Journal of Trace Elements in Medicine and Biology 31 (2015) 107–112

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Pharmacology

Mangiferin ameliorates aluminium chloride-induced cognitive dysfunction via alleviation of hippocampal oxido-nitrosative stress, proinflammatory cytokines and acetylcholinesterase level

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ARTICLE INFO

Article history:
Received 17 January 2015
Accepted 13 April 2015

Keywords:
Neurotoxicity
Oxido-nitrosative stress
Mangiferin
Aluminium chloride
Cognitive dysfunction

ABSTRACT

Mangiferin is a phytochemical primarily present in the stem, leaves and bark of *Mangifera indica*. It offers neuroprotection mainly through inhibition of oxidative stress, and decreasing proinflammatory cytokines level in the brain. Aluminium has been reported to cause oxidative stress-associated damage in the brain. In the present investigation, protective effect of mangiferin against aluminium chloride ($AlCl_3$)-induced neurotoxicity and cognitive impairment was studied in male Swiss albino mice. $AlCl_3$ (100 mg/kg) was administered once daily through oral gavage for 42 days. Mangiferin (20 and 40 mg/kg, p.o.) was given to mice for last 21 days of the study. We found cognitive dysfunction in $AlCl_3$-treated group, which was assessed by Morris water maze test, and novel object recognition test. $AlCl_3$-treated group showed elevated level of oxidative stress markers, proinflammatory cytokines level and lowered hippocampal brain-derived neurotrophic factor (BDNF) content. Mangiferin (40 mg/kg) prevented the cognitive deficits, hippocampal BDNF depletion, and biochemical anomalies induced by $AlCl_3$-treatment. In conclusion, our data demonstrated that mangiferin offers neuroprotection in $AlCl_3$-induced neurotoxicity and it may be a potential therapeutic approach in the treatment of oxido-nitrosative stress and inflammation-associated neurotoxicity.

Introduction

Aluminium is a nonessential element and one of the most abundant metals present in the earth's crust. Extensive use of aluminium in water purification, cooking ware, beverages, food packing, cosmetics, and pharmaceutical industries enhance the chance of human exposure to the aluminium. Prolonged half-life of aluminium (i.e. 150 days in rats) raises the risk of accumulation that eventually leads to neurotoxicity [1,2]. Aluminium exposure leads to neurobehavioural, neurochemical, and neuropathological anomalies [3]. These anomalies accompanied with the release of cytochrome c from mitochondria which eventually leads to upsurge in the production of free radicals, proinflammatory cytokines that results in oxidative stress, and neuroinflammation [4]. Aluminium is a non-redox metal, however numerous studies showed its pro-oxidant effect in the biological system. Aluminium exerts its pro-oxidant effect mainly through influencing the membrane lipids, pro-oxidants and oxidants action [5]. Evidences from the previous studies suggested that chronic administration of aluminium results in the accumulation of aluminium in various brain regions [6]. However, hippocampus is the main target for aluminium accumulation compared to the other brain regions [7]. High accumulation of aluminium in hippocampus contributes to the cognitive dysfunction mainly through inhibition of long-term potentiation (LTP) by disrupting the glutamate–nitric oxide–cyclic guanosine mono-phosphate pathway.

Mangiferin is a well known natural C-glucosylxanthone phytochemical mainly present in the bark, leaves and root of *Mangifera indica*. Mangiferin has been found to exhibit antioxidant, anti-inflammatory, anti-anxiety, anti-depressive, and neuroprotective activities [8–10]. Previous experimental study provides the evidence for the blood brain barrier (BBB) crossing ability of mangiferin in the gerbils [11]. Moreover, the Log *P* value of mangiferin is +2.73 which further supports its BBB permeability. Furthermore, mangiferin was found to be a non-toxic

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http://dx.doi.org/10.1016/j.jtemb.2015.04.002
0946-672X/

compound in the rodents [12]. In the present study, we investigated whether mangiferin treatment could affect the aluminium chloride ($AlCl_3$)-induced neurotoxicity and cognitive dysfunction in mice. Hippocampal oxido-nitrosative stress estimation, proinflammatory cytokines, brain-derived neurotrophic factor (BDNF) and acetylcholinesterase (AChE) level were determined to probe the mechanism involved.

Materials and methods

Materials

$AlCl_3$ (catalogue no. 449598; 99.99% purity) and mangiferin (catalogue no. M 3547; ≥98% purity) were purchased from Sigma–Aldrich, St. Louis, MO, USA. All other chemicals used in the experimental study were of analytical grade. $AlCl_3$ (100 mg/kg) was dissolved in drinking water and administered once daily through oral gavage for 6 weeks. Mangiferin was dissolved in dimethyl sulfoxide and administered orally 1 h after $AlCl_3$ administration for last 3 weeks. Mangiferin (20 and 40 mg/kg) doses were selected for the treatment based on our previous experimental study [9]. Interleukin-1β (IL-1β) (Thermo Fisher Scientific, India), and tumour necrosis factor-α (TNF-α) (Invitrogen Co., Carlsbad, CA, USA) ELISA kits were used to determine proinflammatory cytokines level. Nitrite level was measured using Griess reagent, purchased from Sigma–Aldrich, St. Louis, MO, USA. BDNF Emax® ImmunoAssay kit (Promega, Madison, WI, USA) was used to determine BDNF level.

Experimental design

Male Swiss albino mice (weight: 25–30 g) were used in the present experimental study. Animals were procured from College of Veterinary Sciences, Khanapara, Guwahati and acclimatized to laboratory conditions for 7 days before commencement of the experiment. Mice were caged in groups of 4 and given food and water ad libitum. Experiments were performed in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India guidelines, after approval by the Institutional Animal Ethics Committee (IAEC), Gauhati Medical College & Hospital (IAEC approval no. MC/32/2013/13) (CPCSEA Registration No. 351, 3/1/2001). The animals were kept at room temperature (24 ± 1 °C), with 65 ± 5% humidity and 12 h light and dark cycles. After acclimation for one week, mice were randomly divided into six experimental groups and treated as follows:

Group A: administered drinking water only.
Group B: administered orally $AlCl_3$-100 mg/kg only for 42 days.
Group C: administered orally $AlCl_3$-100 mg/kg for 42 days and mangiferin-20 mg/kg for last 21 days.
Group D: administered orally $AlCl_3$-100 mg/kg for 42 days and mangiferin-40 mg/kg for last 21 days.
Group E: administered orally mangiferin-20 mg/kg only for 21 days.
Group F: administered orally mangiferin-40 mg/kg only for 21 days.

Cognitive function was assessed by performing the Morris water maze (MWM) test and novel object recognition test (NORT) on 42nd day after $AlCl_3$ and drug treatment. MWM test and NORT was performed in a dimly illuminated and isolated room. Different animal groups were taken for behavioural and biochemical estimation to avoid the probable effect of behavioural experiments on biochemical parameters. The animals were killed by cervical dislocation and hippocampus was quickly dissected out from the isolated brain and homogenized in ice cold phosphate buffer saline (pH 7.4), centrifuged and the supernatants were stored at −80 °C until oxido-nitrosative stress, proinflammatory cytokines assessment were performed.

Cognitive assessment

Morris water maze (MWM) test

MWM test is a useful tool to assess the learning and memory function in rodents [13]. In brief, a circular tank (120 cm diameter, 50 cm height) consisted of 4 equal quadrants; containing opaque water (25 ± 1 °C) was used. The platform (10 cm) was placed 1 cm above the water level in acquisition phase. The animal was kept in the pool and allowed to locate the platform for 120 s during the acquisition phase. If the animal was failed to locate the platform in 120 s then animal was placed on the platform for 30 s. Four trials were given daily to each animal for 5 days and 5 min time interval was maintained between the subsequent trials. In each trial, animal was kept at different quadrants of the circular tank. In the retention phase, platform was kept 1 cm below the water level in the pool. Animal was kept in the quadrant facing towards the tank wall and evaluated the retention latency on 5th day through measuring the time taken by the animal to locate the hidden platform.

Moreover, separately on 6th day a probe trial was conducted to determine reference memory in the same water-filled circular tank which was devoid of platform. Animals were trained for initial 5 days in a similar manner that was previously used to determine retention latency. Animal was kept at novel place, facing towards the tank wall. Reference memory was evaluated by calculating the number of crossing over the platform region by the animal.

Novel object recognition (NORT) test

NORT was performed to investigate the recognition memory according to the method described by Leger et al. [14]. NORT consisted of three sessions: habituation, familiarization, and test session. A black coloured open field box (36 cm × 50 cm × 36 cm) was used in this test. In habituation phase, each mouse was habituated to the empty open field by placing it in the open field arena and allowed to explore for 5 min twice in a day. After 3 days of habituation phase, familiarization phase was performed by placing the two objects (a rectangular wooden block and a small rubber ball) at left and right position in the open field. Mouse was placed in open field with its head position opposite to the objects and allowed to explore freely for 10 min. After 24 h, test session was conducted by replacing wooden box with a novel object (a plastic box). Each mouse was allowed to explore for 3 min in the open field. In all phases of the test, objects and open field arena were repeatedly cleaned with alcohol (70%, v/v) to avoid the olfactory cues. Time spent to explore the familiar and novel object was recorded during the experiment. Recognition index, a ratio of the time spent in exploring novel object over the total time spent in exploring the both objects during test session was calculated and results were represented as recognition index in percentage. Total Exploratory period was also measured to confirm that the observed recognition index was not due to stimulation or suppression of sensorimotor function.

Acetylcholinesterase (AChE) activity measurement

Hippocampus was quickly isolated and homogenized in ice cold 0.1 M phosphate buffer. 0.4 mL of homogenate was mixed with 2.6 mL phosphate buffer (0.1 M, pH 8) and 100 μL of DTNB. The contents were mixed in the cuvette thoroughly by bubbling. Then, a 20 μL of acetylthiocholine was added and change in absorbance was recorded for a period of 5 min at intervals of 1 min. The enzyme activity was calculated using the formula: