Effect of Aluminum Induced Toxicity on Behavioral and Hematological Parameters Under the Influence of Manasamitra Vatakam (An Ayurvedic Formulation) In Rats

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Summary

To investigate the effect of Aluminum induced toxicity on behavioral and hematological parameters under the influence of *Manasamitra Vatakam* (*MMV)*, (an Ayurvedic formulation) in rats

Wistar rats were selected for the present study and the animals were divided into four groups containing six animals in each. The Group I[st] served as control and received only vehicle solution whereas, group II[nd] rats received Al chloride (100 mg/kg bwt/day). The group III[rd] rats were treated with Al chloride (100 mg/kg body weight/day) and simultaneously the *MMV* drug (100 mg/kg bwt/day) was also provided to these group animals. The group IV rats were administered *MMV* only at the dose of (100 mg/kg bwt/day) for 90 days. At end of study, behavioral and hematological parameters were investigated. We observed a significant alteration in the performance of Plus Maze, Active avoidance along with gross alteration in the hematological parameters after Al treatment .Whereas, after *MMV* treatment a significant recovery was observed in the behavioral alteration as well as hematological parameters. The present study concludes that the oral administration of *MMV* could very well prevent the behavioral alteration as well as Al induced toxicity in the peripheral system.

Keywords: Aluminium Chloride, Toxicity, Manasamitra Vatakam.

Introduction

Aluminium metal (Al) is ubiquitous in the environment and may cause certain disease such as Alzheimer's disease, dementia and Parkinsonism [1]. Aluminium is a very common toxin and affects the proper functioning of central nervous system through different ways [2]. The daily intake of aluminium was estimated to be approximately 10–20mg from cooking utensils, food additives and medicines such as, antacids or deodorants [3]. 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. Aluminium causes changes in skeletal, digestive, nervous and haemopoietic systems of an organism [4,5,6]. Strong et al. [6] reported that Al exposure caused impairments in glucose utilization, agonist-stimulated inositol phosphate accumulation. Aluminium salts may bind to DNA, RNA and may inhibit the activity of enzymes such as hexokinase and alkaline phosphatases, phosphooxidase and phosphodiesterase [7]. Aluminium is known to enhance the peroxidative damage of lipids and

decreases the antioxidant status in different parts of the rat brain [9]. In addition, an aluminium compound has been reported to affect the metabolism of lipids & proteins by enhancing the peroxidative damage and decreasing the antioxidant enzymes [9].

On the other hand, various natural antioxidants have been used against toxic stress and these drugs maintain the proper functioning of the body by improving the antioxidant status [11]. However, the detail mechanism of action of these drugs are yet to be evaluated. The *Manasamitra vatakam* (*MMV*) is a herbo mineral drug used in the Ayurvedic system of medicines for cognitive deficits. We have already reported the presence of phytoconstituents like alkaloids, steroids, protein, tannins, phenols, flavanoids, saponins, amino acid, glycosides in the MMV. Further, we observed that MMV affects the synthesis and release of a specific neurotransmitter enzyme acetylcholineestrase [11]. It was also observed that MMV influences the different antioxidants parameters and showed very good free radical scavenging activities by affecting the activity of various key enzymes such as DPPH, NO and free radicals [10a]. Thus, keeping above fact in view, the present study has been planned to investigate the effect of Aluminum induced toxicity on behavioral and hematological parameters under the influence of *Manasamitra Vatakam (MMV)* in rats.

Materials and Methods

Chemicals

Manasamitra vatakam was purchased from Kotakkal arya vidya sala, Kerala, India whereas Aluminium chloride was purchased from Merck, Chennai, India. The Scopolamine (standard) was purchased by Sigma-aldrich pvt.ltd., Bangalore, India. These compounds were pure and the efficiency was greater than 99%. The 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.

Experimental animals

Male healthy adult Wistar albino rats (200–220 gm) were housed in clean polypropylene cages and maintained at the room temperature 23^{o}C-25^{o}C with alternate 12 h light and dark cycles. The animals were fed standard pellet diet and drinking water *ad libitum*. All the procedures were carried out in accordance with the guidelines for care and use of laboratory animals and protocols were approved by the Intuitional Ethical Committee on experimental animals (IAEC No. 14/18/IAEC/24/07/07), C.L.Baid Metha College of pharmacy, Chennai, India.

Acute toxicity study

Acute toxicity study was carried out using OECD guide lines No. 423. Three mice of the same age group and weight were taken in a single dose (*MMV*) up to the highest dose 2000 mg/kg orally. The animals were observed for 1 h continuously and then hourly for 4 h, and finally after every 24 h up to 15 days for any mortality or gross behavioral changes [12].

Test Drug

Ayurvedic proprietary formulation, Manasamitra Vatakam (*MMV*) was obtained from Kotakkal arya vidya sala, Kerala. The drug *MMV* (100 mg/kg bw) was weighed and dissolved in distilled water and used for the animal studies.

Experimental design

Rats were divided into four groups containing six animals in each. The Group Ist served as control and received only vehicle solution. Whereas group IInd rats received aluminium chloride (100 mg/kg bwt/day) diluted in pure drinking water. The group IIIrd rats were treated with aluminium chloride (100 mg/kg body weight/day) and simultaneously the drug *MMV* (100 mg/kg body weight) was also provided to these group animals. The group IV rats were administered *MMV* only at the dose of 100 mg/kg body weight. The *MMV* drug and Aluminium chloride were administered orally and entire experiment was conducted for 90 days. The body weighed of animals was recorded twice in a month where as behavioral observations were recorded before and after the entire length of drug treatment [13].

Behavioral test

Plus Maze

The elevated plus maze is a pharmacologically validated model for assessment of anxiety state in the rodent [(19),] and consist of two open arm (40 cm x 10 cm) and two enclosed arms of the same size with 40 cm high walls. The entire maze was elevated 50 cm above the ground and placed in a quiet dimly lit room. Experimental rats were placed individually in center of the maze facing the closed arms and observed for 5 min. and the following parameter were measured: Number of arm entries, time spent on the open arms and number of closed arm entries. Subsequently the percentage of open arm entries (OAE) and time spent in open arms were calculated.

Active avoidance

Cognitive behavior was assessed by the number of times, the animal escapes in the 10 test trials. The apparatus for this test consisted of one chamber with pole climb (Cooks Pole climb apparatus) (Kulkarni,. 1999). One chamber was in mirror lit. The animals were put into inside of the lit compartment. After 10 Sec, the buzzer was set on and after another 5 Sec, an electric shock at 80 V was given. If the animals jumped in to uphold pole climb Shock free zone (SFZ), as soon as the buzzer was set on, it means that animal has avoided the test and if tries to hide some other means, this is termed escapism. A total of 10 trials were given to every animal in a single day and to qualify, the animals had jumped and avoid test at least 7 trials out of 10 trials.

Blood collection

At the end of the experimental period, all the animals were anaesthetized with ketamine and blood samples were collected through retro-orbital sinus in plain vial as well as along with heparin as anticoagulant. All the parameters were such as RBC counts, hemoglobin (Hb), hematrocrit (Hct), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were analyzed using an electronic hematology analyzer (Advia 120, adivia 60, cobas micros 60, Sysmex).

Statistical analysis

Statistical analysis was carried out by using Graph Pad Prism software (version 4.03). One way ANOVA was used, followed by Newman-Keuls multiple comparison test. The data were represent mean ± SEM and the minimum level of significance was set at $p \leq 0.001$.

Results

Effect of *MMV* on behavioral parameters

The anxiety level of the animals was found significantly increased in Al treated animals as compared with control and the level of stress were confirmed by the plus maze experimental findings. On the other hand, the level of anxiety was found significantly decreased in *MMV* treated rats as compared with Al treated rats. Also the number of entries in the open arm was decreased in *MMV* treated rats as compared with the Al exposed rats where the number of entries were increased Further it was observed that Al treated animals spend very less time as compared to *MMV* along with Al treated rats. (Figure 1)

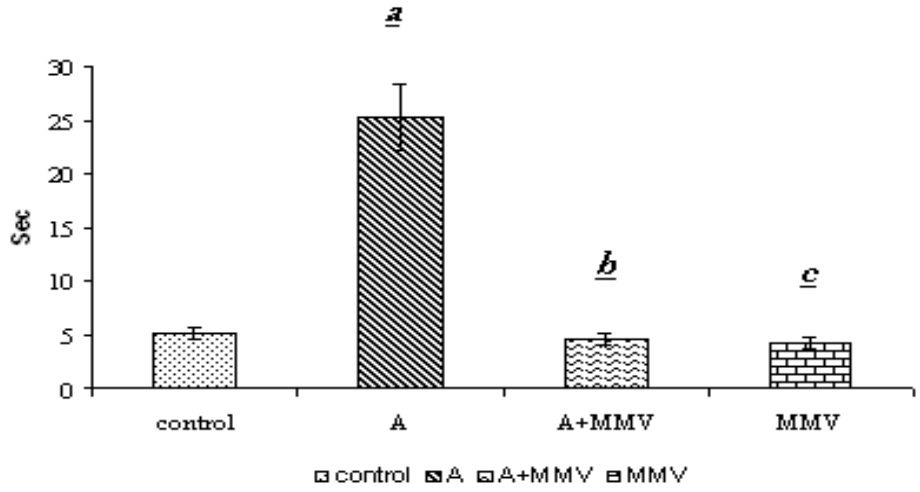

Figure 1: Effect of *MMV* on behavioral changes (Plus maze) in Al treated rats

(Values are mean ± S.E.M): **Comparison:** Control vs Al treated ($^{a}P < 0.001$),

Al treated vs Al + *MMV* treated ($^{b}P < 0.001$), Control vs *MMV* treated (c - non-significant).

The effect of Al and *MMV* treatment on behavioral changes of active avoidance was observed. The test was conduct to assess the cognitive behavioral activities of learning and memory impairment in terms of animals treated with Al. The principle of the mechanism of the test was to observe the animals from escaping the shock on the basis of memory loss or gain. We observed that the number of escaping from the shock under the treatment of *MMV* was significantly decreased ($P \leq 0.001$) as compared with Al treated rats. (Figure 2)

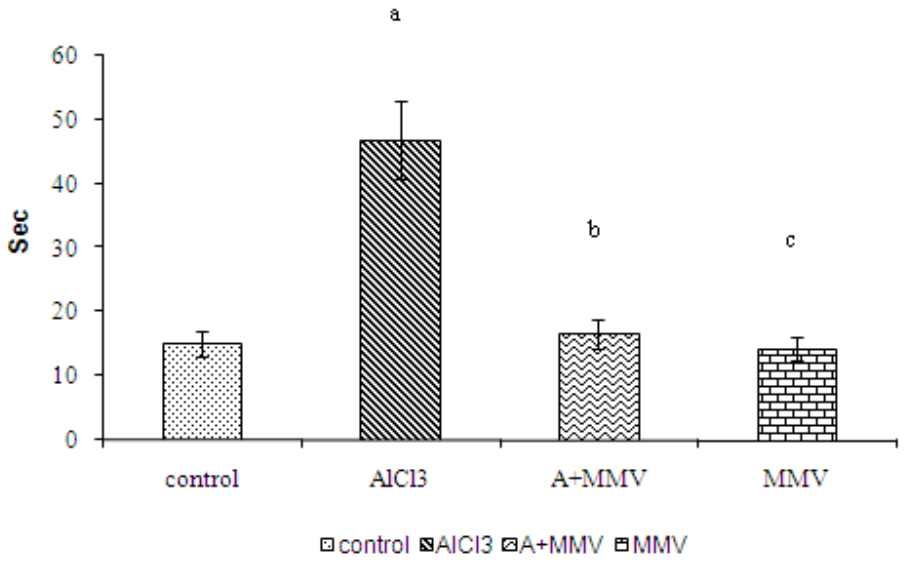

Figure: 2 Effect of *MMV* on behavioral changes (active avoidance) in Al treated rats

(Values are mean ± S.E.M); **Comparison:** Control vs Al treated (^{a} P < 0.001), Al treated vs Al + *MMV* treated (^{b} P < 0.001), Control vs *MMV* treated (c - non-significant)

Hematological parameters

The haemoglobin and RBC were significantly ($p \leq 0.001$) decreased in Al treated rat's as compared with normal control (Fig. 3 and 4). Whereas, in combined treatment (*MMV* with Al), the haemoglobin and RBC level were significantly ($p \leq 0.001$) increased as compared with Al treated rats. The levels of HBS and RBC were within the normal limit in *MMV* alone treated rats. The other hematological parameters such as MCH, MCHC, PCV and hematocrit (Hct) were significantly ($p \leq 0.001$) decreased in Al induced rats (Figure 5). On the other hand, the MCH, MCHC, PCV and hematocrit (Hct) level were significantly ($P \leq 0.001$) increased in *MMV* along with Al treated rats. There was no any significant change in the MCH, MCHC, PCV and hematocrit (Hct) level in *MMV* alone treated rats and the values were within the normal limit.

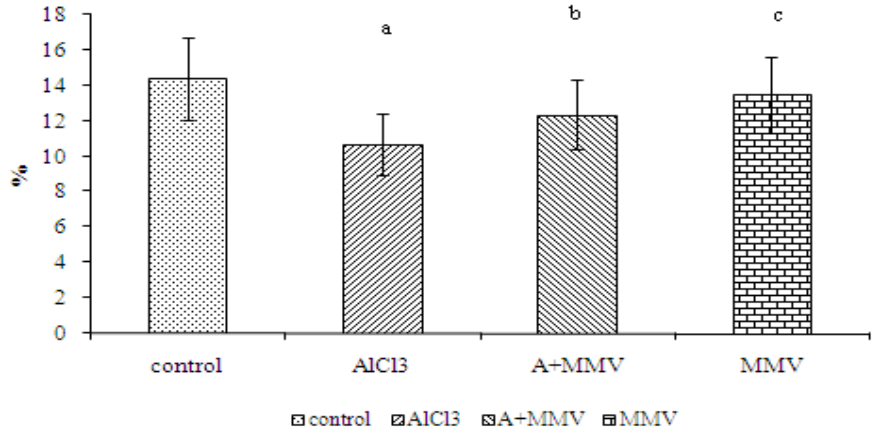

Figure 3: Effect of *MMV* on hemoglobin changes in Al treated rats

(Values are mean ± S.E.M); **Comparison:** Control vs Al treated ([a] $P < 0.001$),

Al treated vs Al + *MMV* treated ([b] $P < 0.001$), Control vs *MMV* treated (c - non-significant).

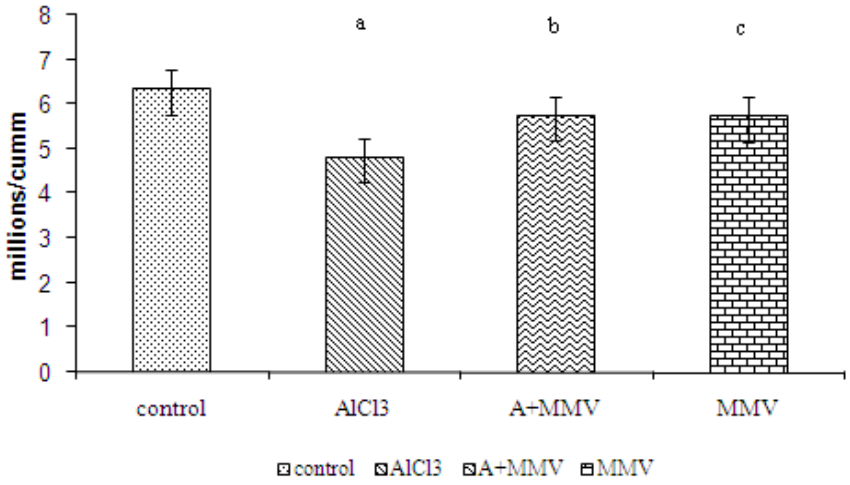

Figure 4: Effect of *MMV* on RBC changes in different group of rats

(Values are mean ± S.E.M); **Comparison:** Control vs Al treated ([a] $P < 0.001$),

Al treated vs Al + *MMV* treated ([b] $P < 0.001$), Control vs *MMV* treated (c - non-significant)

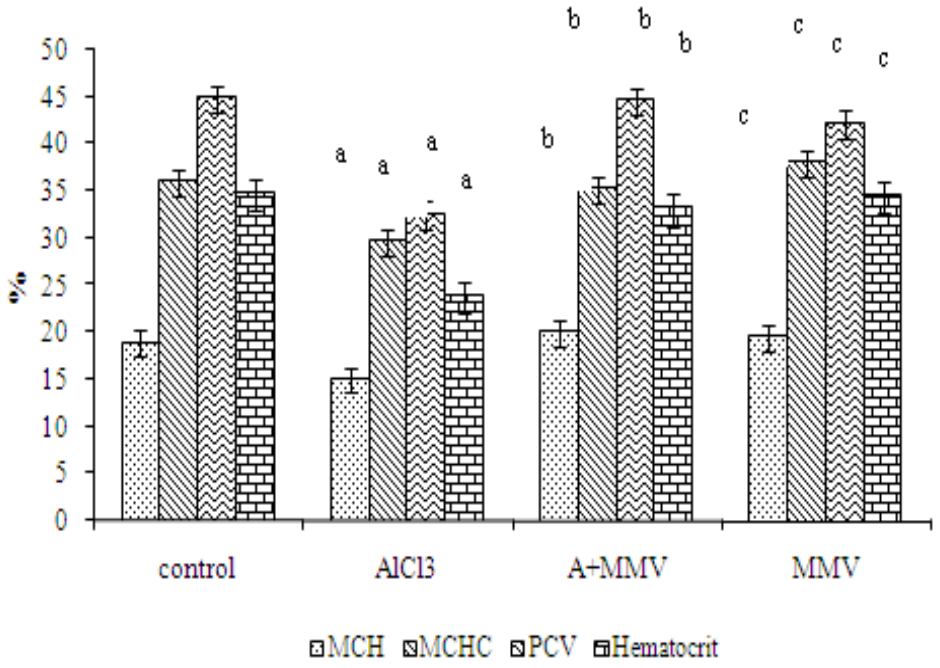

Figure 5: Effect of *MMV* on hematological parameters in Al treated rats

(Values are mean ± S.E.M): **Comparison:** Control vs Al Treated ($^{a}P < 0.001$)

Al treated vs Al + *MMV* treated ($^{b}P < 0.001$), Control vs *MMV* treated (c - non-significant)

Discussion

The animals treated with Al revealed altered behavioral changes suggesting a link between chronic Al induced toxicity with these parameters. We observed long term memory impaired performance under the influence of Al exposure. The animals spent short time in arms after exposure of Al and thus depicting increased anxiety levels [15] and the reentries of animal's were increased which was being considered as error and proved the disturbed cognitive behaviors of animals.

On the other hand, animals treated with *MMV* showed improvement in the cognitive function as well as long term memory significantly. The *MMV* treatment showed an easy identification of previous visited arms after Al exposure may be due to correction in cholinergic neurotransmission, which is linked to altered memory function. Punita Bhalla, et al., [16] also reported the mechanism of action of Al induced toxicity and concluded that it causes defect in cognitive function. The hippocampus and cerebral cortex are the key structures of memory function. Because 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 [17, 18, 19]. 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 [20].

Al is known to concentrate in the water-lipid interface of membrane and interact with the phosphates of the external hemilayer, thus diminishes the membrane external surface area. Additionally, Al can disrupt the bilayer structure, causing a redistribution of membrane lipids, leading to shape change [21]. The Al results in significant hemorheological and hematological changes in rats such as a decrement in RBC deformability at low shear stress levels, aggregation process and an increment in whole blood viscosity at both native and standard Hct. The MCV, Hb and Hct of rats exposed to Al2 (SO4)3 has been also found to be decreased. There is evidence that anemia is associated with Al accumulation in the plasma and /or bone tissue which is known to cause chronic renal insufficiency [22]. The present results indicated that Al treatment resulted in a significantly decreased haemoglobin (Hb), total erythrocytic count (TEC) and packed cell volume (PCV) along with increased while total leukocyte count (TLC). Vittori et al. [23] have reported morphological changes in the erythrocyte after Al toxicity and finally the cell loses their typical biconcave shape. They also suggested that aluminium may disturb erythropoiesis through combined effects on mature erythrocytes and cellular metabolism in late erythroid progenitors. Also, the inhibition in erythropoiesis and iron metabolism due to aluminium treatment probably hinders haemoglobin synthesis and erythroid cell maturation [24, 25]. On the other hand, significant improvement in these parameters after *MMV* treatment it self suggests the protective role of this drug against Al induced toxicity.

The increasing use in preparation and storage of food in Al vessels, cans, and foils may increase the Al content, particularly in the food that are salty, acidic, or alkaline. There has been little concern about toxic consequences of Al ingestion because the bioavailability was considered to be poor [26] and the gastrointestinal tract normally represented a barrier to Al absorption under normal circumstances but this barriercan be breached [27]. It has been clearly shown that individuals ingesting large amounts of Al compound do absorb significant amounts resulting elevate plasma levels [26, 27]. In spite of this, a persistent intoxication would cause compensating mechanisms to be triggered, leading to restoring the hematocrit and hemoglobin concentration with a concomitant persistence of microcytosis and a decrease MCH [28]. The effects of Al on erythroid progenitors and on mature erythrocytes and the toxic effects on erythropoiesis may be responsible for the decrease of hemoglobin and hematocrit levels [29]. It is already known that Al disorganizes the erythrocyte membrane by altering its mechanical properties, suggesting a reduction of the mean lifespan of circulating erythrocytes, which could play a major role in the anemia [30]. Thus, the present findings revealed that the *MMV* can very well protect the cells against the toxic stress caused by Al and it may be due to the presence of active molecules such as saponin and flavanoids [Thirunavukkarasu, et al., 2010] which are well known antioxidants..

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