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# Coordination impairment induced in male albino rats by methylmercury chloride

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## ABSTRACT

Behavior is an important end point for studying environmental toxicants in mammals because it can reveal effects on the nervous system. Therefore present study was designed as a model to analyze the long lasting effects of Methylmercury chloride in male animals with a focus on emotional behavior. Male albino rats of wistar strain were exposed orally to a dose of 2mg/kg of Methylmercury chloride, 100mg/kg Vitamin-E and 100mg/kg Acetyl-L-Carnitine for 28 days. During this defined experimental period, control and all the treated animals were subjected to standard Open Field Apparatus for motor coordination on 0, 7th, 15th, 22nd and 29th day for 5 minutes and four observations were recorded. Exploratory activity was significantly declined in rats treated with Methylmercury chloride as compared to control animals, while as it was enhanced statistically in animals subjected to vitamins. These results indicate that short-term, low doses of Methylmercury in male albino rats can be detrimental to motor, emotional or locomotor coordination. 

## KEYWORDS

Methylmercury chloride;
Emotional behavior;
Vit-E;
Acetyl-L-Carnitine;
Rats.

## INTRODUCTION

Mercury (Hg) is a ubiquitous and hazardous environmental contaminant found in ocean and freshwater fish, shellfish, and plants[4, 19, 23-25]. The organic or methylated form of Hg (methylmercury; MeHg) accounts for most of the Hg to which humans are exposed[16]. Hg as MeHg gets rapidly absorbed from gastrointestinal tract and is readily transported into the brain where it is sequestered and gradually converted into inorganic Hg. MeHg is a potent neurotoxicant known to cause neuronal degeneration, and neocortical and cerebellar granule neurons, in particular, are very sensitive to MeHg exposure[2, 10, 14, 18, 30]. One of the most severe intoxications ever reported in humans was due to eating contaminated fish and shellfish from the Minimata Bay in Japan in the 1950s[3, 7, 11]. Autopsies of affected adults revealed extensive damage of the cerebellum and cortical sulci[11]. In the early 1970s, a second significant episode of MeHg poisoning occurred in Iraq. Numerous individuals exposed to MeHg exhibited symptoms comparable to the residents OF Minimata Bay[1].

In 1997, the U.S. Environmental Protection Agency (EPA) recommended a reference dose of no more than

0.1 µg/kg body weight/day for MeHg exposure in the human population[3, 29]. This translates into limiting consumption of food sources with MeHg contamination, including freshwater fish and ocean fish such as Tuna[10, 17]. The persistent contamination of our environment with mercury indicates that mercury will remain bioavailable as MeHg for decades, affecting current as well as future generations[6].

Studies using rodent animal models have proven useful in simulating neurobehavioral effects of MeHg exposure[26, 27]. However most neurological effects observed in rodents have been reported from studies that utilized prenatal exposure to MeHg[5, 8, 9, 12, 13, 15, 20-22, 28]. Still, comparatively little is known about the behavioral effects of MeHg exposure on young adults at low to moderate exposure levels.

In the present study, male wistar rats were exposed to MeHgCl via gavage using a dose of 2mg/kg body weight. They were compared with age matched and weight matched control (vehicle only), rats using standard Open Field Behavior Test for emotional coordination. It is well known from the literature that the central nervous system (CNS) effects of MeHg are typically delayed in onset[17].

## MATERIALS AND METHODS

### Animals

Fifty male adult albino rats (Rattus norvegicus) of the wistar strain (3 months old) weighing 200±20g were elicited from the Central Animal Breeding House, J N Medical College, AMU, Aligarh and were domiciled in polyethylene plastic cages with paper cutting as bedding and open wire tops. Five animals were harboured per cage. Rats were fed rat chow for 28 days of acclimation. Rats were fortuitously compartmentalized into five weight- matched groups (10 rats per group; mean weights 200±20g), rats were endowed with tap water and their designated diets ad libitum. Treatment groups were aboded in an animal dexterity with ambient room temperature maintained at 24±2ºC, humidity 50±5% with a 12 h light/ 12 h dark cycle. Rat robustness, body tonnage changes and daily feed intake by rats were monitored daily until termination of the experiment. Animals were used according to the guidelines of the committee on care and use of experimental animal resources. The ethics protocol was countenanced by the laboratory animal's subsistence and usage committee of J N Medical College, AMU, Aligarh.

### Exposure to methylmercury and vitamins

Rats were separated into five groups of 10 animals each. Group-1 received 0.9% normal saline by gavage, Group-2 received methylmercury chloride. Methylmercury chloride was dissolved in saline (1.25 mg/ml) and orally administered (2mg/ kg body weight) once a day to 14 days and for the next 14 days rats were kept untreated. Group-3 received 2mg/kg MeHgCl for 14 days and for the next 14 days they were treated with 100mg/kg body weight of vitamin E. Group-4 received 2mg/kg MeHgCl for 14 days and for the next 14 days they were treated with 100mg/kg body weight of Acetyl-L-Carnitine. Group-5 received 2mg/kg MeHgCl for 14 days and for the next 14 days they were treated with vitamin E plus Acetyl-L-Carnitine in combination. Antioxidants were always injected at a gap of 30 minutes as per[34]. The total treatment time was 28 days.

The dose of MMC was selected based on recent estimate of daily ingestion in an environmentally exposed population[31]. We also followed the paper of[32].

### Chemicals

Methylmercury chloride (CAS: 115-09-03) was purchased from sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade or purest quality purchased from Merck, Fluka, Himedia or Loba.

After treatment, each animal of both the treated and control groups was exposed on 0 day, 7$^{th}$ day, 15$^{th}$ day, 22$^{nd}$ day and 29$^{th}$ day for 5 minutes in the apparatus, and the ambulation, preening, rearing and center crossing responses were recorded by a three-channeled hand operated counter.

### Open field apparatus

OFB apparatus used in present study was similar to that used by[51]. Briefly it consisted of a wooden, circular open arena (82 cm diameter) surrounded by a wall (31 cm high). The wooden floor was marked with three centric circles which were divided into segments

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by lines radiating from the centre. These 25 units of approximately equal size were used to score ambulation of the animals during the test. Two types stimuli were presented to the animals: while noise (78 dB, Ref. Intensity 2 x $10^{-4}$ dyn / $cm^2$) was produced by an oscillator through four loud speakers; and light (165 FC) was shown by four lamps. A translucent glass screen enclosed the arena on all sides, the front side having a glass door through which the animals were placed in the arena.

### Statistical analysis

The results were expressed as mean ± SEM. Differences between means of control and treatment rats were analyzed using paired samples t-test. The accepted level of significance in all the cases was $p<0.05$. Mean ± SEM was analyzed by using SPSS package program, version 10.01, SPSS, Chicago, IL.

## RESULTS

The low dose of 2mg/kg Methylmercury chloride produced overt signs of toxicity in treated rats. The MeHgCl-treated rats showed signs of significantly altered behavior compared to control rats. To evaluate the rats for more subtle changes in activity levels, we used a standard behavior test named open field activity.

Open field behavior study was observed with MeHgCl toxicosis on the following parameters

(i)Ambulation (ii) Preening (iii) Rearing and (iv) Center crossing

### Ambulation

Significant decline ($p< 0.05$, 0.001 and 0.01) was observed from the 7th day of toxication. On 22nd and 29th day the decrease noticed was maximum (-70.68%) and (-95.59%) respectively, TABLE 1. In Group-3 and 4 after 15th day when MeHg treated rats were given vitamin E and Acetyl L-Carnitine respectively for the next 14 days, a remarkable elevation was found in ambulation score of rats, Figure 1.

### Preening

Preening activity also showed remarkable decrement ($p< 0.05$, 0.001 and 0.01) from 7th upto last day of toxicosis, TABLE 2. Preening activity was also increased with the inoculation of antioxidants after 15th day in Group-3 and 4, Figure 2.

**TABLE 1 : Perturbation in the Ambulation score of the rats treated with Methylmercury Chloride (MMC) 2mg/kg body weight, gavage for 28 days: Protection by Vitamin-E and Acetyl-L-Carnitine (ALCAR).**

| Four observations daily for 10 rats | | | | | |
|---|---|---|---|---|---|
| Control | Days | MMC | MMC+ Vit-E | MMC+ ALCAR | MMC+ Vit-E+ ALCAR |
| 21.80±0.49 | 0 | 21.37±0.33 (NS) | 21.50± 0.15 (NS) | 21.72± 0.12 (NS) | 21.75± 0.35 (NS) |
| 23.75±0.64 | 7 | 16.95± 0.27*** (-28.63%) | 17.08± 0.22** (-28.08%) | 17.95± 0.15*** (-24.42%) | 16.99± 0.28** (-28.46%) |
| 22.43±0.31 | 15 | 13.49± 0.30** (-39.85%) | 16.98± 0.39* (-24.29%) | 17.37± 0.45** (-22.55%) | 14.52± 0.21* (-35.26%) |
| 22.99±0.42 | 22 | 6.39± 0.19* (-72.20%) | 18.20± 0.42*** (+20.83%) | 17.15± 0.50* (+25.40%) | 19.91± 0.17*** (+13.39%) |
| 23.95±0.29 | 29 | 0.96± 0.26* (-95.99%) | 20.33± 0.25*** (+15.11%) | 20.18± 0.21*** (+15.74%) | 21.73± 0.42 (+9.26%) |

***p < 0.001 ** p < 0.01 *** p < 0.05 NS= Non significant**

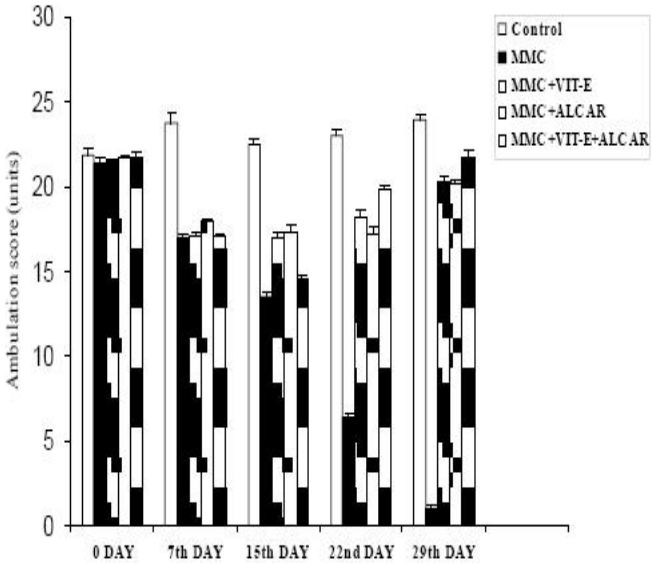


**Figure 1 : Effect of MMC on Ambulation activity in an open field chamber. Values represent Mean±SEM of 10 animals.**

### Rearing

The rearing score was reported to show significant depletion ($p< 0.05$, 0.001 and 0.01) from 15th day and the maximum change was observed on the last day of treatment (-73.55%) TABLE 3. After 15th day the increase in the rearing score in Group-3 and 4 by vita-

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**TABLE 2 : Perturbation in the Preening score of the rats treated with Methylmercury Chloride (MMC) 2mg/kg body weight, gavage for 28 days: Protection by Vitamin-E and Acetyl-L-Carnitine (ALCAR).**

| Four observations daily for 10 rats | | | | | |
|---|---|---|---|---|---|
| Control | Days | MMC | MMC+ Vit-E | MMC+ ALCAR | MMC+ Vit-E+ ALCAR |
| 6.30±0.19 | 0 | 6.13±0.35 (NS) | 6.20± 0.14 (NS) | 6.42± 0.24 (NS) | 6.27± 0.25 (NS) |
| 6.59±0.21 | 7 | 2.16± 0.50*** (-67.22%) | 3.31± 0.23** (-49.77%) | 2.85± 0.39** (-56.75%) | 2.63± 0.29** (-60.09%) |
| 6.85±0.12 | 15 | 1.03± 0.29** (-84.96%) | 1.42± 0.19* (-79.27%) | 1.36± 0.34* (-80.14%) | 1.22± 0.33* (-82.18%) |
| 6.97±0.30 | 22 | 0.86± 0.45* (-87.66%) | 5.49± 0.42*** (+21.23%) | 5.32± 0.48*** (+23.67%) | 5.99± 0.30*** (+14.06%) |
| 7.02±0.29 | 29 | 0.65± 0.26* (-90.74%) | 6.72± 0.35 (NS) | 6.44± 0.36 (NS) | 6.98± 0.36 (NS) |

***p < 0.001 ** p < 0.01 *** p < 0.05 NS= Non significant**

**TABLE 3 : Perturbation in the Rearing score of the rats treated with Methylmercury Chloride (MMC) 2mg/kg body weight, gavage for 28 days: Protection by Vitamin-E and Acetyl-L-Carnitine (ALCAR).**

| Four observations daily for 10 rats | | | | | |
|---|---|---|---|---|---|
| Control | Days | MMC | MMC+ Vit-E | MMC+ ALCAR | MMC+ Vit-E+ ALCAR |
| 7.60±0.25 | 0 | 7.74±0.52 (NS) | 7.66± 0.69 (NS) | 7.68± 0.29 (NS) | 7.72± 0.18 (NS) |
| 7.72±0.29 | 7 | 7.42± 0.63 (NS) | 7.28± 0.11 (NS) | 7.30± 0.35 (NS) | 7.39± 0.21 (NS) |
| 7.95±0.30 | 15 | 5.21± 0.51*** (-34.46%) | 4.94± 0.20** (-37.86%) | 5.17± 0.23** (-34.96%) | 5.09± 0.46** (-35.97%) |
| 7.89±0.62 | 22 | 4.14± 0.42** (-47.52%) | 6.87± 0.26*** (+12.92%) | 6.63± 0.42*** (+15.96%) | 6.99± 0.53*** (+11.40%) |
| 7.92±0.47 | 29 | 2.01± 0.29* (-74.62%) | 7.52± 0.30 (NS) | 7.39± 0.50 (NS) | 7.70± 0.29 (NS) |

***p < 0.001 ** p < 0.01 *** p < 0.05 NS= Non significant**

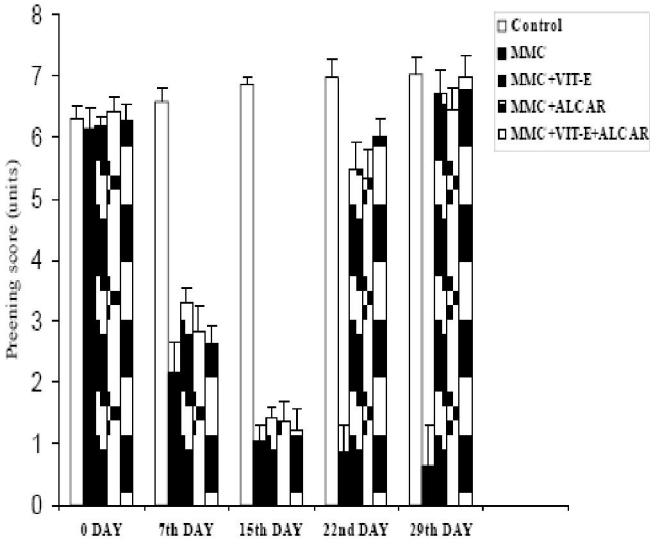


**Figure 2 : Effect of MMC on Preening activity in an open field chamber. Values represent Mean±SEM of 10 animals.**

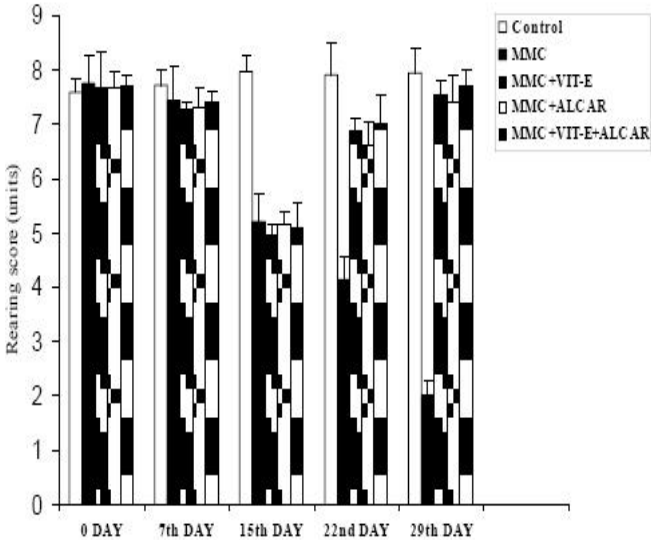


**Figure 3 : Effect of MMC on Rearing activity in an open field chamber. Values represent Mean±SEM of 10 animals.**

mins was maximum on 29th day, Figure 3.

## Center crossing

Center crossing activity also showed a noticeable change from 7th up to 29th day of toxicosis ($p < 0.05$, 0.001and 0.01) (-46.20%) upto (-79.30%) TABLE 4, Figure 4.

Ameliorative effect of vitamin E(100mg/kg) plus Acetyl L-Carnitine(100mg/kg) in combination was also observed simultaneously on the above mentioned open field parameters, the results analyzed were better as compared to vitamins given separately.

## DISCUSSION

The results of the present study show that 2mg/kg of methylmercury chloride induced marked decreases in locomotor activities in the open field. A salient observation was that even the lowest dose of the metal strongly decreased locomotor activity in the open field test. In the present study, we monitored assessment of motoric behaviour in young male rats orally exposed to 2mg/kg

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**TABLE 4 : Perturbation in the Center crossing score of the rats treated with Methylmercury Chloride (MMC) 2mg/kg body weight, gavage for 28 days: Protection by Vitamin-E and Acetyl-L-Carnitine (ALCAR).**

| Four observations daily for 10 rats | | | | | |
|---|---|---|---|---|---|
| **Control** | **Days** | **MMC** | **MMC+ Vit-E** | **MMC+ ALCAR** | **MMC+ Vit-E+ ALCAR** |
| 4.30±1.21 | 0 | 4.45±0.29 (NS) | 4.29± 0.33 (NS) | 4.32± 0.60 (NS) | 4.42± 0.40 (NS) |
| 4.35±1.25 | 7 | 4.23± 0.32 (NS) | 4.16± 0.30 (NS) | 4.22± 0.52 (NS) | 4.36± 0.44 (NS) |
| 4.52±0.92 | 15 | 2.31± 1.11*** (-48.89%) | 2.29± 0.25* (-49.33%) | 2.11± 0.54* (-53.31%) | 2.18± 0.28** (-51.76%) |
| 4.49±0.35 | 22 | 1.11± 1.26** (-75.27%) | 3.76± 0.21*** (+16.25%) | 3.53± 0.36*** (+21.38%) | 3.92± 0.36*** (+12.69%) |
| 4.85±0.41 | 29 | 0.89± 0.45* (-81.64%) | 3.97± 0.42** (+18.14%) | 3.82± 0.29*** (+21.23%) | 4.39± 0.22 (NS) |

***p < 0.001 ** p < 0.01 *** p < 0.05 NS= Non significant**

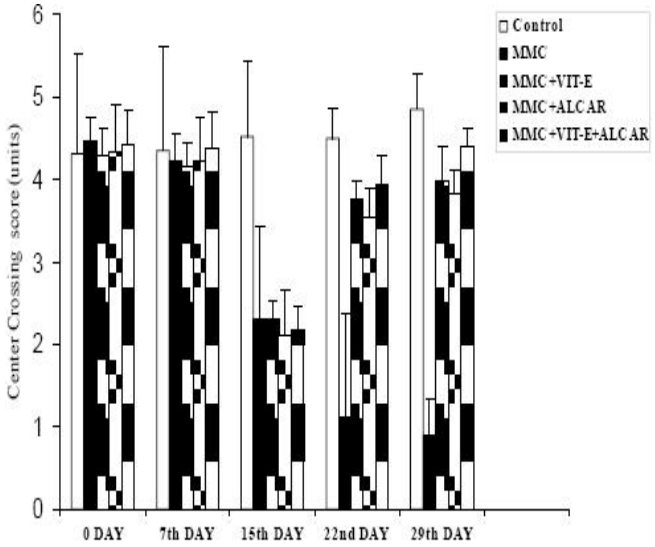


**Figure 4 : Effect of MMC on Center Crossing activity in an open field chamber. Values represent MeanSEM of 10 animals.**

MeHgCl and compared to age-matched control rats. We observed significant differences in all the four parameters of open field test in this study when MeHg-treated rats were compared to control ones.

Mice, when exposed to a novel environment, will typically explore the new environment during the first few minutes to get acquainted with the new space and/or try to find ways to escape[50]. In our experiment, the first 5 min of open field testing revealed decreased rate of locomotor and exploratory activities for 2mg/kg MeHg treated rats. These data are consistent with other rodent studies reported in the literature that examined horizontal exploration after exposure to MeHg[5, 9, 28, 35, 38, 39].

Reduced locomotor activity observed in rats in open field suggests a lowered general arousal or increased fearfulness. In case of this test, the direction of the change found, i.e. a reduction of locomotor activity is the same as that observed by other authors. According to some reports, perinatal MeHg exposure in rats results in reduced locomotor activity in males[41]. In mice, however, females are the affected gender[5].

Important issue in our study is the link between MeHg exposure and altered locomotion parameters. Taking into account that it has been shown that a decreased locomotor and exploratory activities reflects, at least in part, neurological damage induced by MeHg exposure[35, 42], our data suggest neurological damage in MeHg-exposed rats through our protocol of intoxication. There is evidence that cerebellar cells are targeted selectively by mercury compounds in vivo[43] and that MeHg neurotoxicity affects the motor system[44]. In fact, the relationship between MeHg-induced motor deficits and MeHg-induced cerebellar damage is a well-described phenomenon[45]. In this regard, reported decreased locomotor activity in animals exposed to MeHg during adulthood[46, 47] and the early postnatal period[48] is evident.

In our study, the frequencies of excrement defecation and urine traces were also increased by exposure to MeHg. It is considered as a significant factor in this test. Our results are in agreement with a study made by[49]. The distance walked was also reduced. There is a possibility that the distance walked in the open- field test reflects lower spontaneous locomotion activity. However, no significant difference in spontaneous locomotion activity evaluated in the home cage among the treatment groups was found. We considered that the result in the open-field test is independent of spontaneous locomotion activity, and is caused presumably by emotional stress.

## CONCLUSION

In conclusion, it was clearly demonstrated that continuous oral administration of MeHgCl (2.0 mg/Kg) for

14 days caused behavioral changes in rats. Thus it is evident that this behavioral test can be used as effective tool to measure subtle motor and coordination deficits that result from exposure to moderate to low doses of neurotoxicants. We found that MeHg produces adverse effects in individuals that were exposed to the neurotoxicant during early adulthood. It would be interesting in future experiments to determine the affect of such exposures in aged populations, as we know that fish forms a major source of protein in the ageing population, and fish is a major source of MeHg. Moreover, it is rather likely that this chemical exerts its effect differently and that the developmental or behavioral changes it induces are based on the integration of individual functions, not the interactive effect on a single function. Vitamin E and Acetyl L-Carnitine by virtue of their antioxidant properties decreased oxidative stress caused by MeHgCl that in turn improved emotional behavior in rats.

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