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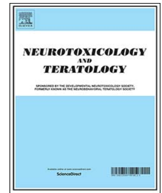
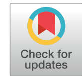


Full length article

# Neuroprotective effect of morin on lead acetate- induced apoptosis by preventing cytochrome *c* translocation *via* regulation of Bax/Bcl-2 ratio



Sumathi Thangarajan[a,*], Aishwarya Vedagiri[a], Shanmathy Somasundaram[b], Rathika Sakthimanogaran[b], Mahalakshmi Murugesan[a]

[a] Department of Medical Biochemistry, Dr. ALM Post Graduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, Chennai 600 113, Tamil Nadu, India

[b] Department of Biotechnology, Rajalakshmi Engineering College, Rajalakshmi Nagar, Thandalam, Chennai 602 105, Tamil Nadu, India

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ABSTRACT

Lead (Pb) intoxication is a prevalent type of environmental toxicity as well as minimal amount of lead exposure is liable for neurobehavioral or perhaps intelligence defects. The present study was undertaken to investigate the beneficial effects of morin in protecting the lead acetate (PbAc)-induced oxidative stress in rat brain. PbAc intoxication resulted in motor deficit, memory impairment and oxidative stress Further, PbAc administration alters Bax/Bcl-2 expression thereby increases cytochrome *c* release from the mitochondria. Treatment with morin at a dose of 40 mg/kg b.wt. significantly restored back the abnormal changes that were noticed in PbAc intoxicated rats. Histopathological sections of cortex, cerebellum and hippocampus showed the extent of neuronal loss in PbAc induced rats and its restoration upon administration of morin. These outcomes imply that morin might be employed therapeutically to chelate toxic metals like Pb, thus possibly lowering PbAc-induced neurotoxicity and tissue damage.

## 1. Introduction

Lead (Pb) is an extensively used heavy metal in nature as well as it is one of the oldest metals recognized by the humans. Ancient people generally made use of Pb for producing water pipes, lining baths as well as for fashioning decorative objects. Considering the industrial revolution in the middle of 18th century, manufacturing and usage of lead have drastically increased globally ensuing in intensive release and accumulation of this undestroyable metal in the atmosphere (Seddik et al., 2011; Flora et al., 2012). Exposure to Pb may have undesirable health consequences such as behavioral, neurological, respiratory, hematological, immunological, renal, hepatic and reproductive disorder (Patra et al., 2001; Mansouri and Cauli, 2009; Xia et al., 2010).

Pb toxic conditions exerts its most extreme implications in the developing brain (Burdette and Goldstein, 1986; Guilarte et al., 2000) and also its harmful effects on central nervous system (CNS) are identified as lead encephalopathy or perhaps lead neuropathy (Cloues et al., 2000). Pb is a widely identified neurotoxicant leading to impairments in both the human and experimental animals that typically causes functional and structural abnormalities in the brain (Lidsky and Schneider, 2003; White et al., 2007).

Experimental corroboration suggests that the cellular damage mediated through free radicals might be associated with the pathology involve in Pb poisoning (Verina et al., 2007; Flora et al., 2007). It is certainly demonstrated that Pb modifies the lipid metabolism consequently increases lipid peroxidation, and enhances brain thiobarbituric acid reactive substances (TBARS) *via* the suppression of superoxide dismutase (SOD) in different brain regions (Shukla et al., 1987; Skoczynska et al., 1990; Antonio et al., 1998; Wang et al., 2006). Hindered antioxidant defenses by cells might be an outcome of the inhibitory effects of Pb on several enzymes that triggers the cells to be most vulnerable to oxidative stress (Gurer et al., 1998).

Ample studies evidenced that the regulation of mitochondrial membrane permeability might be controlled *via* a family of proto-oncogenes. The Bcl-2 family of proto-oncogenes is anti-apoptotic (Bcl-2) or pro-apoptotic (Bad, Bax) (Tsujimoto and Shimizu, 2001). Bax activation causes its insertion into the mitochondrial membrane thereby increases membrane permeability (Eskes et al., 1998), this in turn leads to release of cytochrome *c*, activation of several caspases and cleavage of downstream death effector proteins. This ultimately results in apoptotic cell death (Lidsky and Schneider, 2003). The anti-apoptotic protein namely Bcl-2, suppresses the ability of Bax thereby increases membrane potential (Gross et al., 1999). As such, the stability of these essential proteins may ascertain the cell fate.

* Corresponding author.
*E-mail address:* sumathi.doctor@unom.ac.in (S. Thangarajan).

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Morin is a well-recognized naturally occurring bioflavonoid. It could possibly be isolated from natural herbs as well as fruits that belongs to Moraceae family such as onion, almond (*P. guajava* L.), fig (*Chlorophoratinctoria*), seed weeds, Osage orange, mill (*Prunusdulcis*) and red wine (Nandhakumar et al., 2012). Experimentally morin has showed different pharmacological potentials particularly antioxidant (Prahalathan et al., 2012; Merwid-Lad et al., 2012) and also anti-inflammatory (Fang et al., 2003). Reports have revealed in which morin exerted neuroprotective effects on 1-methyl-4-phenylpyridinium ion (MPP+)- induced neuronal apoptosis in PC12 cells and also in a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) animal model of Parkinson disease (PD) (Zhang et al., 2010). Studies have also evaluated the potential prophylactic role of morin on experimental diabetic neuropathy model in male wistar albino rats (AlSharari et al., 2014).

Being a naturally occurring polyphenol, morin, is identified to be an effective antioxidant, however their neuroprotective potential has not yet been well characterized. Hence the present study aimed at examining the possible free radical scavenging activity of morin and for the first time whether morin can protect against lead acetate (PbAc)-induced oxidative stress associated sensorimotor impairment in male wistar rats.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Lead acetate was obtained from HIMEDIA. Morin was acquired from Sigma Aldrich. Glutathione reductase, glutathione (GSH)-reduced form, 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB) were purchased from SRL. β-Nicotinamide adenine dinucleotide phosphate reduced (NADPH) was obtained from CDH. Primers were bought from Synergy scientific. Every other reagent utilized was of analytical quality.

### 2.2. Animals

Male Wistar rats weighing about 250 - 300 g had been acquired from the Central Animal House, Dr. ALMPGIBMS, University of Madras, Taramani campus, Chennai - 113, Tamil Nadu, India. Animals had been maintained separately in polypropylene cages as well as provided with standard pellet diet and housed under hygienic environment. Animals had been maintained on a 12 h light and dark cycles with free accessibility to water. All experiments and methods outlined in the current study were sanctioned by the Institutional Animal Ethics Committee (IEAC) of Dr. ALM PGIBMS, University of Madras, Taramani campus, Chennai - 113, Tamil Nadu, India.

### 2.3. Drugs and treatments

The animals were randomly divided into four groups of 15 animals each and assigned to different treatments.

Group I (Control): Rats were given physiological saline (0.9% NaCl) intraperitoneally for 14 days. Group II (PbAc): Rats were given PbAc (20 mg/kg b.wt.) intraperitoneally for 14 days. Group III (PbAc + Morin): Rats were given Morin (40 mg/kg b.wt.) orally 2 h after the administration of PbAc (20 mg/kg b.wt. i.p.) for 14 days. Group IV (Morin alone): Rats were given Morin (40 mg/kg b.wt.) alone orally for 14 days.

Experimental doses adopted in this study were as reported by; PbAc (Nehru and Kanwar, 2004) and Morin (Zhang et al., 2010).

### 2.4. Behavioral studies

All the behavioral tests had been conducted at room temperature in a peaceful place devoid of outside disturbance. All the experiments were conducted around 10.00 am and 6.00 pm.

#### 2.4.1. Rotarod activity

Motor coordination as well as the grip strength had been evaluated using rotarod apparatus on 7th and 14th day. Rats had been subjected to training session for about 300 s to acclimatize them on the rotarod prior to evaluation of drug treatments. Animals were positioned on the rotating rod having a diameter of about 3 cm (speed 20 rpm). The cut off time was basically 120 s. The average time of fall was documented and denoted as count per 2 min (Kulkarni, 1999).

#### 2.4.2. Water maze task

The Morris water maze had been carried out according to the previously described method (Morris, 1984). The experimental apparatus comprised of a spherical water tank (diameter-100 cm; height-35 cm), that contains water at 28 °C to a depth of about 15 cm and made cloudy by adding powdered milk. A platform (diameter 4.5 cm; height 14.5 cm) was immersed at about 0.5 cm under the surface of water and positioned at the midpoint of one quadrant. The platform will be maintained in a fixed position throughout the training session. Each rat was subjected to four successive trials during which they were permitted to escape on to the hidden platform as well as allowed to stay there for about 20 s. Escape latency time to discover the hidden platform in water maze was recorded as an index of acquisition or learning. In case the animal was not able to discover the platform within 120 s, rat was removed out and positioned on the platform for 20 s. After a few trials, the test was performed on the 7th and 14th day of the experimental period. On the 14th day, a spatial probe trial was carried out by taking off the platform and the time spent by each rat in target quadrant exploring for the hidden platform was observed as an index of retrieval and calculated.

#### 2.4.3. Open field test

Exploratory behavior was examined in an open field paradigm. The open field was made of plywood which comprised of a floor (96 cm × 96 cm) with high walls. The apparatus was painted completely with black except for 6 mm thick white lines which separated the floor into 16 equal squares. Each animal was then positioned at one corner of the apparatus and for the following 5 min the animals were observed for their ambulation (in s).The floor was wiped clean with wet sponge and the dry paper towel in between each trial (Ramirez et al., 2005).

#### 2.4.4. Forced swim test

Forced swim test is done to evaluate depression. All animals have been subjected to forced swim test and this test was conducted in accordance with the previously described method (Rojas et al., 2011). Following the open field test, animals were positioned separately in Plexiglas cylinder (height: 40 cm diameter: 18 cm) that contains 25 cm water, maintained at about 23–25 °C. Rats were then removed from the cylinder just after 15 min and dried out before they were returned back to their home cages. On the next day, animals were again positioned in the cylinders for about 5 min and the total time period of immobility was assessed. An animal was deemed to remain immobile when it is floating passively in the water.

#### 2.4.5. String test for grip strength

The latency to maintain the grip on the horizontal wire by the animals is regarded as an indirect measure to evaluate grip strength. The rats were allowed to hold (with the forepaws) a steel wire (2 mm in diameter and 35 cm in length), extended horizontally at a height of about 50 cm over a cushion support (Shear et al., 1998). The length of time period by which the rats were able to hold the wire was documented.

#### 2.4.6. Adhesive removal test

Adhesive removal test was carried out in order to examine the sensorimotor deficits in rats (Bouet et al., 2007). Two tiny adhesive-

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