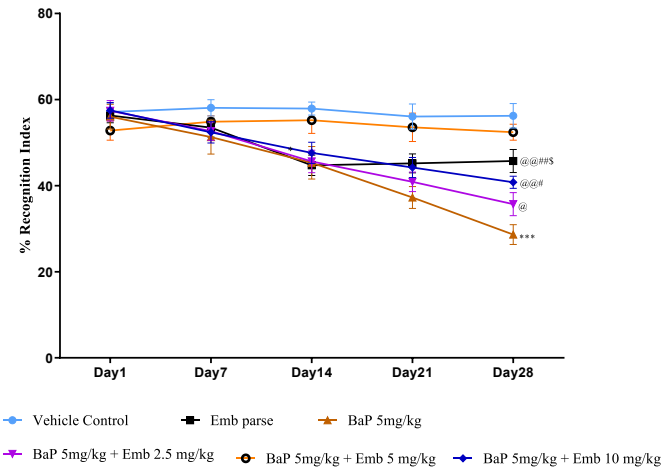

Fig. 4. Effect of Emb on memory performance in Novel object recognition task in BaP administered mice

Effect of Emb on memory performance in Novel object recognition task in BaP induced AD in mice. Values expressed as mean ± Standard deviation (SD), ***p < 0.001 versus vehicle control; @ p < 0.05, @@p < 0.01 and @@@ p < 0.001 versus BaP 5 mg/kg; # p < 0.05 and ## p < 0.01 versus Emb 2.5 mg/kg; $ p < 0.05 versus Emb 5 mg/kg [two-way Analysis of variance (ANOVA) followed by post hoc multiple comparison test Bonferroni].

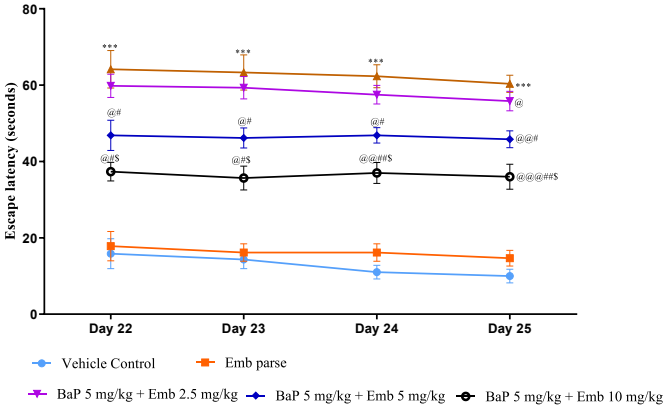

Fig. 5. Effect of Emb on escape latency in Morris water maze task in BaP administered mice

Effect of Emb on escape latency in Morris water maze task in BaP induced AD mice. Values expressed as mean ± Standard deviation (SD), ***p < 0.001 versus vehicle control; @ p < 0.05, @@p < 0.01 and @@@ p < 0.001 versus BaP 5 mg/kg; # p < 0.05 and ## p < 0.01 versus Emb 2.5 mg/kg; $ p < 0.05 versus Emb 5 mg/kg [two-way Analysis of variance (ANOVA) followed by post hoc multiple comparison test Bonferroni].

performance compared to vehicle control. Treatment with Emb at doses 2.5, 5 and 10 mg/kg significantly and dose-dependently showed improved memory performance in BaP-administered mice. Moreover, in the treatment of Emb (10 mg/kg), treated mice showed more improvement in memory performance than Emb (2.5, 5 mg/kg). (Fig. 7).

7.6. Effect of Emb on of the expression levels of NF-κB protein

The expression level of NF-κB protein was significantly augmented in BaP-intoxicated mice as compared to the vehicle control group. Treatment with Emb at different doses, 2.5, 5 and 10 mg/kg, significantly decreased expression levels of NF-κB protein compared to the treated group. Among the selected doses, Emb 10 mg/kg was most effective in ameliorating BaP-induced increased expression levels of NF-κB protein in BaP-administered mice. The values are expressed as pg/ml protein

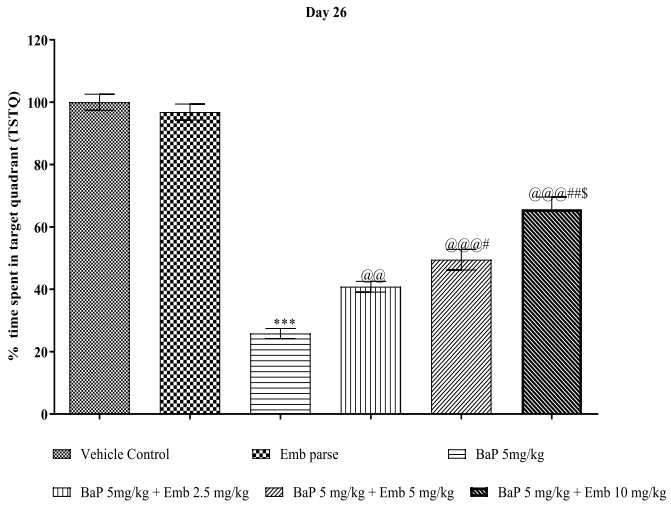

Fig. 6 a). Effect of Emb on time spent in target quadrant (TSTQ) in Morris water maze task in BaP administered mice

Effect of Emb on TSTQ in Morris water maze task in BaP induced AD in mice. Values expressed as mean ± Standard deviation (SD), ***$p < 0.001$ versus vehicle control; $^{@@}p < 0.01$ and $^{@@@}p < 0.001$ versus BaP 5 mg/kg; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus Emb 2.5 mg/kg; $^{\$}p < 0.05$ versus Emb 5 mg/kg [two-way Analysis of variance (ANOVA) followed by post hoc multiple comparison test Bonferroni].

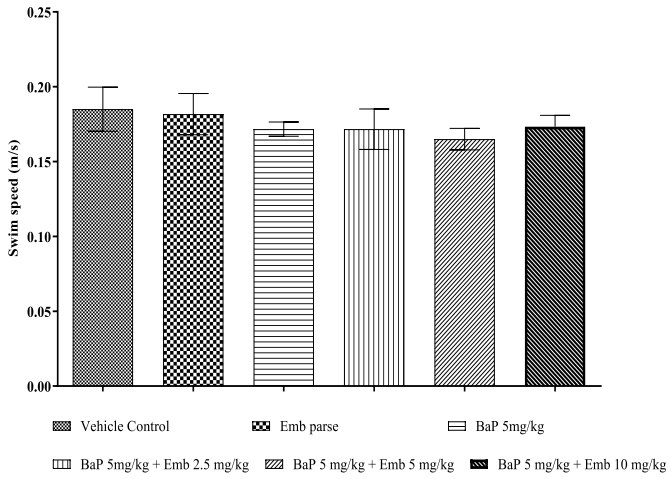

Fig. 6 b). Effect of Emb on average swim speed in Morris water maze task in BaP administered mice

Effect of Emb on average swim speed in Morris water maze task in BaP induced AD in mice. The data has shown no significant change in swim speed in BaP group as compared to vehicle control and Emb parse groups. Similarly, none of the Emb treatment doses (2.5, 5,10 mg/kg) caused any significant alteration in the swim speed of all mice which underwent BaP treatment.

(Jamali-Raeufy et al., 2020). (Fig. 8).

7.7. Neuroprotective effect of Emb on amelioration of inflammatory cytokines in BaP- treated mice

Neuroinflammatory cytokines such as TNF-α, IL-6 and IL-1β levels were significantly increased in BaP-intoxicated mice compared to vehicle control. Chronic treatment with Emb at different doses 2.5, 5, 10 mg/kg significantly decreased inflammatory cytokines compared to the treated group. Among the selected doses, Emb 10 mg/kg was most effective in administering BaP-induced increased levels of inflammatory cytokines in BaP-administered mice **(Table 1).**

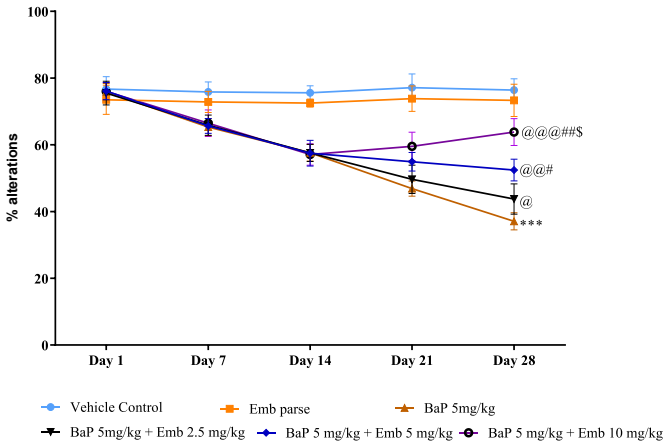

Fig. 7. Effect of Emb on learning and memory using Y-maze test in BaP administered mice

Effect of Emb on learning and memory in BaP induced AD in mice. Values expressed as mean ± Standard deviation (SD),***$p < 0.001$ versus vehicle control; $^{@}p < 0.05$, $^{@@}p < 0.01$ and $^{@@@}p < 0.001$ versus BaP 5 mg/kg; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus Emb 2.5 mg/kg; $^{\$}p < 0.05$ versus Emb 5 mg/kg [two-way Analysis of variance (ANOVA) followed by post hoc multiple comparison test Bonferroni].

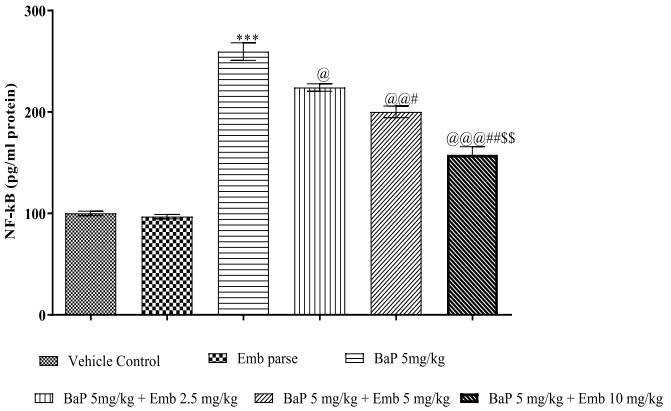

Fig. 8. Effect of Emb on of the expression level of NF-kB protein in hippocampus of mice brain

Effect of Emb on NF-&Kgr; B protein in BaP induced AD in mice. Values expressed as mean ± Standard deviation (SD), ***$p < 0.001$ versus vehicle control; $^{@}p < 0.05$, $^{@@}p < 0.01$ and $^{@@@}p < 0.001$ versus BaP 5 mg/kg; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$ versus Emb 2.5 mg/kg; $^{\$\$}p < 0.01$ versus Emb 5 mg/kg [One-way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test].

7.8. Neuroprotective effect of Emb on reduction of oxidative stress markers in BaP-treated mice

Oxidative stress markers like LPO, GSH, AchE, Nitrite, p-Tau and catalase were quantified. There were LPO, p-Tau, nitrite, and AchE levels that were increased significantly and reduced in the level of reduced GSH and catalase in chronic BaP-administered mice compared to vehicle control. Chronic administration with Emb remarkably and dose-dependently decreased LPO, p-Tau, Nitrite, and AchE and restored the antioxidant defence system by increasing reduced GSH and catalase levels compared with BaP, BaP-administered mice. Among the selected doses of Emb, a high dose of Emb 10 mg/kg was significantly effective in reducing BaP-induced oxidative stress markers in mice **(Table 2).**

Table 1
Neuroprotective effect of Emb inflammatory cytokines in BaP administered mice.

Groups	Neuroinflammatory markers		
	TNF-α (pg/ml protein)	IL-1β (pg/ml protein)	IL-6 (pg/ml protein)
Vehicle Control	36.49 ± 3.31	75.89 ± 4.88	54.40 ± 5.42
Emb Parse	35.12 ± 2.42	75.74 ± 3.80	51.22 ± 4.25
BaP 5 mg/kg	382.3 ± 7.42***	427.9 ± 6.86***	213.1 ± 4.85***
Emb 2.5 mg/kg	323.1 ± 4.76$^{@}$	374.6 ± 9.12$^{@}$	184.5 ± 6.31$^{@}$
Emb 5 mg/kg	252.8 ± 6.55$^{@@\#}$	268.3 ± 7.56$^{@@\#}$	152.5 ± 3.66$^{@@\#}$
Emb 10 mg/kg	123.3 ± 8.82$^{@@@\#\#\$\$}$	151.4 ± 4.06$^{@@@\#\#\$\$}$	126.2 ± 7.69$^{@@@\#\#\$}$

Effect of Emb on pro-inflammatory cytokines in BaP induced AD mice. Values expressed as mean ± Standard deviation (SD), ***p < 0.001 versus vehicle control; $^{@}$ p < 0.05, $^{@@}$p < 0.01 and $^{@@@}$ p < 0.001 versus BaP 5 mg/kg; $^{\#}$p < 0.05 and $^{\#\#}$p < 0.01 versus Emb 2.5 mg/kg; $^{\$}$ p < 0.05 and $^{\$\$}$ p < 0.01 versus Emb 5 mg/kg [One-way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test].

7.9. Neuroprotective effect of Emb on amelioration of neurotransmitters level in BaP treated mice

Neurotransmitters such as Ach, dopamine, NE, serotonin, GABA and glutamate were assessed at the end of the protocol schedule. BaP resulted in a significant decrease of Ach, dopamine, NE, serotonin, and GABA levels, whereas increased glutamate concentration in mice brains compared to vehicle control. On the other hand, chronic treatment with Emb significantly increased Ach, dopamine, NE, serotonin, and GABA levels and decreased glutamate levels in mice brain homogenate compared with BaP-administered mice. Furthermore, Emb 10 mg/kg was most effective in restoring dopamine, NE, serotonin, Ach, GABA, and glutamate neurotransmitter changes in BaP-administered mice. (**Tables 3 and 4**).

7.10. Effect of Emb on Aβ-42 level in BaP-treated AD in mice

Vehicle control animals did not produce any significant changes in the level of Aβ-42 as compared with the normal group. The BaP-induced cognitive impairment mice showed an increase in the level of Aβ-42 within the brain compared to a vehicle control group. However, treatment with Emb at different doses 2.5, 5 and 10 mg/kg, significantly decreased the level of Aβ-42 compared to BaP-treated mice. Moreover, the oral administration of Emb (10 mg/kg) instigated a significant reduction in the level of Aβ-42 compared to a low dose of Emb (2.5, 5 mg/kg). (Fig. 9).

8. Discussion

The present research showed that Emb has a neuroprotective ability against BaP-induced neurotoxicity in Swiss albino mice by directly targeting the NF-κB pathway. Intraperitoneal (i.p) infusion of BaP has affected behavioral, biochemicals, neurotransmitters and molecular changes seen in AD (Maciel et al., 2014). In this study, the administration of BaP has induced cognitive dysfunction, cholinergic deficit, an increased proinflammatory cytokines, oxidative stress and neurotransmitters alterations in mice brains. The reported improvements are aligned with available studies demonstrating significant behavioral and biochemical changes following the injection of BaP into mice (Kandimalla and Reddy, 2017). However, the exact mechanism of cognitive impairments induced by BaP is unclear. However, BaP-induced cholinergic dysfunction, cerebro energetic failure, mitochondrial dysfunction, consequent glial cell activation, and majorly oxidative stress further contribute to the upregulation of the NF-κB pathway and glutamate excitotoxicity, correlated with cognitive dysfunction as well as other signs of AD.

Even some clinical studies reported the same i.e. altered lower limb locomotion in AD patients was observed. There have been increasing reports of non-cognitive symptoms, including loss of motor function, associated with the incidence of AD (Buchman and Bennett, 2011). Since AD pathology is related to loss of cognition along with motor incoordination-specifically in brain regions like the hippocampus and entorhinal cortex (Buchman and Bennett, 2011). It has been suggested that β-amyloid and its precursors are abnormally and specifically

Table 3
Neuroprotective effect of Emb on amelioration of neurotransmitters level (Dopamine, Serotonin, Norepinephrine) in BaP administered mice.

Groups	Neurotransmitters level		
	Dopamine (ng/mg protein)	Serotonin (ng/mg protein)	Norepinephrine (ng/mg protein)
Vehicle Control	102.0 ± 1.58	27.83 ± 0.80	34.91 ± 0.90
Emb Parse	99.23 ± 1.23	25.23 ± 0.67	33.45 ± 0.45
BaP 5 mg/kg	24.82 ± 2.05**	5.01 ± 0.64**	7.40 ± 0.32**
Emb 2.5 mg/kg	32.98 ± 1.05$^{@}$	10.93 ± 0.63$^{@}$	12.85 ± 0.69$^{@}$
Emb 5 mg/kg	46.73 ± 2.48$^{@@\#}$	16.41 ± 1.02$^{@@\#}$	20.93 ± 0.87$^{@\#}$
Emb 10 mg/kg	58.49 ± 1.29$^{@@@\#\#\$}$	23.18 ± 0.65$^{@@@\#\#\$}$	28.84 ± 0.57$^{@@@\#\#\$}$

Effect of Emb on neurotransmitters level in BaP induced AD in mice. Values expressed as mean ± Standard deviation (SD), **p < 0.01 versus vehicle control; $^{@}$ p < 0.05, $^{@@}$p < 0.01 and $^{@@@}$ p < 0.001 versus BaP 5 mg/kg; $^{\#}$p < 0.05 and $^{\#\#}$p < 0.01 versus Emb 2.5 mg/kg; $^{\$}$ p < 0.05 versus Emb 5 mg/kg [One-way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test].

Table 2
Neuroprotective effect of Emb on reduction of oxidative stress markers in BaP administered mice.

Groups	Catalase (μM H_2O_2 decomposed/mg of tissue/min)	AchE (U/mg protein)	MDA (μmol/mg protein)	NO (μmol/mg protein)	GSH (μmol/mg protein)	p-Tau (pg/g) tissue
Vehicle Control	16.25 ± 0.27	14.93 ± 0.41	1.57 ± 0.01	1.56 ± 0.01	0.086 ± 0.0017	8.55 ± 0.14
Emb Parse	15.15 ± 0.22	14.24 ± 0.23	1.63 ± 0.02	1.53 ± 0.02	0.081 ± 0.0015	8.51 ± 0.13
BaP 5 mg/kg	44.89 ± 0.67**	53.59 ± 0.57***	3.40 ± 0.04**	0.48 ± 0.01**	0.015 ± 0.0024*	28.25 ± 3.20*
Emb 2.5 mg/kg	35.87 ± 0.32$^{@}$	45.88 ± 0.57$^{@}$	2.97 ± 0.02$^{@}$	0.67 ± 0.009$^{@}$	0.027 ± 0.0018$^{@@}$	24.10 ± 2.19$^{@@}$
Emb 5 mg/kg	29.80 ± 0.27$^{@@\#}$	38.26 ± 0.46$^{@@\#}$	2.55 ± 0.01$^{@@\#}$	0.88 ± 0.01$^{@@\#}$	0.040 ± 0.0019$^{@@\#}$	14.10 ± 1.17$^{@@\#}$
Emb 10 mg/kg	22.03 ± 0.22$^{@@@\#\#\$}$	27.06 ± 0.18$^{@@@\#\#\$}$	2.08 ± 0.01$^{@@@\#\#\$}$	1.16 ± 0.01$^{@@@\#\#\$}$	0.064 ± 0.0033$^{@@@\#\#\$}$	10.32 ± 1.23$^{@@@\#\#\$}$

Effect of Emb on oxidative stress in BaP induced AD in mice. Values expressed as mean ± Standard deviation (SD), *p < 0.05 and **p < 0.01 and ***p < 0.001 versus vehicle control; $^{@}$ p < 0.05, $^{@@}$p < 0.01 and $^{@@@}$ p < 0.001 versus BaP 5 mg/kg; $^{\#}$p < 0.05 and $^{\#\#}$p < 0.01 versus Emb 2.5 mg/kg; $^{\$}$ p < 0.05 versus Emb 5 mg/kg [One-way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test].

Table 4
Neuroprotective effect of Emb on amelioration of neurotransmitters level (GABA, Glutamate, Ach) in BaP administered mice.

Groups	Neurotransmitters level		
	GABA (ng/mg protein)	Glutamate (ng/mg protein)	Ach (ng/mg protein)
Vehicle Control	76.76 ± 1.00	75.83 ± 0.80	8.84 ± 0.51
Emb Parse	73.34 ± 0.54	73.89 ± 0.34	7.32 ± 0.34
BaP 5 mg/kg	27.32 ± 1.28**	175.70 ± 0.82***	1.19 ± 0.63**
Emb 2.5 mg/kg	35.80 ± 1.54$^{@}$	155.30 ± 0.80$^{@}$	2.87 ± 0.27$^{@}$
Emb 5 mg/kg	47.21 ± 1.37$^{@@\#}$	134.70 ± 0.80$^{@@\#}$	4.85 ± 0.32$^{@@\#}$
Emb 10 mg/kg	57.16 ± 1.45$^{@@@\#\#\$}$	104.70 ± 0.88$^{@@@\#\#\$}$	6.94 ± 0.42$^{@@@\#\#\$}$

Effect of Emb on neurotransmitters level in BaP induced AD in mice. Values expressed as mean ± Standard deviation (SD), **p < 0.01 and ***p < 0.001 versus vehicle control; $^{@}$ p < 0.05, $^{@@}$p < 0.01 and $^{@@@}$ p < 0.001 versus BaP 5 mg/kg; $^{\#}$p < 0.05 and $^{\#\#}$p < 0.01 versus Emb 2.5 mg/kg; $^{\$}$ p < 0.05 versus Emb 5 mg/kg [One-way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test].

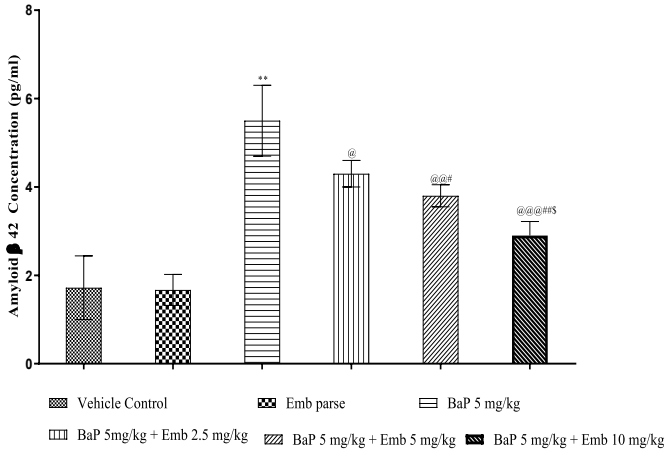

Fig. 9. Effect of Emb on Aβ-42 in BaP induced AD rats
Effect of Emb on Aβ-42 in BaP induced AD in mice. Values expressed as mean ± Standard deviation (SD), **p < 0.01 versus vehicle control; $^{@}$ p < 0.05, $^{@@}$p < 0.01 and $^{@@@}$ p < 0.001 versus BaP 5 mg/kg; $^{\#}$p < 0.05 and $^{\#\#}$p < 0.01 versus Emb 2.5 mg/kg; $^{\$}$ p < 0.05 versus Emb 5 mg/kg [One-way Analysis of variance (ANOVA) followed by Tukey's multiple comparison test].

present in inclusion body myositis muscle fibers, which is associated with non-cognitive (muscle incoordination) AD pathology (Vattemi et al., 2009). Therefore, in the present study, we found that lansoprazole could impair memory function in MWM and EPM might have resulted from impaired locomotor activity. In the present study, BaP caused a decline in locomotor activity in mice as observed in OFT at different time intervals and caused significant learning and memory impairment as observed in MWM, Y-Maze and NORT similarly at different time intervals. It is known that neurotransmitters produce an impact on neuronal functioning and are strongly enriched with cholinergic, glutaminergic, GABAergic, and monoaminergic axon terminals, and these neurotransmitters are believed to play a vital role in the encoding, storage, and expression of memory (Luján et al., 2005).

In this study, we have reported significant changes in neurochemistry after infusion of BaP in the brain of mice. BaP has caused a significant elevation in acetylcholinesterase activity (AchE) and a deficit in monoamine levels. AchE is responsible for the acetate and choline synthesis from Ach, thus, the elevated AchE level suggests an enhanced Ach metabolism. Therefore, the neurotransmitter's extracellular concentration decline can be due to their increased metabolism. On the other side, BaP causes significant reductions in GABA and elevation in glutamate levels in mice's brains. Such differences in GABA levels and glutamate have been documented to contribute to excitotoxicity-induced neuronal damage and cognitive impairment (Gupta et al., 2013). Epidemiological studies have shown that BaP exposure is associated with learning and memory deficits in healthy adults and coke oven workers, possibly due to the alteration of neurotransmitters (Niu et al., 2010). Chronic exposure to BaP has been shown to modulate the levels of several neurotransmitters, including dopamine and serotonin (Zhang et al., 2016).

Similarly, our study found that the BaP injected group has lower GABA, Ach, dopamine, serotonin, norepinephrine and higher glutamate levels. However, Emb treatment prevented neuronal death and balanced various neurotransmitter levels in a dose-dependent way. Emb restored brain monoamine and GABA levels, whereas it reduced glutamate hyperactivity in BaP-injected mice, suggesting its neuromodulator role. The previously reported indicate that the positive benefits of Emb may be due to its ability to improve the levels of neurotransmitters in the hippocampus. Emb restored hippocampal monoamine, GABA levels, and AchE activity while reducing glutamate hyperactivity in STZ-infused rats, suggesting its neuromodulator action. Our result agrees with earlier results (Arora and Deshmukh, 2017).

Excitotoxicity due to glutamate is also reported to occur in brains with AD. It is shown with the support of ELISA that BaP is also responsible for the increase in the expression of NF-κB and is often stated to occur in AD brains. In this study, we found that BaP showed significant changes in the neurotransmitter concentration in the mice's brain. BaP causes reductions in GABA, and elevated glutamate levels; such differences in GABA levels and glutamate contribute to excitotoxicity-induced neuronal damage and cognitive impairment. Emb therapy balanced the glutamate and GABA levels in check in a dose-dependent manner. Nevertheless, the current research demonstrates the neuromodulatory function of Emb and its immense capacity to down-regulate the NF-κB pathway responsible for AD. Our results showed that BaP-treated mice have impaired spatial learning and memory functions, enhanced oxidative stress, damaged the cholinergic system, and activated the NF-κB signalling pathway in the hippocampus. The neuroprotective effect of Emb against BaP-induced AD inhibits the AChE activity and reduces the oxidative damage, and proinflammatory cytokines (TNF-α IL-6, IL-1β) markedly of brain tissue via suppressing the NF-κB signalling pathway. Thus, the observed increase in cognitive functioning in the present study may be related to Emb's ability to restore the neurotransmitters of the brain and target the NF-κB pathway. Evidence shows that oxidative stress and neuroinflammatory cytokines play a critical role in several neurodegenerative diseases (Liu et al., 2015). There have also been records of mitochondrial dysfunction, increased production of free radicals, a compromised antioxidant defence, and neuroinflammation in AD (Block et al., 2007). Microglial cells function as the brain immune defence against brain injury; however, overactivation of microglia cells is harmful to the survival of neuronal cells and may contribute to neurotoxic events triggering the release of inflammatory cytokines and increased free radical production (Zawia et al., 2009). ROS and peroxides, on the contrary, play a significant role in host defence and may influence the number of transcription factors, such as the production of NF-κB-dependent cytokine and activated protein 1, resulting in the output of proinflammatory cytokines triggering neuroinflammation (Raj et al., 2021).

Likewise, elevated cytokine levels are also related to mitochondrial dysfunction and enhanced ROS production in neurodegenerative pathologies (Bourgognon and Cavanagh, 2020). Cytokines (TNF-α and IL-1β) were associated with cholinergic dysfunction and cognitive decline, as seen in ageing and AD. Enhanced formation of ROS and RNS, known to react with biomolecules including proteins, lipids, carbohydrates, DNA, and RNA, contribute to their cellular dysfunction and oxidative damage (Li et al., 2015). There has also been proof of the

overactivation of microglia cells, enhanced cytokine production, and oxidative stress following BaP administration in mice. In the current study, BaP has substantially increased MDA, nitrite, and proinflammatory cytokine levels (TNF-α, IL-1β, and IL-6) and decreased GSH levels in mice's brains. In the current investigation, Emb inhibited BaP-induced oxidative stress and reduced the burden of the proinflammatory cytokine, indicating its antioxidant and anti-inflammatory capabilities.

Furthermore, our study found that down-regulation of the NF-κB pathway after dosing with Emb was dose-dependent. Indeed, Emb has been reported to possess antioxidant activity and anti-inflammatory ability and show its neuroprotective role by downregulating the NF-κB pathway. Previous studies have reported that Emb is an inhibitor of X-linked antiapoptotic protein and also blocks the NF-κB signalling pathways, thus leading to the downregulation of various antiapoptotic and metastatic gene products (Ahn et al., 2007). It has also shown *in vivo* anti-inflammatory activity in acute and chronic models of psoriasis or inflammatory skin diseases. Furthermore, it is reported that Emb can block the NF-κB signalling pathway, suppressing NF-κB-regulated anti-apoptotic and metastatic gene products (Li et al., 2019).

Thus, the reported activities in the present study also play a significant role in improving cognitive impairment and memory dysfunction. Further studies are needed to confirm the role of NF-κB pathway in the pathophysiology of AD. However, research is still needed to understand the exact molecular approach and mechanisms of Emb against AD. Although the study mainly focused on the neuroprotective effects of Emb against BaP-induced cognitive impairment, it is important to note that the BaP caused more mortality in rats than other standard models, which is a major limitation of the study. In addition, BaP has taken more time to produce disease progression in rodents.

In conclusion, BaP administration in mice induced significant cognitive and memory impairment, oxidative damage, increased proinflammatory cytokines, altered levels of neurotransmitters, and triggered upregulation of the NF-κB pathway. Treatment with the Emb dose-dependently attenuated cognitive dysfunction and other neurotoxic effects induced by BaP. The cognitive improvement in BaP-administered mice found after Emb therapy could be attributed to its antioxidant and anti-inflammatory function and its ability to down-regulate the NF-κB pathway, which is responsible for memory and cognitive impairment. Nonetheless, our findings indicate that the Emb would be a beneficial candidate drug molecule for restoring cognitive function associated with AD by directly targeting the NF-κB pathway.

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Author contributions

Akansh Goal: Conducted the experiment and wrote the manuscript. Khadga Raj: collected data and analyzed by taking the help of Dr. Shamsher Singh. Dr. Shamsher Singh and Rimpi Arora: Designed, reviewed, and edited manuscript.

Ethics approval and consent to participate

The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (ISFCP/IAEC/CPCSEA/meeting NO.25/2019/Protocol 411) and performed according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) for the use and care of experimental animals.

Consent for publication

Not applicable.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Peer Review Overview and Supplementary data

A Peer Review Overview and (sometimes) Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.crneur.2023.100122.

References

Abraham, W.C., Jones, O.D., Glanzman, D.L., 2019. Is plasticity of synapses the mechanism of long-term memory storage? NPJ. Sci. Learn. 4 (1), 1, 0.
Ahn, K.S., Sethi, G., Aggarwal, B.B., 2007. Emb, an inhibitor of X chromosome-linked inhibitor-of-apoptosis protein, blocks nuclear factor-κB (NF-κB) signaling pathway leading to suppression of NF-κB-regulated antiapoptotic and metastatic gene products. Mol. Pharmacol. 71 (1), 209–219.
Arora, R., Deshmukh, R., 2017. Emb attenuates intracerebroventricular streptozotocin-induced behavioral, biochemical, and neurochemical abnormalities in rats. Mol. Neurobiol. 54 (9), 6670–6680.
Ayyappan, P., Palayyan, S.R., Kozhiparambil Gopalan, R., 2016. Attenuation of oxidative damage by Boerhaavia diffusa L. against different neurotoxic agents in rat brain homogenate. J. Diet. Suppl. 13 (3), 300–312.
Block, M.L., Zecca, L., Hong, J.S., 2007. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. Nat. Rev. Neurosci. 8 (1), 57–69.
Bourgognon, J.M., Cavanagh, J., 2020. The role of cytokines in modulating learning and memory and brain plasticity. Brain and Neurosci. Adv. 2398212820979802.
Buchman, A.S., Bennett, D.A., 2011. Loss of motor function in preclinical Alzheimer's disease. Expert Rev. Neurother. 11 (5), 665–676.
Calderon-Garciduenas, L., Reed, W., Maronpot, R.R., Henriquez-Roldan, C., Delgado-Chavez, R., Calderon-Garciduenas, A., 2004. Brain inflammation and Alzheimer 's-like pathology in individuals exposed to severe air pollution. Toxicol. Pathol. 32, 650–658.
Carola, V., D'Olimpio, F., Brunamonti, E., Mangia, F., Renzi, P., 2002. Evaluation of the elevated plus-maze and open-field tests for the assessment of anxiety-related behaviour in inbred mice. Behav. Brain Res. 134 (1–2), 49–57.
Cummings, J., Lee, G., Ritter, A., Sabbagh, M., Zhong, K., 2019. Alzheimer's disease drug development pipeline: 2019. Alzheimer's Dementia: Transl. Res. & Clin. Interventions 5, 272–293.
Donzanti, B.A., Yamamoto, B.K., 1988. An improved and rapid HPLC-EC method for the isocratic separation of amino acid neurotransmitters from brain tissue and microdialysis perfusates. Life Sci. 43 (11), 913–922.
Ellman, G.L., 1959. Tissue sulfhydryl groups. Arch. Biochem. Biophys. 82 (1), 70–77.
Fan, P., Tyagi, A.K., Agboke, F.A., Mathur, R., Pokharel, N., Jordan, V.C., 2018. Modulation of nuclear factor-kappa B activation by the endoplasmic reticulum stress sensor PERK to mediate estrogen-induced apoptosis in breast cancer cells. Cell Death Discov. 4 (1), 1–4.
Galasko, D., Montine, T.J., 2010. Biomarkers of oxidative damage and inflammation in Alzheimer's disease. Biomarkers Med. 4 (1), 27–36.
Goel, A., Singh, S., 2020. Emerging Approaches for the Treatment of Alzheimer Disease: Targeting NF-Kb Pathway.
Green, L.C., Wagner, D.A., Glogowski, J., Skipper, P.L., Wishnok, J.S., Tannenbaum, S.R., 1982. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal. Biochem. 126 (1), 131–138.
Gupta, G., Kazmi, I., Afzal, M., Upadhyay, G., Singh, R., Habtemariam, S., 2013. Antidepressant-like activity of Emb isolated from Embelia ribes. Phytopharmacol. 4 (1), 87–95.
Jayasekara, S., Sharma, R.P., Drown, D.B., 1992. Effects of benzo [a] pyrene on steady-state levels of biogenic amines and metabolizing enzymes in mouse brain regions. Ecotoxicol. Environ. Saf. 24 (1), 1–2.
Jha, N.K., Jha, S.K., Kar, R., Nand, P., Swati, K., Goswami, V.K., 2019. Nuclear factor-kappa β as a therapeutic target for Alzheimer's disease. J. Neurochem. 150 (2), 113–137.
Jones, S.V., Kounatidis, I., 2017. Nuclear factor-kappa B and Alzheimer disease, unifying genetic and environmental risk factors from cell to humans. Front. Immunol. 8, 1805.
Kandimalla, R., Reddy, P.H., 2017. Therapeutics of neurotransmitters in Alzheimer's disease. J. Alzheim. Dis. 57 (4), 1049–1069.
Khazdair, M.R., Anaeigoudari, A., Hashemzehi, M., Mohebbati, R., 2019. Neuroprotective potency of some spice herbs, a literature review. J. Tradit. Complementary Med. 9 (2), 98–105.
Kundap, U.P., Bhuvanendran, S., Kumari, Y., Othman, I., Shaikh, M., 2017. Plant derived phytocompound, Emb in CNS disorders: a systematic review. Front. Pharmacol. 8, 76.

Li, J.Y., Chen, R.J., Huang, L.T., Lee, T.Y., Lu, W.J., Lin, K.H., 2019. Emb as a novel inhibitor of PKC in the prevention of platelet activation and thrombus formation. J. Clin. Med. 8 (10), 1724.

Li, Y., Fan, J., Hu, Z., Shao, Q., Zhang, L., Yu, H., 2015. Influence of land use patterns on evapotranspiration and its components in a temperate grassland ecosystem. Adv. Meteorol. 2015, 1.12.

Liu, D., Zhao, Y., Qi, Y., Gao, Y., Tu, D., Wang, Y., Gao, H.M., Zhou, H., 2020. Benzo (a) pyrene exposure induced neuronal loss, plaque deposition, and cognitive decline in APP/PS1 mice. J. Neuroinflammation 17 (1), 1–7.

Liu, P.P., Xie, Y., Meng, X.Y., Kang, J.S., 2019. History and progress of hypotheses and clinical trials for Alzheimer's disease. Signal Transduct. Targeted Ther. 4 (1), 1–22.

Liu, Z., Li, T., Li, P., Wei, N., Zhao, Z., Liang, H., Ji, X., Chen, W., Xue, M., Wei, J., 2015. The Ambiguous Relationship of Oxidative Stress, Tau Hyperphosphorylation, and Autophagy Dysfunction in Alzheimer's Disease. Oxidative Medicine and Cellular Longevity 2015.

Luján, R., Shigemoto, R., López-Bendito, G., 2005. Glutamate and GABA receptor signalling in the developing brain. Neuroscience 130 (3), 567–580.

Maciel, E.S., Biasibetti, R., Costa, A.P., Lunardi, P., Schunck, R.V., Becker, G.C., Arbo, M. D., Dallegrave, E., Gonçalves, C.A., Saldiva, P.H., Garcia, S.C., 2014. Subchronic oral administration of Benzo [a] pyrene impairs motor and cognitive behavior and modulates S100B levels and MAPKs in rats. Neurochem. Res. 39 (4), 731–740.

Morris, R, 1984. Developments of a water-maze procedure for studying spatial learning in the rat. J. Neurosci. Methods 11 (1), 47–60.

Niu, Q., Zhang, H., Li, X., Li, M., 2010. Benzo [a] pyrene-induced neurobehavioral function and neurotransmitter alterations in coke oven workers. Occup. Environ. Med. 67 (7), 444–448.

Oeckinghaus, A., Ghosh, S., 2009. The NF-κB family of transcription factors and its regulation. Cold Spring Harbor Perspect. Biol. 1 (4), a000034.

Raj, K, GD, G, Singh, S, 2021. Spermine protects aluminium chloride and iron-induced neurotoxicity in rat model of Alzheimer's disease via attenuation of tau phosphorylation, Amyloid-β (1–42) and NF-κB pathway. Inflammopharmacology 29, 1777–1793.

Rojas-Gutierrez, E., Muñoz-Arenas, G., Treviño, S., Espinosa, B., Chavez, R., Rojas, K., Flores, G., Díaz, A., Guevara, J., 2017. Alzheimer's disease and metabolic syndrome: a link from oxidative stress and inflammation to neurodegeneration. Synapse 71 (10), e21990.

Shaikh, A., Dhadde, S.B., Durg, S., Veerapur, V.P., Badami, S., Thippeswamy, B.S., Patil, J.S., 2016. Effect of Emb against lipopolysaccharide-induced sickness behaviour in mice. Phytother Res. 30 (5), 815–822.

Singh, S., Kumar, P., 2016. Neuroprotective activity of curcumin in combination with piperine against quinolinic acid induced neurodegeneration in rats. Pharmacology 97 (3–4), 151–160.

Spuch, C., Ortolano, S., Navarro, C., 2012. New insights in the amyloid-Beta interaction with mitochondria. J. Aging Res. 2012, 1-9.

Sun, B., Halabisky, B., Zhou, Y., Palop, J.J., Yu, G., Mucke, L., Gan, L., 2009. Imbalance between GABAergic and glutamatergic transmission impairs adult neurogenesis in an animal model of Alzheimer's disease. Cell Stem Cell 5 (6), 624–633.

Vattemi, G., Nogalska, A., King Engel, W., D'Agostino, C., Checler, F., Askanas, V., 2009. Amyloid-β42 is preferentially accumulated in muscle fibers of patients with sporadic inclusion-body myositis. Acta Neuropathol. 117 (5), 569–574.

Vorhees, C.V., Williams, M.T., 2006. Morris water maze: procedures for assessing spatial and related forms of learning and memory. Nat. Protoc. 1 (2), 848–858.

Wideman, C.E., Jardine, K.H., Winters, B.D., 2018. Involvement of classical neurotransmitter systems in memory reconsolidation: focus on destabilization. Neurobiol. Learn. Mem. 156, 68–79.

Wills, E., 1966. Mechanisms of lipid peroxide formation in animal tissues. Biochem. J. 99 (3), 667.

Xu, Y., Yan, J., Zhou, P., Li, J., Gao, H., Xia, Y., Wang, Q., 2012. Neurotransmitter receptors and cognitive dysfunction in Alzheimer's disease and Parkinson's disease. Prog. Neurobiol. 97 (1), 1–3.

Zawia, N.H., Lahiri, D.K., Cardozo-Pelaez, F., 2009. Epigenetics, oxidative stress, and Alzheimer disease. Free Radic. Biol. Med. 46 (9), 1241–1249.

Zhang, R., Xue, G., Wang, S., Zhang, L., Shi, C., Xie, X., 2012. Novel object recognition as a facile behavior test for evaluating drug effects in AβPP/PS1 Alzheimer's disease mouse model. J. Alzheim. Dis. 31 (4), 801–812.

Zhang, W., Tian, F., Zheng, J., Li, S., Qiang, M., 2016. Chronic administration of benzo (a) pyrene induces memory impairment and anxiety-like behavior and increases of NR2B DNA methylation. PLoS One 11 (2), e0149574.

Zou, J., Crews, F., 2010. Induction of innate immune gene expression cascades in brain slice cultures by ethanol: key role of NF-κB and proinflammatory cytokines. Alcohol Clin. Exp. Res. 34 (5), 777–789.