Life Sciences 221 (2019) 196–203

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Aegle marmelos leaf extract ameliorates the cognitive impairment and oxidative stress induced by intracerebroventricular streptozotocin in male rats

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ARTICLE INFO

Keywords:
A. marmelos
Memory impairment
Alzheimer's disease
Oxidative stress
Streptozotocin

ABSTRACT

Aims: *Aegle marmelos* (L.) Correa (*A. marmelos*) has been used in Ayurvedic medicine as a brain tonic however its neuroprotective effect against streptozotocin (STZ) induced cognitive impairment and oxidative stress has not been reported yet in vivo. Therefore, the present study was attempted to investigate the neuroprotective potential of ethanolic extract of *A. marmelos* leaves (AME) on STZ induced memory impairment in male rats.
Main methods: Albino Wistar rats were pre-treated orally with AME at the doses 200 and 400 mg/kg for two weeks, followed by intracerebroventricular (i.c.v.) injection of STZ (3 mg/kg) on day 1 and 3. Two weeks after STZ administration, behavioural parameters were monitored using Morris water maze task. Biochemical and histopathological studies were carried out after three weeks of STZ administration. The levels of oxidative stress markers (malondialdehyde (MDA), glutathione, nitrite, catalase) neuroinflammatory mediators; tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) and acetylcholinesterase (AChE) activity were estimated in hippocampus of rat brain. Donepezil (5 mg/kg) was taken as a standard drug.
Key findings: The levels of MDA, nitrite, TNF-α and IL-6 were significantly increased while glutathione levels were significantly decreased in hippocampus of STZ-treated rats. Further, a significant decrease in the activity of catalase and increase in AChE activity was observed indicating cholinergic hypofunction and neuronal damage in STZ-treated animals. All these alterations were significantly ameliorated by AME in a dose dependent manner.
Significance: The neuroprotective potential of *A. marmelos* against STZ induced oxidative stress and cognitive deficit in rats indicates its therapeutic value in Alzheimer's disease (AD).

1. Introduction

Alzheimer's disease (AD), the commonest form of dementia accounting for > 80% of cases worldwide, is among the leading causes of cognitive impairment in elderly [1]. With a new case being diagnosed in every 65 s, it has become the sixth leading cause of deaths in U.S. Estimated 5.7 million people in U.S. alone are affected with AD and this number is expected to rise to approximately 14 million by 2050 [2]. AD is characterized by a gradual loss of cholinergic neurons in brain regions such as cerebral cortex and hippocampus leading to progressive decline in cognitive functions and memory loss [3]. The pathological hallmarks of AD are intracellular neurofibrillary tangles and extracellular amyloid plaques. While the neurofibrillary tangles are insoluble twisted fibres consisting of hyperphosphorylated microtubule-associated protein known as tau, amyloid plaques are formed by cleavage of amyloid-β peptide from neuronal transmembranous amyloid precursor protein. Accumulation of neurofibrillary tangles and amyloid-β plaques in neuronal cells increases the free radical generation resulting in oxidative stress and apoptosis [1,4,5] Oxidative stress causes impairment of energy metabolism resulting in cholinergic dysfunction and is the important factor involved in the development and progression of AD [6].

The behavioural, cognitive and neuropathological changes observed in AD can be induced by intracerebroventricular (i.c.v.) administration of streptozotocin (STZ) in rats [7] and at subdiabetogenic doses, it is considered as established, standard and reproducible approach to sporadic AD [8]. It causes amyloid β deposition, tau hyperphosphorylation,

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https://doi.org/10.1016/j.lfs.2019.02.032
Received 16 October 2018; Received in revised form 6 February 2019; Accepted 13 February 2019
Available online 14 February 2019

depletion of insulin and insulin like growth factor signaling mechanism resulting in prolonged impairment of memory by inhibiting the synthesis of ATP and acetyl coenzyme A. This in turn results in reduced cholinergic function and increased oxidative stress in hippocampal region of the rat brain [9,10]. Currently available drugs for AD i.e. donepezil, rivastigmine, galantamine and memantine only provide functional relief of symptoms and do not alter the course of disease. Moreover, they have peripheral and central adverse effects, which necessitate the search of safe and natural therapeutic agents for treatment of AD [11,12].

Aegle marmelos (L.) (*A. marmelos*), commonly known as bael, Bengal quince, golden apple and stone apple; belongs to family Rutaceae and is widely distributed in Indian subcontinent and Southeast Asia [13]. It has been extensively used as a traditional medicine in India, Nepal, Bangladesh, Myanmar and Sri Lanka since many years. All the parts of this tree viz. fruits, leaves, bark, roots and seeds have great therapeutic value in Ayurveda. Various studies have reported that different parts of *A. marmelos* possess gastroprotective, anti-diabetic, anti-oxidant, memory enhancing, anti-hyperlipidemic, anti-diarrhoeal, contraceptive, radioprotective, antiproliferative, analgesic, anti-inflammatory, antipyretic and antimicrobial properties [14–22].

A wide range of bioactive compounds such as coumarins, tannins, alkaloids, pectins, flavonoids, carotenoids and terpenes have been isolated from different parts of *A. marmelos*. Diverse phytochemicals present in bael include marmelosin, marmelin, marmelide, psoralen, alloimperatorin, skimmianine, rutaretin, scopoletin, aegelin, fagarine, anhydromarmelin, limonene, betulinic acid, marmesin, imperatorin, luvangentin and auraptene, lupeol, cineole, eugenol, citral and citronellal. Aegeline is the important constituent of *A. marmelos* leaves possessing cardioactive and anti-hyperglycemic activities [23,24]. It also stimulates glucose transport and improves insulin sensitivity of skeletal muscles through Akt and Rac1 signaling pathways as reported in a recent study [25].

A. marmelos bears alternate, trifoliate and ovate leaves which are made up of three to five oval, pointed leaflets. Traditionally, bael leaves have been used in fever, abdominal pain, dysentery, vomiting, dyspepsia and urinary troubles [26]. *A. marmelos* leaf extract has shown anti-diabetic, analgesic, anti-hyperlipidemic, anti-obesity, anti-fungal and anti-oxidant activities in various studies [14,16,27–30]. In addition, *A. marmelos* leaf extract has shown to possess acetylcholinesterase (AChE) inhibitory activity and antioxidant potential in vitro [31]. Although *A. marmelos* has memory enhancing and anti-oxidant properties, yet no study evaluating its anti-Alzheimer's potential using STZ induced AD model has been reported. In view of the above facts, in the present study, we investigated the effect of ethanolic extract of *A. marmelos* leaves (AME) in i.c.v. STZ induced oxidative damage and memory impairment in rats through the estimation of different oxidative stress markers as well as various behavioural, biochemical and neurochemical parameters.

2. Materials and methods

2.1. Chemicals and drugs

STZ, glutathione reduced, glutathione oxidised, 5-5′-dithiobis-2-nitrobenzoic acid (DTNB), thiobarbituric acid and donepezil were purchased from Sigma-Aldrich, USA. Biomarker, aegeline was procured from Clearsynth, Mumbai. All the chemicals used were of analytical grade.

2.2. Animals

Male albino Wistar rats (350–400 g, 6–8 months old) were obtained from Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar. The animals were housed in polypropylene cages with standard housing conditions of 23 ± 2 °C temperature and 60–65% relative humidity in a natural light/dark cycle, provided food and water ad libitum. The protocol was reviewed and approved by Institutional Animal Ethics Committee, Jan Nayak Ch. Devi Lal Memorial College of Pharmacy, Sirsa, India (Approval no. JCDMCOP/IAEC/06/16/36). Ethical standards were maintained for carrying out all the experimental procedures on animals.

2.3. Preparation of extract

Fresh leaves of *A. marmelos* were collected from the herbal garden of Jan Nayak Ch. Devi Lal Memorial College of Pharmacy, Sirsa, India and were authenticated from Raw Materials Herbarium and Museum at National Institute of Science Communication and Information Resources (NISCAIR), New Delhi, India (Ref no. 2017/3104-53-1). A voucher specimen was submitted at NISCAIR. The leaves were washed and shade dried for 7 days and then coarsely powdered and defatted with hexane. Next, the powdered material was soaked in ethanol, and occasional stirring was done for 7 days, later on the day 8, the extract was strained and evaporated under reduced pressure and was stored at 2–4 °C protected from sunlight till further studies. The ethanolic extract of *A. marmelos* obtained was dissolved in saline each time prior to pharmacological studies.

2.4. Standardization of extract

A. marmelos extract was standardized by high performance liquid chromatography (HPLC) using aegeline as marker. The HPLC instrument (Shimadzu, Kyoto, Japan) was equipped with two LC-10 ATVP pumps, SPD-10AVP UV–Visible detector and rheodyne injector with a 50 µl loop. Shimadzu LC-solution version 6.42 software was used for data acquisition and processing. The mobile phase consisted of 10 Mm ammonium acetate and acetonitrile and was pumped at a flow rate of 1 ml/min with detection wavelength of 200 nm. The calibration curve of aegeline was prepared from peak areas obtained using five different concentrations (1–5 µg/ml). A stock solution of AME was prepared at the concentration of 10 mg/ml in methanol.

2.5. Experimental design and grouping of animals

The animals were randomly divided into five groups ($n = 8$). Group I-sham operated, received normal saline; Group II-i.c.v. STZ-treated; Group III-i.c.v. STZ administered and pre-treated with AME (200 mg/kg/day, p.o.) for two weeks; Group IV-i.c.v. STZ administered and pre-treated with AME (400 mg/kg/day, p.o.) for two weeks and Group V-i.c.v. STZ administered and treated with donepezil (5 mg/kg). The doses of AME were selected based on previous study [32].

2.6. Experimental induction of AD

After anesthetizing the animal with ketamine (100 mg/kg, i.p.) and xylazine (5 mg/kg, i.p.), the head was fixed in a stereotaxic apparatus and scalp was shaved. A midline sagittal incision was made in the scalp and a burr hole was drilled on either side of the skull for insertion of cannula into cerebral ventricles according to the stereotaxic atlas: 0.8 mm antero-posterior from bregma; 1.5 mm medio-lateral from midline; 3.6 mm dorso-ventral from the skull [33]. STZ (3 mg/kg) was dissolved in freshly prepared saline and 10 µl was infused slowly into each lateral ventricle using Hamilton syringe needle on day 1 and 3 [34,35]. After surgery, animals were administered gentamicin (5 mg/kg, i.p.) and neomycin powder topically and were kept in aseptic environment with regulated temperature to prevent infection.

2.7. Behavioural assessment

2.7.1. Evaluation of learning and spatial memory

Morris water maze (MWM) task, as described by Morris in 1984, was performed to test the spatial memory of animals, two weeks after

the surgery. A circular water pool of 130 cm diameter and 60 cm height, filled with water (27 ± 1 °C) up to 3/4th level, was divided into four equal quadrants [36,37]. A round platform (10 cm diameter) was submerged 1 cm beneath the water surface in any one of the quadrant and its position was fixed during the entire experiment. The water in the pool was made opaque by using non-toxic paint so that the platform was invisible. Animals were given 4 trials per day (with a gap of 30 s) in a 4 days training period to find the submerged platform. The trial was started by placing the animal in the pool facing the wall of the pool. The maximum time given to the rats to find the platform was 60 s and once found, animals were allowed to rest there for 30 s. The time taken to locate the platform (latency time) was observed in every trial and the rats that could not find the platform within 60 s were trained by the experimenter to reach the platform. A significant decrease in mean latency during training period was considered as successful memory retention [38].

On the day 5 of MWM task, a probe trial for 60 s was conducted to assess the memory by removing the platform and the rats were allowed to swim freely in the pool. Time spent by rats in the target quadrant (in which the submerged platform was placed) was recorded which indicated the degree of memory consolidation after learning sessions.

2.7.2. Spontaneous locomotor activity

The locomotor activity of the animals was assessed on day 21 by using digital actimeter. Each animal was observed for a period of 10 min in photoactometer equipped with infrared beams and photocells connected to a digital counter. Motor activity was estimated as number of counts of beam interruptions.

2.8. Estimation of biochemical parameters

2.8.1. Preparation of brain homogenate

Animals were sacrificed on day 22 following first i.c.v. STZ administration by euthanizing with lethal dose of anaesthesia. Brains were removed quickly and rinsed with ice cold saline. The hippocampus was isolated and hippocampal tissue was homogenized with 0.1 M phosphate buffer (pH 7.4) in a tissue homogenizer. The tissue homogenate (10% w/v) was then centrifuged (Remi cold centrifuge) at 10,000*g* for 15 min and supernatant was collected for further estimation of following biochemical parameters.

2.8.2. Protein estimation

Total protein was estimated by the method of Lowry et al. [39] with slight modification. The absorbance was measured at 750 nm by spectrophotometer. Bovine serum albumin (1 mg/ml) was used to plot a standard curve. The amount of total protein was expressed in mg [39].

2.8.3. Estimation of oxidative stress markers

2.8.3.1. Reduced glutathione (GSH) estimation. GSH in the prepared homogenate was estimated by Ellman's method, 1959. Briefly, 1 ml of 4% sulfosalicylic acid was added to 1 ml supernatant and digested for 1 h at 4 °C. The mixture was subjected to centrifugation at 1200*g* for 15 min. One milliliter of fresh supernatant was mixed with 2.7 ml of phosphate buffer (0.1 M, pH 7.4) and 0.2 ml of DTNB. The yellow colour developed was measured immediately at 412 nm using spectrophotometer. GSH concentration was calculated from the standard curve and expressed as μmol/mg protein [40].

2.8.3.2. Malondialdehyde (MDA) estimation. MDA, an oxidative stress marker, in brain tissue homogenate was estimated by the method reported by Ohkawa et al. [41]. Briefly, to 0.1 ml of tissue homogenate, 1.5 ml of acetic acid (20% v/v, pH 3.5), 0.2 ml of dodecyl sulphate (8.1% w/v) and 1.5 ml of thiobarbituric acid (0.8% w/v) were added and the mixture was heated at 90 °C for 1 h. After cooling, 5 ml of n-butanol/pyridine and 1 ml distilled water were added to the mixture followed by centrifugation at 4000 rpm for 10 min. The absorbance was measured at 532 nm using spectrophotometer (Shimadzu, UV-1700). The concentration of MDA was estimated using the standard curve prepared and demonstrated as nmol/mg protein [41].

2.8.3.3. Nitrite estimation. Estimation of nitrite, an indicator of nitric oxide production, was carried out by colorimetric assay described by Green et al. [42] using Griess reagent (1% sulfanilamide, 2.5% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediaminedihydrochloride). 100 μl each of Griess reagent and supernatant were mixed and incubated for 10 min at room temperature. The absorbance was measured at 542 nm spectrophotometrically. The concentration of nitrite was determined from sodium nitrite standard curve and was expressed as μmol/mg protein [42].

2.8.3.4. Catalase estimation. The activity of catalase, an anti-oxidant enzyme which removes hydrogen peroxide, was determined by Aebi's method, 1974. Briefly, to the 0.05 M phosphate buffer (pH 7.0), 0.019 M hydrogen peroxide and 0.1 ml post mitochondrial supernatant were added. The change in absorbance was recorded at 240 nm and catalase activity was calculated as nmol of hydrogen peroxide (H_2O_2) consumed/min/mg protein [43].

2.8.4. AChE assay

AChE assay was performed by the previously reported method of Ellman et al. [44]. To 0.05 ml of supernatant, 3 ml of 0.01 M sodium phosphate buffer (pH 8), 0.1 ml of AChE iodide and 0.1 ml of Ellman reagent were added. The absorbance was measured immediately at 412 nm using spectrophotometer. The AChE activity in the samples was expressed as nmol/mg protein [44].

2.9. Estimation of neuro-inflammatory mediators

The estimation of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) was carried out as marker of neuro-inflammation. The assay procedure for TNF-α and IL-6 estimation was carried out as per the instructions provided by Quantikine rat TNF-α and Quantikine rat IL-6 (R&D Kit system 2006, Becton Dickinson Biosc., India).

2.10. Histopathological studies

The rat brains were isolated and cut into 3–5 mm sections exposing hippocampus. The tissues were dehydrated and blocks were prepared by embedding tissues in paraffin. The blocks were cut into 5 to 6 μm slices using microtome and staining was done with hematoxylin and eosin.

2.11. Statistical analysis

The data was analyzed by applying one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for biochemical evaluations and two-way ANOVA for behavioural parameters and the results were expressed as mean ± S.D. $P < 0.05$ was considered as significant in all cases.

3. Results

3.1. Quantification of Aegeline in the extract

The concentration of aegeline in ethanolic extract of *A. marmelos* leaves was found to be 25.523 μg/mg of dry extract. Chromatograms of standard aegeline and the ethanolic extract of *A. marmelos* are shown in Fig. 1.

3.2. Effect of AME on STZ induced memory impairment in MWM task

The effect of AME on spatial memory impairment was evaluated two weeks after STZ administration by measuring the escape latencies in

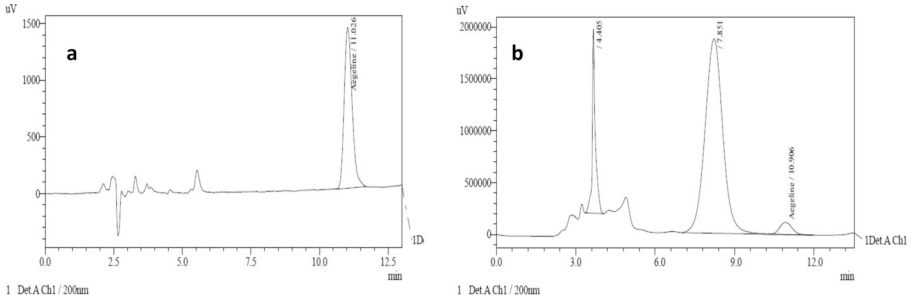

Fig. 1. Chromatogram of a. standard compound aegeline b. ethanolic extract of *A. marmelos* leaves.

MWM task. Two-way ANOVA for escape latency showed that there was significant main effect for drug ($F = 519$, $P < 0.001$, $df = 3$), a significant time effect ($F = 163.5$, $P < 0.001$, $df = 4$) and a significant drug × time interaction effect ($F = 35.09$, $P < 0.001$, $df = 12$). Further, post hoc analysis indicated a decrease in escape latencies in sham group ($P < 0.001$) as compared to STZ group which shows impairment of learning and memory in STZ-treated rats. Pre-treatment with AME decreased the STZ induced memory impairment dose dependently. AME (400 mg/kg) was more effective in reversing the STZ induced memory deficit than AME (200 mg/kg) (Fig. 2a).

3.3. Probe trial

On day 5 of training period, the platform was removed to assess the memory. STZ treated rats failed to remember the location of platform and spent significantly less time ($P < 0.001$) in the selected quadrant as compared to sham group. However, the mean percent time spent in same quadrant was significantly increased in the rats pre-treated with AME (200 and 400 mg/kg) as compared to STZ treated rats ($F = 248.4$, $P < 0.001$, $df = 39$) indicating improvement of learning and memory (Fig. 2b).

3.4. Effect of AME on GSH levels

In the brains of STZ-treated rats, a significant depletion in GSH levels was observed as compared to sham group ($P < 0.001$). However, the reduction in GSH levels was significantly improved by AME pre-treatment (200 and 400 mg/kg) ($F = 162.6$, $P < 0.001$, $df = 39$). The effect of AME (400 mg/kg) was similar to that of donepezil (5 mg/kg) (Fig. 3a).

3.5. Effect of AME on MDA levels

There was a significant rise in MDA levels in STZ-treated rats as compared to sham group ($P < 0.001$). However, pre-treatment with AME (200 and 400 mg/kg) produced a significant decrease in MDA levels in STZ-treated brains as compared to STZ group ($F = 394.6$, $P < 0.001$, $df = 39$) (Fig. 3b). Also, the effect of AME (400 mg/kg) on MDA level was similar to that produced by donepezil (5 mg/kg).

3.6. Effect of AME on nitrite levels

The level of nitrite increased significantly in STZ control rats as compared to sham group animals ($P < 0.001$). AME at 400 mg/kg and 200 mg/kg doses showed a significant decrease in nitrite levels compared to STZ group ($F = 69.79$, $P < 0.001$, $df = 39$). Further, no significant difference ($P > 0.05$) was observed between AME (400 mg/kg) and donepezil (5 mg/kg) treated groups (Fig. 3c).

3.7. Effect of AME on catalase activity

Fig. 3d shows that the catalase activity decreased significantly in STZ treated group ($P < 0.001$) as compared to sham group. There was restoration of enzymatic activity in rats pre-treated with AME (200 and 400 mg/kg) ($F = 450$, $P < 0.001$, $df = 39$) as compared to STZ group.

3.8. Effect of AME on AChE activity

A significant increase in brain AChE activity was observed in STZ group rats as compared to sham group ($P < 0.001$). AME (200 and 400 mg/kg) and donepezil (5 mg/kg) administration in STZ infused rats significantly decreased the enhanced AChE activity compared to STZ control rats ($F = 121.6$, $P < 0.001$, $df = 39$). The higher dose of AME was more effective in ameliorating the effect of STZ (Fig. 4a).

3.9. Spontaneous locomotor activity

The difference in spontaneous locomotor activity among the groups was not significant ($F = 1.259$, $P > 0.05$, $df = 39$) (Fig. 4b).

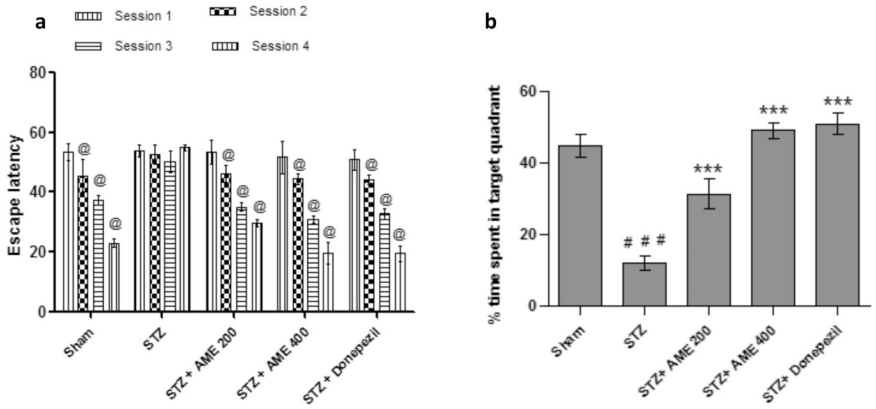

Fig. 2. Effect of AME on a. escape latency, Data was analyzed by two-way ANOVA. $^{@}P < 0.001$ compared to session 1, b. percent time spent in target quadrant. Data are expressed as mean ± S.D. ($n = 8$). $^{\#\#\#}P < 0.001$ vs sham group, $^{***}P < 0.001$ vs STZ group.

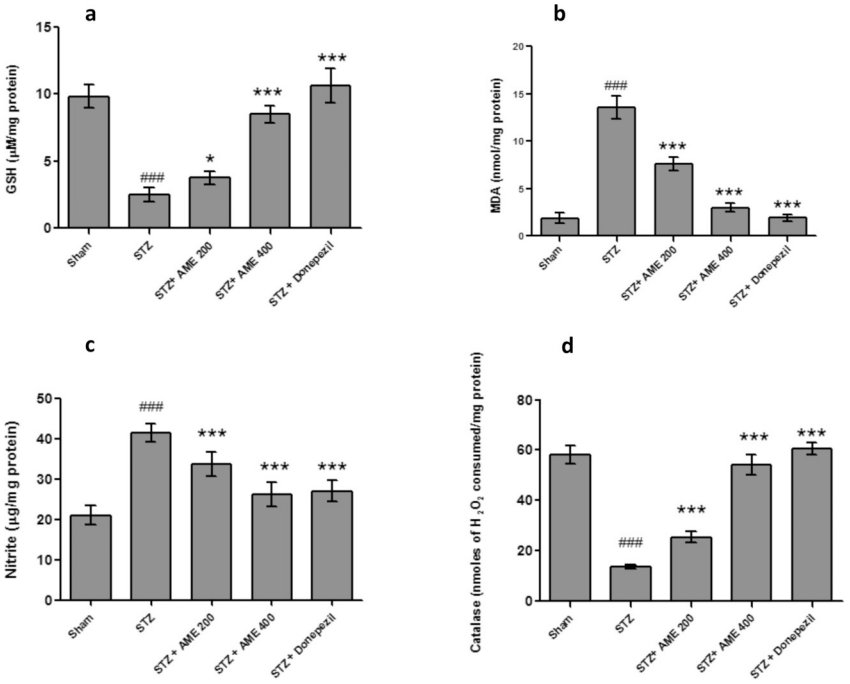

Fig. 3. Effect of AME on a. GSH level, b. MDA level, c. Nitrite level, d. Catalase activity. Data are expressed as mean ± S.D. $^{###}P < 0.001$ vs sham group, $^{*}P < 0.05$ vs STZ group, $^{***}P < 0.001$ vs STZ group.

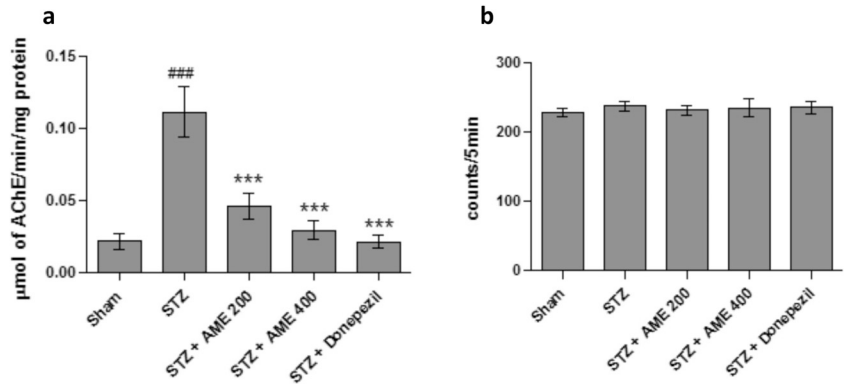

Fig. 4. Effect of AME on a. AChE activity b. locomotor activity. Data are expressed as mean ± S.D. $^{###}P < 0.001$ vs sham group, $^{*}P < 0.05$ vs STZ group, $^{***}P < 0.001$ vs STZ group.

3.10. Effect of AME on neuroinflammatory mediators: TNF-α and IL-6 levels

Increased levels of cytokines including TNF-α and IL-6 in the hippocampus of STZ-treated rats indicated neuroinflammation. The changes were significant as compared to sham group (Fig. 5). AME pre-treatment at 200 mg/kg and 400 mg/kg significantly decreased the levels of TNF-α ($F = 4271$, $P < 0.001$, $df = 39$) and IL-6 ($F = 1460$, $P < 0.001$, $df = 39$) as compared to STZ group. The effects of AME at higher dose and donepezil (5 mg/kg) were similar.

3.11. Effect of AME on histopathological changes

Histopathological changes in hippocampus region of brain of STZ-treated rats and AME pre-treated rats are shown in Fig. 6. STZ administration resulted in neuronal damage whereas it was prevented by AME pre-treatment. In STZ group, there was shrinkage of neuronal cell bodies and nuclei were hyperchromatic with irregular shape. However, pre-administration of AME in STZ-treated rats reversed these changes as compared to STZ group.

4. Discussion

Though the etiology of AD is unknown, oxidative stress is considered as important factor in progression of AD [45,46]. Administration of i.c.v. STZ results in oxidative stress and progressive decline of memory and is considered as most appropriate animal model for sporadic AD. The oxidative damage and memory deficit caused by STZ in the current study is in line with earlier findings [47,48]. Administration of i.c.v. STZ in male rats at subdiabetogenic doses caused memory impairment which was evaluated by MWM task, the widely accepted model of spatial memory. In MWM task, STZ administration caused impairment of learning and memory in rats which is in accordance with previous reports [49–51]. AME significantly reversed these changes dose-dependently. STZ induced cognitive decline was confirmed in probe trial as STZ-treated rats stayed in selected quadrant for less duration. Nevertheless, AME treatment significantly increased the duration of stay of rats in the selected quadrant indicating memory retention. Spontaneous locomotor activity of animals was also evaluated to rule out the possibility that modulation of cognitive function was due to changes in locomotor activity. Further, any significant

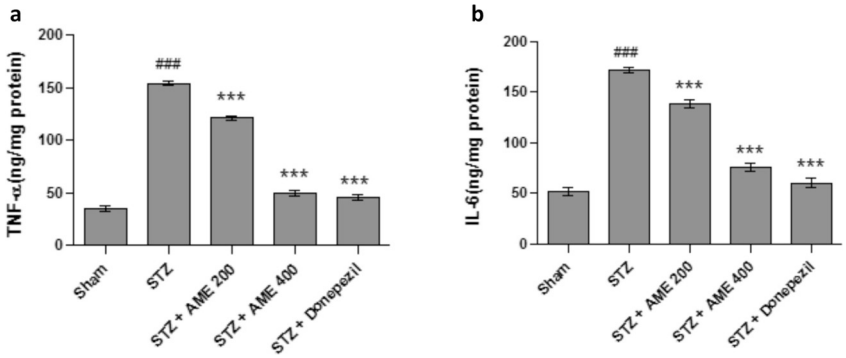

Fig. 5. Effect of AME on a. TNF-α level in STZ treated rats; b. IL-6 level in STZ treated rats. Data are expressed as mean ± S.D. $^{###}P < 0.001$ vs sham group, $^{***}P < 0.001$ vs STZ group.

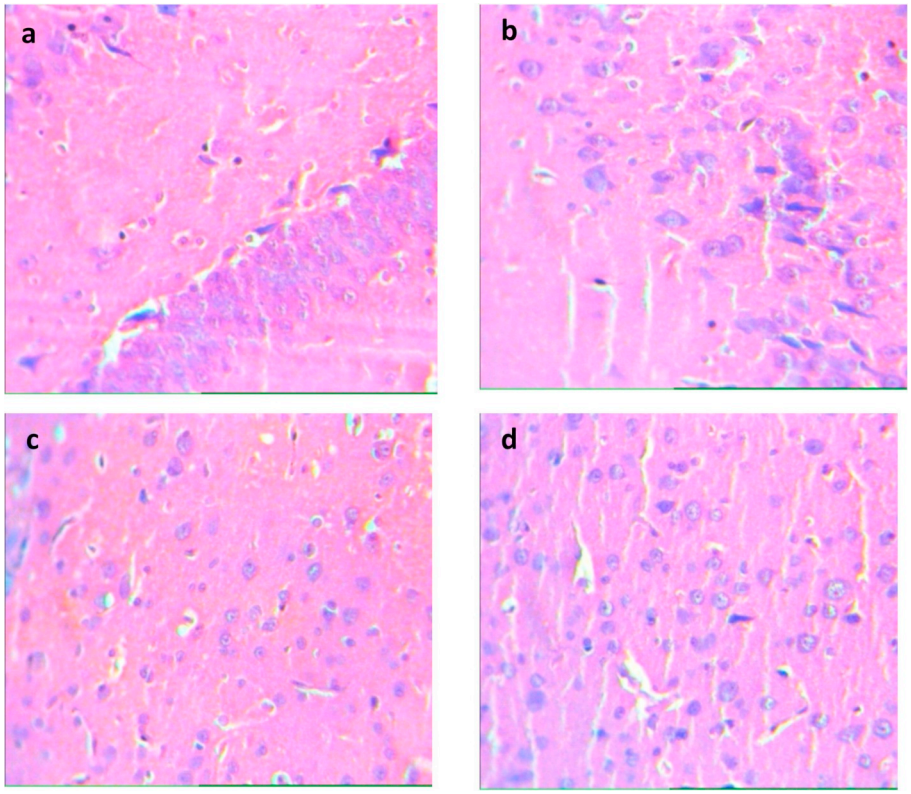

Fig. 6. Photomicrographs of hippocampal region of rat stained with hematoxylin and eosin. a. Sham control, hippocampal area showing intact neuronal layer with large, clear nucleus, b. STZ control, neurons are shrunken with irregular shape and hyperchromatic nuclei, c. STZ + AME 200, neuronal shrinkage was not observed, d. STZ + AME 400, neuronal shrinkage was not observed and round nuclei were seen.

difference in locomotor activity among the groups was not observed in the present study indicating that locomotion had no role in learning and memory function.

Growing evidences indicate that the decreased activity of free radical scavenging enzymes such as catalase results in accumulation of free radicals like H_2O_2. It has been suggested that GSH detoxifies the free radicals generated in brain and increases the H_2O_2 clearance. However, STZ decreases the activity of antioxidant enzyme catalase and GSH levels in brain resulting in decreased elimination of H_2O_2 and formation of OH which causes oxidative damage. As the level of H_2O_2 increases, the peroxidation of polyunsaturated fatty acids is induced resulting in formation of MDA. Furthermore, STZ also increases the nitrite stress resulting in increased nitrite levels. In the present study as well, STZ decreased the catalase activity and levels of GSH while increased the levels of MDA and nitrite; AME pre-treatment reversed these effects dose dependently.

The cerebral cortex, hippocampus and amygdala are the major regions of brain involved in pathogenesis of AD and are more prone to oxidative damage. Cholinergic transmission in these brain regions, especially hippocampus, plays an important role in learning and memory. However, the loss of cholinergic neurons at this site results in cholinergic hypofunction which eventually results in cognitive impairment in AD patients. Thus, inhibition of AChE improves the symptoms of cognitive deficit in AD by elevating the levels of acetylcholine. Currently, only three cholinesterase inhibitors: donepezil, rivastigmine and galantamine are approved by FDA for the treatment of AD. They only treat symptoms of AD and none of these alter the progression of AD. In past few years, great attention has been focused on natural bioactive compounds for AD therapy owing to their anti-oxidant potential, lesser side effects and ability to modify the disease process. Natural antioxidants, melatonin and resveratrol have shown considerable improvement in cognitive function and oxidative damage in STZ treated rats in previous studies [34,52,53]. In the same line in vitro antioxidant and anti-cholinesterase activities of *A. marmelos* leaves have also been demonstrated previously [31]. Similar effect was observed in the present study, where AME pre-treatment improved the cholinergic function by significantly decreasing the AChE activity in rat brains which was previously increased by STZ administration.

The inflammatory mediators, TNF-α and IL-6 are expressed in hippocampus and involved in memory acquisition, consolidation and retrieval [54]. These are produced by activated microglia and are involved in pathogenesis of AD. Administration of STZ activates microglia and increases the number of activated astrocytes in hippocampus and thus the levels of neuroinflammatory mediators [55]. In the present study, STZ administration increased the levels of TNF-α and IL-6 in hippocampal region of rat brain However, pre-treatment with AME significantly reduced the levels of these inflammatory mediators indicating the anti-inflammatory effect of. *A. marmelos*.

Histopathological studies of hippocampal region of rat brain identified large neurons with well-defined shape, cytoplasm and prominent nuclei in sham group. There was shrinkage of neuronal cell bodies with irregular shape and hyperchromatic nuclei indicating neuronal damage in STZ treated group. Although, there was significant improvement in size and shape of neurons in AME treated group.

The results of the present study reveal that *A. marmelos* exhibits neuroprotective effect against STZ induced oxidative stress and cognitive impairment in male rats. This effect may be attributed to its antioxidant activity. In addition to suppression of oxidative stress, *A. marmelos* also suppresses AChE activity and neuroinflammatory mediators. It is postulated that *A. marmelos* has potential to treat AD and other neurodegenerative disorders and further studies may be designed to explore the underlying mechanism of its neuroprotective effect.

5. Conclusion

In the current work, the effect of AME on oxidative damage and cognitive deficit induced by i.c.v. STZ was investigated in rats. It was found that i.c.v. STZ produced neurotoxic effects through the generation of free radicals. However, pre-treatment with *A. marmelos* leaf extract ameliorated oxidative stress, neuroinflammation, cholinergic hypofunction and memory impairment induced by STZ, dose dependently. Overall *A. marmelos* has the potential to ameliorate the cognitive impairment caused by STZ.

Acknowledgement

The authors are thankful to Jan Nayak Chaudhary Devi Lal Memorial college of Pharmacy, Sirsa and IKG Punjab Technical University, Jalandhar, India for providing necessary facilities to complete this research work.

Conflict of interest

The authors declare no conflicts of interest.

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