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Effect of Methylmercury on Depression like Behavior in Rats: a Study Mitigated by Exogenous Vitamins

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ABSTRACT

Depression is a heterogenous, multifaceted disorder with symptoms manifested at the psychological, behavioral and physiological level. Therefore, present study was designed as a model to analyze the long lasting effects of methylmercury chloride in male rats with a focus on depression like behavior. Male albino rats of Wistar strain were exposed orally to a dose of 2 mg/kg of methylmercury chloride, 100 mg/kg vitamin-E and 100 mg/kg acetyl-L-carnitine alone or in combination for 14 days. The total treatment time was 28 days. On 29th day, the animals were tested for tail suspension test and force swim test. Methylmercury-exposed rats displayed significantly longer immobility time (passive floating without limb movements) in both the tests than control animals. Vitamins significantly reduced the immobility period in rats thus offered protection against methylmercury-induced increment in depression like behavior. These findings point to early exposure to environmental contaminants as a possible risk factor for neurodevelopmental disorders.

Keywords: *Methylmercury, Depression, Rats, Vitamins*

The rat is the most frequently used animal for behavioral experiments, and several methods can be used to assess behavioral changes that result from modifications to its nervous system. Objective neurological tests can be used to establish a "behavioral baseline" for a normal animal, and to study the effects of drugs or a lesion on behavior. Neurological tests should be administered much like a doctor would administer a battery of tests after a trauma, with objective observation, precise documentation, and repeated measurements by the same experimenter.

Methylmercury (MeHg) is known to be an environmental neurotoxicant potentially causing neuropsychological disorders in humans [1]. Furthermore, epidemiological and experimental studies have clearly shown that the developing nervous system is particularly vulnerable to MeHg toxicity. Severe neurotoxic effects of prenatal exposure to high doses of MeHg were established in humans after MeHg disasters in Japan and Iraq [2,3] and confirmed in animal studies [4]. Later, developmental exposure to low doses of methylmercury contained in seafood was found to be a risk factor for cognitive disorders (e.g. memory, attention, and language problems) in children and adolescents in the fish-eating population of the Faroe Islands [5,6]. This fact raised researchers interest in studying effects of prolonged low-dose exposure in animal models, representing a chronic pattern of exposure in humans.

Depression is a heterogeneous, multifaceted disorder with symptoms manifested at the psychological, behavioral and physiological level. This is perhaps why it is so difficult to mimic the disorder in the laboratory [7]. Many of the human symptoms of depression, as described in the Diagnostic and Statistical Manual of the American Psychiatric Association (DSM IV) (such as recurring thoughts of death or suicide or having excessive thoughts of guilt) are impossible to be modeled in mice or rats. The question, therefore, remains impenetrable as to whether we can ever assume a mouse or rat is 'depressed'. Evolutionary theories have been proposed for psychiatric disorders [8,9],

which would plausibly predict that also lower animal species can exhibit behaviors useful in modeling human depression. However, such hypotheses are heavily debated and are difficult to address empirically [10,11]. Another difficulty in assessing depressive states in rodents is that the underlying pathophysiology in depression is still unresolved. Further, the mode of action of clinically-effective antidepressants is not yet understood beyond the fact that they primarily alter monoamine neurotransmission [12-15].

Despite the difficulties in translating the complexities of human affective disorders in its entire spectrum into relevant tests in mice, numerous attempts have been made to create so-called animal models of depression, or at least models of some of the core aspects of depression. Such models include those paradigms where various stress, pharmacological, lesion, environmental or genetic manipulations are applied [12, 16-21].

The goal of the current experiment was to examine whether exposure to low dose of methylmercury chloride would exacerbate the effects on depression like behavior in male rats. The two most commonly used tests for depressants and antidepressants are the Porsolt forced swim test (FST), and the tail suspension test (TST) [16]. Therefore, the FST and TST behavioral assays were used in this work to elucidate the potential of MeHg to produce effects on depression like behavior in rats.

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 harbored 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 (2 mg/ kg body weight) once a day to 14 days and for the next 14 days rats were kept untreated. Group-3 received 2 mg/kg MeHgCl for 14 days and for the next 14 days they were treated with 100 mg/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 2 mg/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 [22]. 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 [23].

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 both control and treated rats were exposed to these tests and the immobility time was noted accordingly.

Depression: Forced Swim Test (FST)

The forced swim test was performed according to the method of [24]. A vertical glass cylinder (25cm high, 14cm in diameter) was filled with water (30ºC) to a depth of 20 cm. The water depth was adjusted so that the animals must swim or float without their hind limbs or tail touching the bottom. For testing, each animal was placed in the cylinder for 6 min, and the latency to float, and the duration of floating (i.e. the time during which rat made only the small movements necessary to keep their heads above water) was scored. As suggested by Porsolt, only the data scored during the last 4 min were analyzed and presented.

In the forced swim procedure, rats are forced to swim in un-escapable situation. After a period of vigorous struggling, the animal becomes immobile, or makes only those movements necessary to keep its head above the water. The immobility observed in this test is considered to reflect a state of despair. The forced swim test has a high degree of pharmacological validity as reflected by its sensitivity to major classes of antidepressants, including tricyclics antidepressant and Selective Serotonin Reuptake Inhibitors (SSRIs) [25].

Depression: Tail Suspension Test (TST)

We used the test essentially as described by [26]. A short piece of paper adhesive tape (about 6 cm) was attached along half the length of the tail (3 cm). The free end of the tape was attached to a 30 cm long rigid tape which hung from a horizontal bar clamped to a heavy laboratory support stand. Suspended animals were surrounded by a white wooden enclosure (45 cm high, 40 cm wide and 40 cm deep) such that the rat's head

Table 1. Methylmercury Chloride (MMC) 2 mg/kg body weight influencing immobility time in Forced Swim Test (FST): Protection displayed by Vitamin-E (100 mg/kg body weight) and acetyl-L-carnitine (ALCAR).

	Mean Immobility Time, Sec ± SEM (N)				
TEST GROUP	Control	MMC	MMC+Vit-E	MMC+ALCAR	MMC+Vit-E + ALCAR
Alterations in Seconds	148±0.15 (10)	244±0.19* (10) (+64.86%)	107±0.30*** (10) (-31.08%)	116±0.22*** (10) (-21.62%)	96±0.14** (10) (-35.13%)

Values expressed as Mean ± SEM of 10 animals, Figures in parentheses indicate percent change compared to controls. * $p<0.001$ ** $p<0.01$ *** $p<0.05$, N= number of animals

was about 20cm above the floor. For testing each rat was suspended by its tail and observed for 6 min. an observer scored the total duration of a passive, "dead weight" hanging (immobility), between the periods of wriggling of the animal to avoid aversive situation.

The tail suspension test is among the most widely utilized rodent model of depression [25]. In this paradigm, the rat is suspended by the tail to a tail hanger. Following an initial period of vigorous struggling, the animal gradually abates into immobility. The duration of immobility has been inferred as an index of behavioural despair (the animal has given up hope of escaping). The tail suspension test has been shown to be sensitive to various classes of antidepressants, including tricyclics antidepressants and SSRIs [25]. Furthermore, marked genetic differences in baseline immobility were found among inbred and outbred strains of mice in this test [25,27].

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

Effect of methylmercury chloride in force swim test (FST) in male rats

This paradigm evaluates animal's response to inescapable aversive situation (placement in a beaker of water), inducing an active (swimming, climbing on the walls) or inactive (floating) behavior. The latter is interpreted as a measure of depression like behavior [24]. Methylmercury exposed rats displayed significantly longer immobility time (passive floating without limb movements) than control animals ($p< 0.05$ and $p<0.001$, one-way ANOVA) (Fig 1). Vitamins significantly reduced the immobility period in rats thus offered protection against methylmercury-induced increment in depression like behavior. Results are interpreted in Table 1.

Effect of methylmercury chloride in tail suspention test (TST) in male rats

The effect of methylmercury in tail suspension test is shown in Table 2 and Fig 2. The total duration of a passive, "dead weight" hanging (immobility) was scored as a measure of depression like behavior [26]. Methylmercury-chloride-intoxicated rats showed significant increase in dead weight than control animals. ANOVA revealed the significance at $p<0.05$, 0.001 and 0.01. Vitamin-E and acetyl-L-carnitine treatment offered significant protection against methylmercury-induced cognitive deficits.

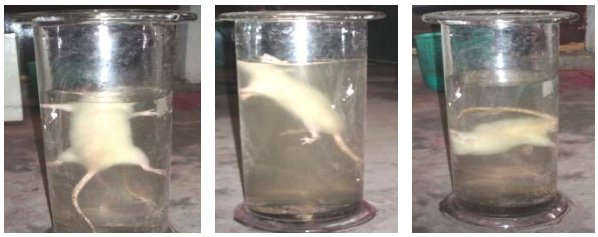
DISCUSSION

In the present study, we analyzed the effects of methylmercury on emotional behavior. We used the forced swim test and tail suspension test, generally considered as animal models of depression [24,26] respectively. In these tests, methylmercury induced male rats showed significantly longer immobility time that represented a behavioral despair response to an aversive situation and therefore a depression-like type of behavior. Depressive syndromes have been reported in humans later in life after occupational exposure to inorganic mercury [28]. The neurochemical basis of methylmercury-induced behavioral alterations may be due to disturbances in a number of neurotransmitter systems, initially occurring during exposure and followed by long-lasting changes in brain functioning [29]. Abnormalities in neuronal functioning, in turn, can be due to intracellular methylmercury toxicity, which includes alteration in Ca^{2+} homeostasis, cytoskeletal damage, and induction of oxidative stress [30,31].

Table 2. Methylmercury Chloride (MMC) 2mg/kg body weight influencing immobility time in Tail Suspension Test (TST): protection displayed by vitamin-E (100mg/kg body weight) and Acetyl-L-Carnitine (ALCAR) 100mg/kg body weight.

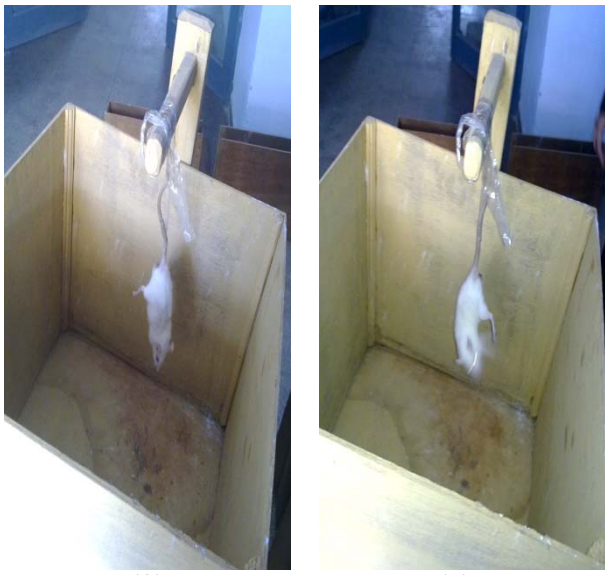
	Mean Immobility Time, Sec ± SEM (N)				
TEST GROUP	Control	MMC	MMC+Vit-E	MMC+ALCAR	MMC+Vit-E + ALCAR
Alterations in Seconds	150±0.14 (10)	270±0.31* (10) (+80.00%)	104±0.27** (10) (-30.66%)	119±0.18*** (10) (-20.66%)	100±0.33** (10) (-33.33%)

Values expressed as Mean ± SEM of 10 animals, Figures in parentheses indicate percent change compared to controls. * $p<0.001$ ** $p<0.01$ *** $p<0.05$, N= number of animals



(A) (B) (C)

Fig 1. Vertical glass cylinder demonstrating a rat treated with MMC and appraised for depression like behavior in Forced Swim Test. A) Rat showing vigorous movements to escape from aversive situation. B) Rat floating to keep its head above water. C) Rat displaying immobility to reflect a state of despair.



(A) (B)

Fig 2. Tail Suspension apparatus hanging a MMC treated rat evaluated for depression like behavior. A) Rat showing "passive hanging" inferred as an index of behavioral despair. B) Rat showing "vigorous wriggling" to avoid aversive situation.

Many hypotheses have been advanced to explain the physical adaptation that is the immobility response observed in the FST and TST [18]. The posture of immo bility in the context of the FST was originally coined 'behavioral despair' by [24], largely based on the assumption that the animals have 'given up hope of escaping'. In other words, the immobility represents a failure of persistence in escape-directed behavior. Other investigators have contended that the behavioral responses comprise an evolutionary preserved coping strategy [32] in which immobility behaviors represent the psychological concept of "entrapment" described in clinical depression [33-35]. Thus, the development of immobility disengages the animal from active forms of coping with stressful stimuli [35]. Further, immobility in the TST is due to inability or reluctance to maintain effort rather than a generalized hypoactivity, as evidenced by the fact that animals can adopt this posture quickly and drugs which may suppress activity [36] counter the immobility response. As such, this immobility may be analogous to the clinical observations that depressed patients often lack sustained expenditure of effort reflected in a pronounced psychomotor impairments [37].

In conclusion, we have shown in this study that low level of methylmercury induces alterations in depression like behavior and this effect was prevented by post treatment with combination of vitamin-E and acetyl-L-carnitine. These findings point to early exposure to environmental contaminants as a possible risk factor for neurodevelopmental disorders.

The TST has great utility as a model to assess antidepressant-related behavior; however its use is best exploited when used in battery style fashion with other depression models such as FST. Therefore, one can conceive that these relatively-robust paradigms will be useful in the future unraveling of the genetic, molecular and neurochemical pathways relevant to depression and antidepressant action and that dietary supplementation of vitamin-E and acetyl-L-carnitine might be useful in populations that are occupationally exposed to methylmercury chloride.

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REFERENCES

1. Gilbert SG, Grant-Webster KS. Neurobehavioral effects of developmental methylmercury exposure. *Environ Health Perspect* 1995; 103: 135-42.
2. Amin-Zaki L, Majeed MA, Elhassani SB, Clarkson TW, Greenwood MR, Doherty RA. Prenatal methylmercury poisoning: Clinical observations over five years. *Am J Dis Child* 1979; 133: 172-7.
3. Harada M. Minamata disease: Methylmercury poisoning in Japan caused by environmental pollution. *Crit Rev Toxicol* 1995; 25: 1-24.
4. Burbacher TM, Rodier PM, Weiss B. Methylmercury developmental neurotoxicity: A comparison of effects in humans and animals. *Neurotoxicol Teratol* 1990; 12:191-202.
5. Debes F, Budtz-Jorgensen E, Weihe P, White RF, Grandjean P. Impact of prenatal methylmercury exposure on neurobehavioral function at age 14 years. *Neurotoxicol Teratol* 2006; 28:363-75.
6. Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, Murata K, Sorensen N, Dahl R, Jorgensen PL. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. *Neurotoxicol Teratol* 1997; 19: 417-28.
7. American Psychiatric Association. Diagnostic and statistical manual of mental disorders (4th Eds.). Washington, DC: Author 1994.
8. Jones I, Blackshaw JK. An evolutionary approach to psychiatry. *ANZ J Psychiat* 2000; 34: 8-13.
9. Nesse RM. Is depression an adaptation? *Arch Gen Psychiat* 2000; 57: 14-20.
10. Dubrovsky B. Evolutionary psychiatry: Adaptationist and nonadaptationist conceptualizations. *Prog Neuropsychopharmacol Biol Psychiat* 2002; 26: 1–19.
11. McLoughlin G. Is depression normal in human beings? A critique of the evolutionary perspective. *Int J Ment Health Nurs* 2002; 11: 170-3.

12. Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of Depression Review. *Neuron* 2002; 34: 13-25.
13. Frazer A. Pharmacology of antidepressants. *J Clin Psychopharmacol* 1997; 17:2S-18S.
14. Richelson MDE. Pharmacology of Antidepressants. *Mayo Clin Proc* 2001; 76:511-27.
15. Blier P. Possible neurobiological mechanisms underlying faster onset of antidepressant action. *J Clin Psychiat* 2001; 62: 7-11.
16. Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. *Trends Pharmacol Sci* 2002; 23: 238-45.
17. Willner P, Mitchell PJ. The validity of animal models of predisposition to depression depression. *Behav Pharmacol* 2002; 13: 169-88.
18. Cryan JF, Mombereau C. In search of a depressed mouse: utility of models for studying depression-related behavior in genetically modified mice. *Mol Psychiat* 2004; 9: 326-57.
19. Geyer M, Markou A. Animal models in psychiatric disorders. In: Psychopharmacology, the Forth Generation of the Progress. (Eds. Bloom, F.E. and Kupfer, D.J.). Raven Press, New York. 2001; pp. 155-173.
20. Weiss JM, Kilts CD. Animal models of depression and schizophrenia. In: American Psychiatric Press Textbook of Psychopharmacology. (Eds. 2nd Nemeroff, C.B. and A.F. Schatzberg, A.F.). American Psychiatric Press. Washington, DC. 1998; pp. 89-131.
21. McKinney WT. Overview of the past contributions in animal models and their changing place in psychiatry. *Sem Clin Psychiat* 2001; 6: 68-78.
22. Sood PP, Bapu C, Sinha N, Rao AP. Cholesterol and triglyceride fluctuations in mice tissues during methylmercury intoxication and monothiols and vitamin therapy. *J Nutri Environ Med* 1997; 7:155-62.
23. Passos CJS, Sampaio DS, Lemire M, Fillion M, Guimaraes JRD, Lucotte M, Mergler D. Daily mercury intake in fish-eating populations in the Brazilian Amazon. *Exp Sci Environ Epidemiol* 2008; 18:76-87.
24. Porsolt RD, Le Pichon M, Jalfre M. Depression: a new animal model sensitive to antidepressant treatments. *Nature* 1977; 266:730-2.
25. Dalvi A, Lucki I. Murine models of depression. *Psychopharmacol* 1999; 147: 14-6.
26. Steru L, Chermat R, Thierry B, Simon P. The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacol* 1985; 85:367-70.
27. Liu X, Gershenfeld HK. Genetic differences in the tail-suspension test and its relationship to imipramine response among 11 inbred strains of mice. *Biol Psychiat* 2001; 49:575-81.
28. Grum DK, Kobal AB, Arneric N, Horvat M, Zenko B, Dzeroski S, Osredkar J. Personality traits in miners with past occupational elemental mercury exposure. Environ. *Health Perspect* 2006; 114: 2906.
29. Castoldi AF, Coccini T, Ceccatelli S, Manzo L. Neurotoxicity and molecular effects of methylmercury. *Brain Res Bull* 2001; 55: 197-203.
30. Sarafian T, Verity MA. Oxidative mechanisms underlying methyl mercury neurotoxicity. *Int J Dev Neurosci* 1991; 9:147-53.
31. Yee S, Choi BH. Methylmercury poisoning induces oxidative stress in the mouse brain. *Exp Mol Pathol* 1994; 60:188-96.
32. Thierry B, Steru L, Chermat R, Simon P. Searching-waiting strategy: a candidate for an evolutionary model of depression. *Behav Neural Biol* 1984; 41:180-9.
33. Dixon AK. Ethological strategies for defence in animals and humans: their role in some psychiatric disorders. *Br J Med Psychol* 1998; 71:417-45.
34. Gilbert P, Allan S. The role of defeat and entrapment (arrested flight) in depression: an exploration of an evolutionary view. *Psychol Med* 1998; 28:585-98.
35. Lucki I. A prescription to resist proscriptions for murine models of depression. *Psychopharmacol* 2001; 153:395-8.
36. Cryan JF, Mombereau C, Vassout A. The tail suspension test as a model for assessing antidepressant activity: Review of pharmacological and genetic studies in mice. *Neurosci Biobehav Rev* 2005; 29:571-625.
37. Weingartner H, Silberman E. Models of cognitive impairment: cognitive changes in depression. Psychopharmacol Bull 1982; 18:27-42.

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