

Research Article

Effect of *Manasamitra vatakam*, an Ayurvedic Formulation, on Aluminium-Induced Neurotoxicity in Rats

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Abstract

Purpose: To investigate the effect of *Manasamitra vatakam* (MMV), an Ayurvedic formulation, on aluminium (Al)-induced neurotoxicity in rats.

Methods: Wistar rats were selected for the present study and were divided into four groups of six animals each. Group I (control) received tap water while group II received aluminium chloride (AlCl₃, 100 mg/kg/day). Group III was treated with concurrently Al chloride (100 mg/kg/day) and MMV drug (100 mg/kg/day). Group IV were administered MMV (100 mg/kg/day) for 90 days. Behavioral tests were performed on the rats during the experiment. At the end of the experiment various biochemical, neurochemical and histopathological histopathological was assessments were also carried out on the blood and brain of the rats.

Results: Following MMV treatment, there was a significant recovery in the performance of the radial maze and muscle grip strength of the rats, as well as the levels of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and acetylcholinesterase (AChE) in different parts of the rat's brain. The neuroprotective efficacy of MMV was supported by histopathological observations. Furthermore, MMV afforded protection against oxidative damage in the rats.

Conclusion: MMV prevents Al-induced toxicity in peripheral as well as in central nervous systems of rats. Therefore, further studies are required to ascertain its usefulness in humans.

Keywords: Rat brain, Aluminium chloride, Behavioral studies, Neurotoxicity, *Manasamitra vatakam*.

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INTRODUCTION

Aluminium metal (Al) is ubiquitous in the environment and may be linked to some diseases such as Alzheimer's disease, dementia and Parkinsonism. The daily intake of aluminium is estimated to be approximately 10 – 20 mg from cooking utensils, food additives and medicines such as, antacids or deodorants [1]. Since a variety of biomolecules are able to bind aluminium, and it can displace other biological cations (such as calcium and magnesium) from their binding sites, almost every metabolic pathway is a potential target for the adverse effects of aluminium.

Strong *et al* [2] reported that Al³⁺ exposure cause impairments in glucose utilization, agonist-stimulated inositol phosphate accumulation. Aluminium salts may bind to DNA and RNA, may inhibit the activity of enzymes such as hexokinase, alkaline phosphatases, and phosphodiesterase [3]. It is known to enhance the peroxidative damage of lipids and decreases the antioxidant status in different parts of the rat brain. On the other hand, various natural antioxidants have been used as neuroprotective drugs which may increase the antioxidant status and thus maintain the proper functioning of brain, however, the detail mechanism of action of these drugs is not known. *Manasamitra vatakam* (MMV) is an herbo mineral drug used in India's Ayurvedic system of medicines for treating cognitive deficits. We previously reported the presence of phytoconstituents such as alkaloids, steroids, protein, tannins, phenols, flavanoids, saponins, amino acid, glycosides in the *MMV*. We also observed that *MMV* could affect the synthesis and release of a specific neurotransmitter enzyme, acetyl cholinesterase [4] as well as influenced various antioxidants parameters and showed good free radical scavenging activities [4]. However, the detailed mechanism of action as well as the neuroprotective effect of *MMV* are not yet known. Therefore, the objective of this study was to investigate the neuro-

protective effect of *MMV* against Al-induced neurotoxicity and also to determine the relationship between molecular changes in rat brain and behavioral pattern in Al- treated rats.

EXPERIMENTAL

Chemicals

Aluminium chloride was purchased from Merck, Chennai, India. Tritonx-100 solution was purchased from Sigma-Aldrich Pvt Ltd., Bangalore, India. Standard pellet diet was obtained from Hindustan Lever, Bangalore, India. All other chemicals were purchased from Sisco Research Laboratories Pvt. Ltd, India.

Test drug

The Ayurvedic proprietary formulation, *Manasamitra Vatakam* (MMV) was obtained from Kotakkal arya vidya sala, Kerala, India. It was dissolved in distilled water and used for the animal studies.

Animals

Male Wistar albino rats (200 – 220 g) were housed in clean polypropylene cages and maintained at the room temperature 23 -25°C with alternate 12 h light and dark cycles. The animals were fed on standard pellet diet and drinking water ad libitum. All procedures were carried out in accordance with the guidelines for care and use of laboratory animals. The protocols were approved by the institutional animal ethical committee (ref no. 14/18/IAEC/24/07/07) of C.L. Baid Metha College of Pharmacy (Chennai, India).

Acute toxicity study

Acute toxicity study was conducted according to Organization for Economic Cooperation and Development (OECD) guidelines (no. 423). Three mice (aged 5 – 6 weeks and weighing 25 – 30 g) were selected for the study. A single high dose of *MMV* (2,000

mg/kg body weight was given orally to each animal. The animals were observed for 1 h continuously for any mortality or gross behavioral changes, then hourly for 4 h and thereafter, after every 24 h for up to 15 days [5].

Experimental design

The rats were divided into four groups of six animals each. Group I served as control and received the tap water while group II received aluminium chloride (100 mg/kg / day) dissolved in drinking water. Group III was treated simultaneously with aluminium chloride (100 mg/kg /day) and MMV (100 mg/kg). Group IV was administered only MMV (100 mg/kg). MMV and aluminium chloride (Al) were administered orally for 90 days except for group I. The animals were weighed twice a month and behavioral observations were recorded before and at the end of the experiment.

Behavioral assessment

Radial arm maze test

Radial maze is used to evaluate working memory in animals and cognitive tasks as comprehension, learning and reasoning. Each arm (50 x 12cm) of the eight arms radial maze extends from an octagonally shaped central hub of 30 cm diameter. The platform remains elevated up to 40 cm above the floor and small black plastic cups can be mounted at the end of each arm as containers for food. The trial is considered complete when the rats visit all eight arms or spends 10 min in the maze. Entry into an arm which the rat had not previously visited is recorded as a correct response and re-entry is counted as an error. The percentage of successful rats was calculated based on the total running time divided by the total number of choices to give the running time [6].

Rota-rod test

The effect of aluminium as well as MMV treatment on muscle performance was evaluated using Rota-rod (Techno) test. All the rats were given two initial training trials of 300 s, approximately 10 min apart, to maintain posture on the Rota-rod (3 cm in diameter and rotating at a constant 20 rev/min). After the initial training trials, a baseline trial of 120 s was conducted. The time each animal remained on the rota-rod was recorded. The animals that did not fall off the Rota-rod were given a maximum score of 120 s [6].

Biochemical assessment

Blood samples were collected from each animal by retro-orbital sinus puncture at the end of experiment. The blood samples were centrifuged at 4000 rpm for 5 min and the serum separated was stored at -20 °C for evaluation of plasma glucose, Alkaline phosphatase (ALP), Serum glutamyl-oxaloacetate transaminase (SGOT), Serum glutamyl pyruvate transaminase (SGPT) and glucose were assessed using an autoanalyser (Biosystem 250).

The rats were sacrificed by cervical dislocation, the brain of each rat removed, immediately washed well with ice-cold saline to remove blood and stored at -80 °C pending determination of biochemical parameters. The hippocampus and cerebral cortex were micro-dissected following the technique of Paxinos and Watson [7]. The left and right cerebral cortex of the brain of one rat was pooled to make one sample. A total of 10 % homogenate was prepared using phosphate buffer (0.1M, pH 7.4) containing 1mmol ethylene diamine-tetra-acetic acid (EDTA), 0.25M sucrose, 10mM potassium chloride (KCl) and 1mM phenyl methyl sulfonyl fluoride (PMSF) with a Potter-Elvehjem type homogenizer fitted with a teflon plunger, which was centrifuged at 800 g for 5 min at 4 °C in an IEC020 refrigerated centrifuge to yield the supernatant. The

supernatant were used for the estimation of acetyl cholinesterase (AChE). The remaining supernatant was again centrifuged at 12,365 g for 15 min at 4 °C to obtain supernatant, which was used for the determination of brain levels of DNA and RNA.

DNA and RNA assays

Both total tissue DNA and RNA were separately extracted and assayed according to the method described by Sambrook *et al* [8]. and expressed in mg DNA/g tissue and mg RNA/g tissue, respectively.

Evaluation of tissue acetyl cholinesterase (AChE)

The acetylcholinesterase activity of rat brain tissue was determined by the method of Ellman *et al* [8]. An aliquot of the brain homogenate (0.4 ml obtained earlier was added to a cuvette containing 2.6 ml of phosphate buffer (pH 8.0, 0.1M), 100 µl of 2,4 dithionitrobenzene (DTNB) reagent was added to the cuvette followed by the addition of 20 µl of acetylthiocholine iodide (AChI). Thiocholine reacted with DTNB reagent to produce a yellow color which was read spectrophotometrically at 412 nm.

Histopathological studies

Histopathological evaluation was performed by the method of Nakayama *et al* [10]. The brain hippocampus of each animal was fixed with 10 % formalin and further embedded in paraffin with wax. The blocks were processed for sectioning; the sections were then stained with haematoxylin and eosin as nuclear and cytoplasmic stains, respectively. Pathological changes, if any, were viewed under light microscope and recorded.

Statistical analysis

Statistical analysis was carried out using Graph Pad Prism software (version 4.03). One-way ANOVA was applied to process the data, followed by Newman-Keuls multiple

comparison test. The data are presented as mean \pm SEM. The level of statistical significance was set at $p \leq 0.001$.

RESULTS

Effect of Al chloride on mice behavioral characteristics

When the mice were treated with *MMV* up to 2000 mg/kg p.o. there was neither mortality nor any sign of gross behavioral changes after 48 h, and 15 days. However, it was observed that the numbers of entries into radial arms of the rats increased in Al-treated rats, and the animals also spent less time in each radial arm of the apparatus, compared with the normal control. On the other hand, in groups III and IV the behavioural characteristics of learning and reasoning increased (Figure 1).

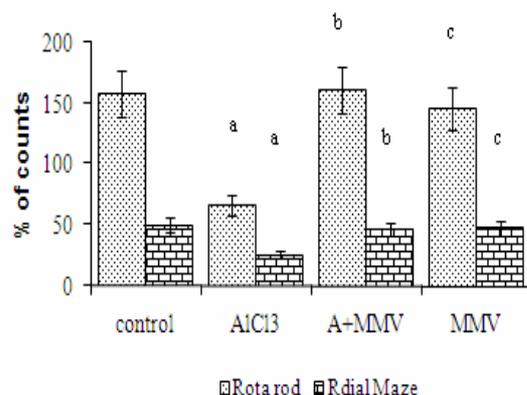


Figure 1: Effect of *MMV* on behavioural characteristics (mean \pm SEM), assessed by Rota rod and Radial Maze analysis; ^a $p < 0.001$, AlCl_3 group compared with control group; ^b $p < 0.001$, *MMV*+ AlCl_3 group compared with aluminium-treated group; ^c not significant ($p < 0.001$), *MMV*-treated group compared with control

A significant decrease ($p < 0.001$) in muscle strength and locomotor activities was observed in Al-exposed rats compared with normal control. On the other hand, significant improvement in the muscle strength and muscle grip occurred in *MMV*-treated rats compared with Al-treated rats (Figure 1).

Biochemical parameters

Figure 2 shows the effect of MMV on plasma parameters. Oral administration of Al (100 mg/kg) for 90 days significantly ($p < 0.001$) increased plasma glucose, ALP, SGOT and SGPT whereas co-administration of MMV with Al significantly ($p < 0.001$) decreased the levels of these parameters in rats. There was no significant alteration in plasma glucose, ALP, SGOT and SGPT when compared with normal control and rats treated with MMV alone.

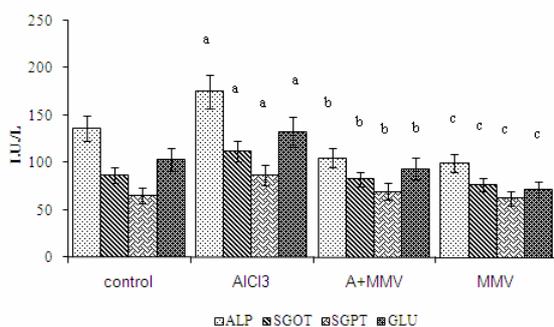


Figure 2: Effect of MMV on biochemical parameters (mean \pm SEM) in Al-treated rats. ^a $p \leq 0.001$, AlCl₃ group compared with control group; ^b $p \leq 0.001$, MMV+AlCl₃ group compared with aluminium-treated group; ^c not significant ($p \leq 0.001$) MMV treated group versus control

The effect of MMV on DNA and RNA levels in blood and brain tissues are shown in Figures 3 and 4. DNA and RNA significantly decreased in Al-treated rats compared with normal control ($p \leq 0.001$) but increased significantly in MMV + Al-treated rats ($p \leq 0.001$) when compared with the Al-treated group. On the other hand, there was no significant ($p \leq 0.001$) alteration of DNA and RNA levels in rats treated with MMV alone.

The level of acetyl cholinesterase decreased significantly ($p \leq 0.001$) in the plasma, brain, cerebral cortex and hippocampus of Al-treated rats (Figure 5). However, the enzyme level increased following co-administration of MMV and AlCl₃ ($p \leq 0.001$).

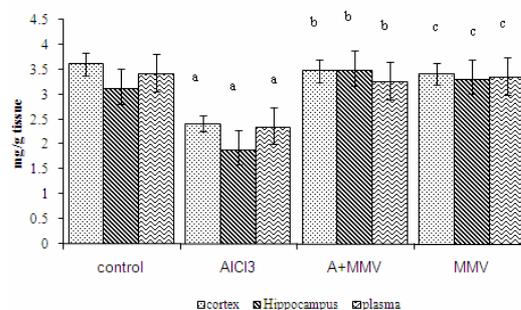


Figure 3: Effect of MMV on DNA content (mean \pm SEM) in of plasma, cerebral cortex and hippocampus of Al- treated rats. ^a $p \leq 0.001$, AlCl₃ group compared with control group; ^b $p \leq 0.001$, MMV+AlCl₃ group compared with aluminium-treated group; ^c not significant ($p \leq 0.001$) MMV treated group versus control

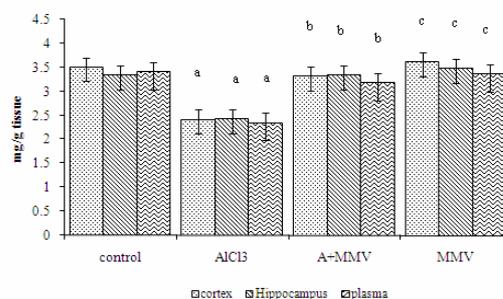


Figure 4: Effect of MMV on RNA contents (mean \pm SEM) of cerebral cortex and hippocampus of Al-treated rats. ^a $p \leq 0.001$, AlCl₃ group compared with control group; ^b $p \leq 0.001$, MMV+AlCl₃ group compared with aluminium-treated group; ^c not significant ($p \leq 0.001$) MMV treated group versus control

Histological observations

We observed normal cellular structure of hippocampus in normal control group which signifies normal working memory of the rats (Figure 6A). Gross histopathological changes, including heavy loss of cortical neurons, lacunae spaces (50 μ m diameter), ghost cells and vacuolated cytoplasm were observed in Al-treated animals (Figure 6B). On the other hand, remarkable improvement was observed in the MMV + Al-treated group. (Figure 6C, D).

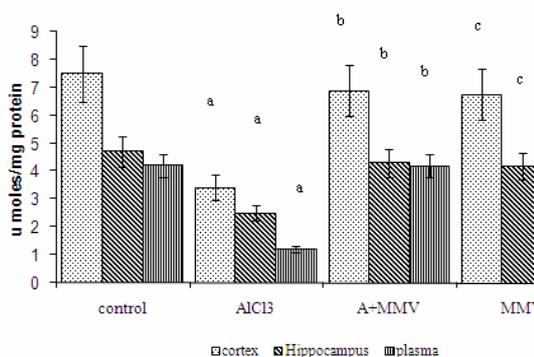


Figure 5: Acetyl cholinesterase (AChE) level (mean \pm SEM) in the plasma and brain of MMV treated and Al- exposed rats. ^a $p \leq 0.001$, by Newman-Keuls multiple comparison test; AlCl₃ (A) group was compared with control group; ^b $p \leq 0.001$, by Newman-Keuls multiple comparison test; MMV+AlCl₃ group was compared with aluminium-treated group; ^c - non-significant (ns) control vs MMV treated group.

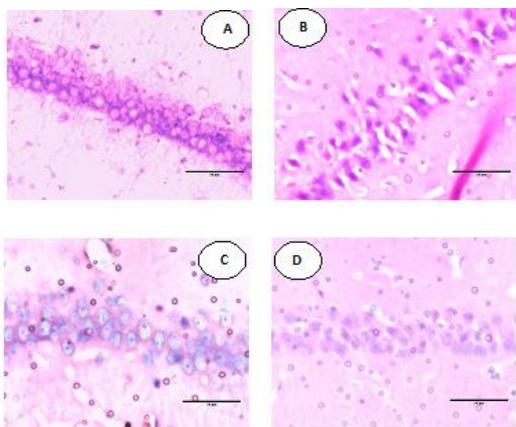


Figure 6: Microphotographs showing histopathological alterations in the hippocampus region of (A) control group, (B) Al-treated group (100 mg/kg), (C) Al-treated (100 mg/kg) + MMV (100 mg/kg) group, (D) MMV-treated (100 mg/kg) group

DISCUSSION

The animals treated with Al revealed altered behavioral changes within 90 days, suggesting a link between chronic Al-induced toxicity and the parameters evaluated. There was long-term memory impaired performance

under the influence of Al exposure. The animals spent a shorter time in the arms of the test apparatus after exposure to Al, thus depicting increased anxiety levels [11]. Re-entry frequency of the animals increased, indicating disturbed cognitive behavior. This observation was also supported by histopathological findings in that Al treatment caused marked alterations such as heavy loss of cortical neurons, lacunae spaces (50 μ m diameter), ghost cells and vacuolated cytoplasm. On the other hand, animals treated with MMV significantly showed improvement in cognitive function and long-term memory.

Following Al exposure, MMV treatment facilitated easy identification of previously visited arms and this may be due to correction in cholinergic neurotransmission, which in turn can be linked to altered memory function. Bhalla *et al* [12] reported the mechanism of action of Al induced toxicity and indicated that it causes defect in cognitive function. Levesque *et al* [13] observed severely damaged glia cells in rat brain following Al treatment. Hippocampus and cerebral cortex are the key structures of memory formation. Since the hippocampus is especially indispensable in the integration of spatial information, a decline in learning ability may be induced by the deterioration of hippocampal function [23].

Hu *et al* [14] has reported that Al-associated toxicity diminished motor activities and grip strength. Thus, improvement in a neurotransmitter enzyme such as AChE, and nuclear materials in rat blood and brain could be responsible for the improvement in learning, memory, cognition and locomotor functions. The importance of neurobehavioral studies in risk assessment lies in the fact that behavior can be regarded as the net output of the sensory, motor and cognitive functions occurring in the nervous system and can serve as potentially sensitive end-points of chemically-induced neurotoxicity.

The increasing preparation and storage of food in Al vessels, cans, and foils may

increase the Al content, particularly in food that are salty, acidic, or alkaline. There has been insufficient concern about toxic consequences of Al ingestion because its bioavailability was considered to be poor and the gastrointestinal tract normally represented a barrier to Al absorption under normal circumstances but this barrier can be breached. It has been shown that individuals ingesting large amounts of Al compounds do absorb significant amounts resulting in elevated plasma levels [15].

Al-exposed rats showed increased blood glucose level which may be due to decreased pyruvate formation that affects the synthesis of acetylcholine. It appears that Al toxic potency, which leads to alteration in acetylcholine level, might have caused neurobehavioral changes. Davis [16] has observed that acetyl choline affects human memory and cognitive function by dramatically decreasing the activity of acetylcholine transferase in the cortex and hippocampus of Alzheimer patients.

Increase in blood glucose level may have disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen, possibly mediated by increase in adrenocorticotrophic and glucagon hormones and/or reduced insulin activity [17]. Sallam *et al* [18] reported that Al accumulation in the liver leads to liver damage as a result of increased enzymes levels of ALP, SGOT, SGPT and glucose in the serum. Increased enzyme levels may cause cellular degeneration or destruction of hepatic cells. Decreased plasma concentration of ALP, SGOT, SGPT and glucose levels in MMV-treated animals suggests that the drug exhibits hepato-protective activity.

Aluminium is a trivalent cation and has high affinity for negatively charged groups such as phosphates which are present in nucleic acids and phosphorelated proteins. Although plasma and brain tissue DNA and RNA levels of Al-exposed rats decreased, MMV-treated rats had these levels restored, thereby showing a positive influence of the plant

extract on either degradation or synthesis of nucleic acids in these animals.

Al causes disturbances in cholinergic neurotransmission and disrupts the cognitive behavior of animals by increasing the stress levels. Studies have shown that Al exposure results in a decrease in hexokinase activity [19], which in turn decreases the pyruvate formation and hence affect the synthesis of acetylcholine and subsequently the enzyme activity of AChE. Kowall *et al* [20] found that Al is a pro-oxidant and indirectly results in the production of free radicals leading to oxidative damage and reduced levels of reactive oxygen species (ROS) which indirectly affects acetyl cholinesterase enzyme activity.

In our earlier study, increased peroxidative damage of lipids and proteins in cells was observed after Al exposure in rats [21]. On the other hand, MMV-treated rats decreased LPO level after drug treatment suggesting that MMV can protect against peroxidative damage. Since oxidative damage is mediated by free radicals, it was necessary to investigate the status of endogenous antioxidant enzymes under the different treatment conditions. The results revealed that tissue damage was prevented by MMV and this may be due to the presence of active molecules such as saponin and flavanoids [4] which are well known antioxidants.

An enzyme, such as SOD, is an important antioxidant, which protects the brain from peroxidase-mediated neuronal damage, and therefore, decreased level of these biomolecules, may lead to increased severity of Al toxicosis in the brain. It was observed that in Al intoxication, the activity of antioxidant enzyme such as SOD was significantly increased compared to control. Usually, decreased antioxidant to oxidant ratio plays a crucial role in generating a condition of oxidative stress. The decreased activity might have resulted from oxidative modification of proteins. Our previous study showed significantly decreased activity of

SOD in MMV + Al-treated group; therefore, in this experimental condition, SOD may be responsible for preventing the tissues from oxidative damage [21]. Evidence for stronger glia activation has been observed in Al-exposed animals and this is claimed to be responsible for pathological and inflammatory events [23]. Inflammatory responses are known to play an important role in neurodegenerative disease such as Alzheimer's disease (AD).

Gross disruption in brain cells was seen during microscopical examination following Al treatments, which indicate adverse effects at sub-cellular levels including changes in the structure of the cerebral cortex due to loss of neurons, ghost cells and gliosis. Struys-Ponsar *et al* [22] also found that Al causes structural changes in the cerebral cortex due to loss of neurons, ghost cells, gliosis, hippocampus and cortex, and this may affect the various enzymes responsible for synthesis as well as degradation of neurotransmitters [19].

CONCLUSION

MMV has a protective effect on the levels of various biochemical and neurochemical parameters in blood and brain. It can also protect against cognitive impairment during chemically-induced stress conditions caused by metals such as aluminium chloride. Thus, MMV is promising for the treatment of cognitive impairment in neurodegenerative disorders.

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