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RESEARCH ARTICLE

Methylmercury Chloride Coaxed Oxidative Stress in Rats

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ABSTRACT

The present experiment was designed to access the effects of Methylmercury chloride (MeHgCl) on antioxidant status, protein concentration, lipid peroxidation and hydroperoxidation in brain parts, spinal cord, lung, heart and pancreas of rats. Twenty male Wistar rats (3 months old) were divided in saline controls (C) and MeHgCl-treated group (MMC). Treated rats were intoxicated with MMC at a dose of 2 mg/kg body weight orally by gavage once a day for 14 consecutive days, for the next 14 days, they were kept intoxicated. Control animals received a corresponding volume of isotonic saline. Health, total feed intake and body weights of rats were monitored daily throughout the study. Both the groups were sacrificed on 29th day. Study revealed an increase in Lipid peroxide (LPO) and Lipid hydroperoxide (LHPO) levels after MeHgCl administration. The levels of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), total sulfhydryl (TSH) and Protein were significantly declined in all the tissues of MMC treated group as compared to controls. In summary, depletion of antioxidant enzymes in tissues increases MeHgCl accumulation and enhances MeHg-induced oxidative stress, especially LPO and LHPO which plays an important role in tissue degeneration process during MeHgCl intoxication. Results support the hypothesis that depletion of antioxidant enzymes is a primary mechanism of organic mercury toxicity.

Keywords: *Methylmercury chloride, Rat oxidative stress, Antioxidants, LPO, LHPO*

Methylmercury is an exemplary paragon of a comprehensive neurotoxic agent that is also a customary and perilous milieu adulterant proficient of attitudinizing jeopardies to human health [1]. It is a hazardous trail metal that is emancipated into the environment from both natural and anthropogenic sources [2,3]. It is a pandemic milieu problem and is listed by the international program of chemical safety as one of the six most dangerous chemicals in the world's environment [4]. Human exposure to Methyl mercury pre-eminently occurs through the consumption of contaminated food such as fish from mercury-polluted waters [5-7] although cataclysmic exposures due to industrial profanation have occurred. Gold mining activities were considered the unique source of mercury pollution into the Amazon for many years [8]. The clinical findings in children from the tragic methyl mercury poisonings in Japan [9] and Iraq [10] have divulged the unambiguous susceptibility of the developing brain and have drawn great attention toward the corollary of prenatal exposure. Mercury inscribes an organism in a medley of chemical forms (elemental, inorganic and organic), brandishing its toxicologic characteristics including neurotoxicity, nephrotoxicity and gastrointestinal toxicity with ulceration and hemorrhage [11-15]. It is one of the most malicious metals in the environment exposure to which conspicuously in organic form, Methyl mercury may cause sullen havoc to nervous [16] immune [17], cardiovascular [18] and the kidney [19] may also induce genetic damage [20].

It is well known that orally administered methylmercury chloride is expeditiously and sleekly imbibed from gastrointestinal tract into the circulation. CH_3Hg^+ is allocated in all tissues but pertaining to its organ distribution and excretion panoramic variations between species have been denounced [21,22]. Most of the inhaled (Hg^o) vapor spawned from metallic mercury is highly rambling and lipid soluble and rapidly oxidizing to bivalent ionic mercury by intricate catalase-hydrogen peroxide in the blood, and is disseminated through blood to various organs. The content of catalase

in tissues and production of hydrogen peroxide are the curbing aspects of oxidizing Hg^{o} to Hg^{2+} and subsequently their discussion through membrane of cells [23]. The oxidation is inhibited by ethanol; oxidized mercury reacts with sulfhydryl groups of proteins, namely enzymes causing their inhibition. The toxicity of mercury and its ability to react with and exhaust free sulfhydryl groups are well known [24]. Depletion of protein-bound sulfhydryl groups results in production of ROS such as superoxide anion, hydrogen peroxide and hydroxyl radical and alters the antioxidant defense system of cells [25] by brindling their sulfhydryl groups (-SH) [26]. ROS are known to arbitrate MeHg-induced neurotoxicity in several experimental models [27-30]. In addition, MeHg has a high kinship for thiols, which results in depletion of intracellular glutathione leading to conglomeration of ROS [31].

CH3Hg+ undergoes biotransformation to inorganic mercury in the body [32,33]. If methyl mercury is putrefied to inorganic mercury (Hg^{2+} and Hg^{o}) and methyl radicals ($CH3^{o}$) in or near the membranes, lipid peroxidation could be graded. Mercury is a potential stimulator of the peroxidation chain reaction, since mercury might catalyze the decomposition of lipid peroxides to lipid radicals [34].

Erstwhile peruse from our laboratory on amendment of lipid peroxidation by [35] and impact on antioxidant deterrence in different parts of brain by the same author exhorted us to scrutinize the leverage of methyl mercury chloride on antioxidant status, lipid peroxidation and hydroperoxidation on different tissues of rat. Although methylmercury chloride and its effects on rats have been investigated in a handful of tissues in primeval studies, there is no itemized and commensurate study investigating the effects of methylmercury chloride on protein concentration, lipid peroxidation, hydroperoxidation and antioxidants in lung, heart and pancreas. The aim of this scrutiny is to inspect the effects of MMC on antioxidant level, protein content and peroxides in Brain parts, spinal cord, Lung, Heart and Pancreas. In addition, we aspired to dig out the most contrived tissue from MMC according to the parameters.

MATERIALS AND METHODS

Animals

Twenty 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 two 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.

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.

Exposure to methylmercury

Rats were separated into two groups of 10 animals each. Group-1 received 0.9% normal saline by gavage and Group-2 received methylmercury chloride. Methylmercury chloride was dissolved in saline (1.25 mg/ml) and orally administered (2mg/kg) once a day to the rats. 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 [36]. We also followed the paper of [37].

Preparation of tissue homogenate

At the culmination of empirical period, rats were robbed of delicacies for 24 h. Rats were immolated by decapitation on the scheduled day between 10:30 and 11:30 am without using anesthesia. The Brain, spinal cord, lung, heart and pancreas were immediately dissected out. Whole tissues were washed with chilled saline (4ºC), blotted and weighed. Brain was sliced into cerebrum, cerebellum and brain stem. Tissues were minced with sharp scissors and then thawed and homogenized with the aid of York's homogenizer fitted with Teflon plunger in a proportion of 1:10 (w/v) ice cold phosphate buffer (50 mM; PH 7.4). Aliquots of homogenates were used for the determination of malondialdehyde (MDA), hydroperoxides, reduced glutathione (GSH) and total sulfhydryl (TSH) levels. The homogenates were centrifuged for 60 min at 10,000 ×g at 4ºC and determination of superoxide dismutase (SOD) and catalase (CAT) activities were carried out with the resultant supernatant. Protein concentration was determined according to the method of [38] using purified bovine serum albumin as standard.

Thiobarbituric acid reactive substance (TBARS) level

Malondialdehyde, a marker of lipid peroxidation was determined as described by [39]. Briefly the reaction mixture consisted of 0.2ml of 8.1% sodium lauryl sulphate. 1.5 ml of 20% acetic acid (PH 3.5) and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid (PH 7.0 by 0.1N NaOH) and 0.2 ml of tissue homogenate.

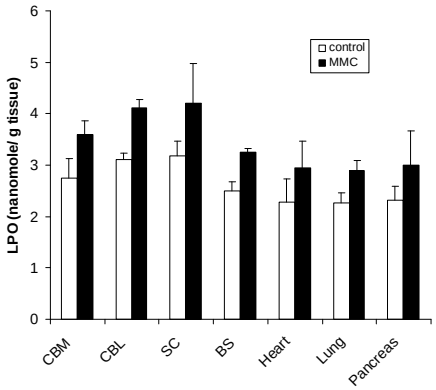

Fig 1: Effect of Methylmercury chloride (MMC) on lipid peroxide activity in different tissues of rat. Values represent Mean±SEM. CBM = Cerebrum, CBL = Cerebellum, SC = Spinal cord, BS = Brain stem

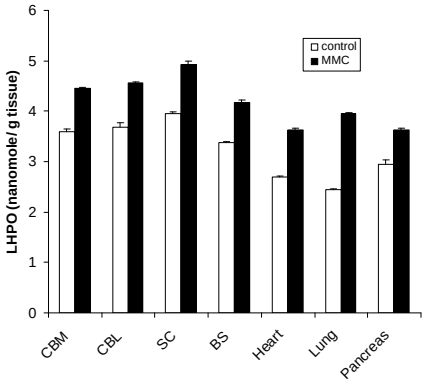

Fig 2: Effect of Methylmercury chloride (MMC) on lipid Hydroperoxide activity in different tissues of rat. Values represent Mean±SEM. CBM = Cerebrum, CBL = Cerebellum, SC = Spinal cord, BS = Brain stem

Lipid hydroperoxide (LHPO) level

Lipid hydroperoxide level was measured by a method described by [40] using cumin hydroperoxide as standard. The formation of Fe $(SCN)_3$ was measured spectrophotometrically by changes in absorbance at 480 nm. Results were given as nmol/g tissue.

Reduced glutathione (GSH) level

GSH was assayed using the method of Ellman with 5,5'-dithiobis-(2-nitro-benzoic acid) (DTNB) [41]. An equal quantity of homogenate was mixed with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.1 ml of this supernatant 2ml of phosphate buffer (PH 8.4), 0.5ml of DTNB and 0.4 ml of double distilled water was added. The mixture was vortexed and absorbance read at 412nm within 15 min. The concentration of reduced glutathione was expressed as µmol/g tissue.

Total sulfhydryl (TSH) level

TSH was measured according to the methods of [42] with DTNB. The absorbance of reduced chromogen was followed spectrophotometrically at 412 nm. TSH level was determined from a standard curve and expressed as µmol/g tissue.

Catalase (CAT) activity

Catalase activity was measured by the method of [43] using hydrogen peroxide as substrate. Briefly 0.1 ml of supernatant was added to a cuvette containing 1.9 ml 0f 50mmol/L phosphate buffer (PH 7.0). The reaction was started by the addition of 1ml of freshly prepared 30 mmol/L H_2O_2. The rate of decomposition of H_2O was followed spectrophotometrically at 240 nm. Enzymatic activity was expressed as units/ mg protein.

Superoxide dismutase (SOD) activiyt

The activity of superoxide dismutase in the supernatants was determined by the method of [44]. The activity of superoxide dismutase to inhibit the autooxidation of pyrogallol makes this reaction a basis for a simple assay of this dismutase. The activity of superoxide dismutase was expressed as units/mg protein.

Protein content

The protein content of the samples was determined by the method of [38] using bovine serum albumin as standard.

Table 1. Effect of Methylmercury chloride (MMC) on Brain parts, spinal cord, Lung, Heart and pancreas superoxide dismutase (SOD), Catalase (CAT) and Total sulfhydryl (TSH) activities of rats in comparison to control animals

	SOD (U/mg protein)			CAT (U/mg protein)			TSH (µmol/g tissue)		
Tissues	Control	MMC	% decrement	Control	MMC	% decrement	Control	MMC	% decrement
Cerebrum	4.62±0.03	3.43±0.04	-25.75***	6.99±0.01	5.10±0.05	-27.03***	16.64±0.93	11.95±0.23	-28.18***
Cerebellum	4.96±0.04	3.67±0.03	-26.00***	6.86±0.05	5.07±0.04	-26.09***	16.73±0.03	12.00±0.41	-28.27***
Brain stem	4.51±0.07	3.41±0.04	-24.39***	6.68±0.05	4.95±0.10	-25.89**	16.33±0.58	11.83±0.50	-27.55**
Spinal cord	4.35±0.06	3.30±0.01	-24.13**	6.75±0.09	5.00±0.01	-25.92**	16.50±0.34	11.87±0.44	-27.95**
Heart	3.85±0.06	2.95±0.03	-23.37**	5.60±0.05	4.32±0.02	-22.85*	15.84±1.09	11.55±0.38	-27.08*
Lung	3.64±0.03	2.81v0.02	-22.80*	5.74±0.05	4.38±0.09	-23.69*	15.67±0.24	11.49±0.65	-26.67*
Pancreas	3.73±0.03	2.87±0.05	-23.05*	5.85±0.07	4.45±0.12	-23.93*	15.35±0.82	11.30±0.18	-26.38*

Values represent Mean and SEM of ten rats per group in brain parts, spinal cord, lung, heart and pancreas. Paired samples t-test was performed to compare the parameters of control (C) and Methylmercury chloride groups (MMC). Values: $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$ are significant. Percent decrement is MMC compared to controls.

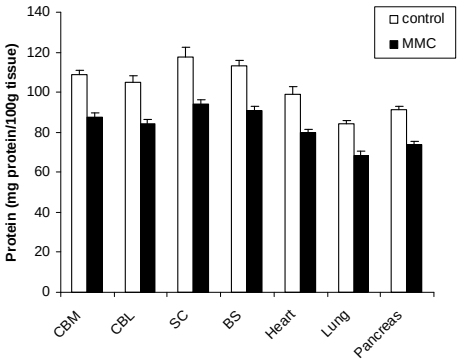

Fig 3: Effect of Methylmercury chloride (MMC) on Protein activity in different tissues of rat. Values represent Mean±SEM. CBM = Cerebrum, CBL = Cerebellum, SC = Spinal cord, BS = Brain stem

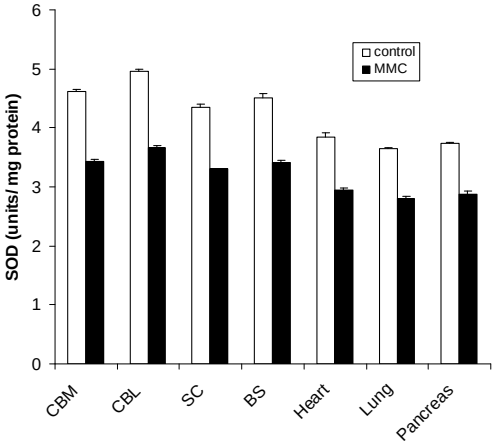

Fig 4: Effect of Methylmercury chloride (MMC) on Superoxide dismutase activity in different tissues of rat. Values represent Mean±SEM. CBM = Cerebrum, CBL = Cerebellum, SC = Spinal cord, BS = Brain stem

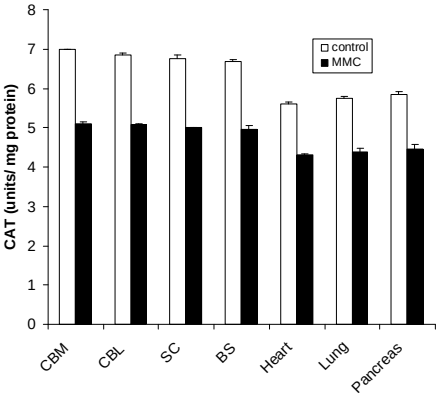

Fig 5: Effect of Methylmercury chloride (MMC) on Catalase activity in different tissues of rat. Values represent Mean±SEM. CBM = Cerebrum, CBL = Cerebellum, SC = Spinal cord, BS = Brain stem

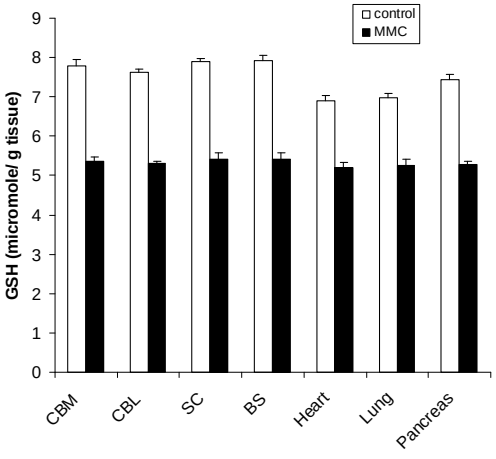

Fig 6: Effect of Methylmercury chloride (MMC) on Free sulfhydryl activity in different tissues of rat. Values represent Mean±SEM. CBM = Cerebrum, CBL = Cerebellum, SC = Spinal cord, BS = Brain stem

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

Antioxidant enzyme activities (SOD, CAT and TSH), Free radical substances (LPO & LHPO) and Subcellular component (protein) activities in Brain parts, spinal cord, pancreas, lung and Heart of all groups are shown in Tables 1 and 2 respectively. The effect of MMC on body weight changes, water intake and feed intake changes in rats during the experiment is summarized in Table 3 and Antioxidant substance (GSH) activity is shown in Table 4. MDA and LHPO activity was enhanced in all tissues ($p<0.001$- $p<.05$, Figs 1 and 2) of treated group against controls. The highest MDA increase was observed in Spinal cord (+32.38%, $p<0.001$). LHPO activity was also highest in Spinal cord (+24.24%, $p<0.001$). Protein level was found declined in all tissues ($p<0.001$-$p<0.05$, Fig 3) of MMC group. The highest decrease was shown by Spinal cord (-20.12%, $p<0.001$).

SOD activities of MMC group were lower in all the tissues in comparison to controls ($p<0.05$- $p<0.001$, Fig 4). The highest decrease of SOD activity was found in cerebellum (-26%, $p<0.001$). Lowest change was observed in lung (-22.80%, $p<0.05$). We saw that CAT activities were declined significantly in all the tissues; cerebrum ($p<0.001$), cerebellum ($p<0.001$), spinal cord ($p<0.001$), brain stem ($p<0.01$), Pancreas ($p<0.01$), Lung ($p<0.01$) and Heart ($p<0.05$). The highest percentage of decreased CAT activity was found in Heart (-22.85%, $p<0.05$, Fig 5).

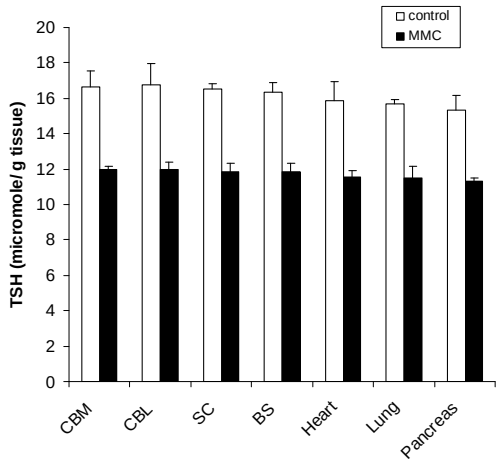

Fig 7: Effect of Methylmercury chloride (MMC) on Total sulfhydryl activity in different tissues of rat. Values represent Mean±SEM. CBM = Cerebrum, CBL = Cerebellum, SC = Spinal cord, BS = Brain stem

GSH and TSH activities of MMC group also showed significant decline in all the tissues when compared with controls (Figs 6 and 7 respectively). The decrement was in the order of Brain stem (-31.56%), Spinal cord (-31.43%), Cerebrum (-31.23%), Cerebellum (-30.53%), Pancreas (-29.26%), Lung (-24.74%) and Heart (-24.31%) for GSH and Cerebellum (-28.27%), Cerebrum (-28.18%), Spinal cord (-27.95%), Brain stem (-27.55%), Heart (-27.08%), Lung (-26.67%) and Pancreas (-26.38%) for TSH.

The body weight changes, water intake and feed intake changes of the rats of metal administered group were statistically significant to controls on the final day of our peruse (p<0.001- p<0.05). The highest impact was shown on water intake parameter.

DISCUSSION

Oxidative stress occurs when the normal balance between the oxidative events and the antioxidant defenses is disrupted either by loss of reducing agents/antioxidant enzymes or by increased amount of oxidizing species. Mercury compounds are known to induce the formation of ROS and there are a number of reports concerning oxidative stress and antioxidant enzyme activities on mercury exposure [29-31,45]. The final effect depends on several factors, such as the nature of the metal, via administration, species, gender, tissue and age. The necessity of developing sensitive procedures to detect toxic effects of heavy metals is given by the fact that human food can be highly

contaminated by heavy metals. For example, up to 10 ppm mercury have been found in European predatory fish. [46] reported that fish from southern Sweden contained 0.02-10 ppm mercury. Up to 3.3 ppm MMC have been found in pike [47] and 0.55 ppm mercury in perch [48,49] detected up to 0.27 ppm mercury in fish from Lake Constance. Mercury in fish exists largely as a methylmercury compound; methylmercury in total mercury averaged 70% in a study of [50] and 91% in investigations of [47].

One notable factor of environmental and occupational pollution is an accumulation of such elements resistant to biological degradation including heavy metals (cadmium, mercury, lead). Both occupational and environmental exposures to heavy metals remain a serious problem [51-53]. Organic and inorganic mercury compounds have been reported to be potent toxic and /or carcinogenic agents in humans and animals, although the exact mode of action is still not certain. Numerous studies indicated that mercuric ions can interact with glutathione (GSH) in the presence of hydrogen peroxide, leading to the generation of reactive oxygen species. However, the mechanism of the generation of radical species including the redox cycle of the metal and the formation of oxidative DNA modification by mercury compounds has not been studied. The reactive oxygen species subsequently induce lipid peroxidation measured by the thiobarbituric acid reaction for malondialdehyde in liver, kidney, lung, testes and serum. Increased MDA was reduced in these target tissues after pre-treatment with antioxidants and chelators in methylmercury treated rats.

The results of this study indicated that the lipid peroxidation is one of the molecular mechanisms for cell injury in acute methylmercury chloride poisoning and is associated with a decrease of cellular protein content. Several in vivo and in vitro studies have demonstrated that both inorganic mercury and

Table 2. Effect of Methylmercury chloride (MMC) on Brain parts, spinal cord, Lung, Heart and pancreas Malondialdehyde (MDA), Lipid hydroperoxide (LHPO) and Protein concentration of rats in comparison to control animals

	LPO (nmol/g tissue)			LHPO (nmol/g tissue)			Protein (mg protein/ tissue)		
Tissues	Control	MMC	% decrement	Control	MMC	% decrement	Control	MMC	% decrement
Cerebrum	2.74±0.39	3.60±0.27	+31.38**	3.60±0.04	4.45±0.03	+23.66***	109.00±2.07	87.41±2.40	-19.80***
Cerebellum	3.11±0.12	4.11±0.16	+32.15***	3.69±0.09	4.57±0.01	+23.84***	105.05±3.20	84.24±0.27	-19.80***
Brain stem	2.50±0.17	3.25±0.08	+30.00**	3.38±0.01	4.17±0.05	+23.60**	113.15±2.95	90.73±2.48	-19.81***
Spinal cord	3.18±0.28	4.21±0.77	+32.38***	3.96±0.02	4.92±0.08	+24.24***	117.78±4.75	94.08±2.26	-20.12***
Heart	2.29±0.44	2.95±0.51	+28.82*	2.69±0.03	3.25±0.03	+20.78*	98.91±3.80	79.84±1.84	-19.28**
Lung	2.26±0.01	2.90±0.19	+28.31*	2.65±0.01	3.15±0.02	+18.25*	84.00±1.59	68.26±2.14	-18.73*
Pancreas	2.32±0.26	3.00±0.67	+29.31*	2.95±0.08	3.62±0.04	+22.61**	91.55±1.60	74.00±1.61	-19.16*

Values represent Mean and SEM of ten rats per group in brain parts, spinal cord, lung, heart and pancreas. Paired samples t-test was performed to compare the parameters of control(C) and Methylmercury chloride groups. Values: $^{***}p$<0.001; $^{**}p$<0.01; $^{*}p$<0.05 are significant. Percent decrement is MMC compared to controls.

Table 3. Effect of Methylmercury chloride (MMC) on Body Weight changes, Feed Intake and Water Intake changes of rats in comparison to control animals

Parameters	Control		MMC		% decrement
	Before Exp	After Exp	Before Exp	After Exp	
Body Weight (g)	304±2.3	334±2.7	300±2.2	399±3.0	+19.46*
Feed Intake (g/100g/day)	2.55±0.29	3.03±0.45	2.95±0.51	3.88±0.79	+28.05***
Water Intake (ml/100g/day)	291±3.4	322±3.1	286±3.0	245±3.2	-23.91**

Values represent Mean and SEM of ten rats per group. Paired samples t-test was performed to compare the parameters of control (C) and Methylmercury chloride (MMC) groups. Values: $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$ are significant. Percent decrement is MMC compared to controls.

methylmercury induce oxidative stress [54-56] by increasing the intracellular levels of ROS and modifying enzyme activities [35,56] reported high concentration of TBARS and decreased protein content in rat brain of methylmercury chloride treated rats. Lipid peroxidation was also increased in plasma of rats treated with mercury [57]. In our study, Methylmercury chloride increased lipid peroxides and hydroperoxides significantly in cerebrum, cerebellum, brain stem, spinal cord, heart, lung and pancreas while decreased protein concentration in the above mentioned tissues. Oxidative stress may contribute to the development of neurodegerative disorders caused by mercury intoxication [58].

It also have been demonstrated that the administration of mercury as Hg^{2+} (1.5 or 2.25 mg $HgCl_2$ / kg) to rats resulted in increased hydrogen peroxide formation, glutathione depletion and lipid peroxidation in kidney mitochondria [59]. The exposure of mice to mercury, chromium or silver results in enhanced production of MDA in liver and kidneys [60]. Lipid peroxidation was also stimulated in liver, kidney and brain of mice administered methylmercury chloride via drinking water [61].

Numerous earlier reports have shown evidence of ROS involvement in methylmercury chloride toxicity in brain parts, liver and kidney [61]. However, the present study is probably the first to suggest the role of ROS in heart, lung and pancreas of rats treated with methylmercury. The increase in LPO and LHPO in cerebrum, cerebellum, brain stem, spinal cord, heart, lung and pancreas after a low dose of methylmercury chloride indicates a condition of oxidative stress. No direct relation between the organ deposition of Hg and the level of lipid peroxidation and hydroperoxidation in these organs was found. This might be due to organ differences in biotransformation of CH_3Hg^+ to inorganic mercury and in the capacity of the antioxidative defense system.

In the present study, along with increase in LPO and LHPO, methylmercury intoxidation leads to a significant decrease in protein concentration as well. This suggests that besides directly affecting proteins through oxyradical stress, methylmercury also indirectly inhibits protein synthesis in nervous and non-nervous tissues on short term low dose exposure. There are various theories which attribute methylmercury inhibition of protein synthesis to direct interaction of protein synthetic machinery [62].

Our results also showed changes in body weight, food intake, water intake and tissue weight after methylmercury treatment. The adverse effects on these parameters may be due to hormonal imbalance inside the body caused by this metal. Thus it is clear that metal induced damage depends on many factors such as the animal species, tissues, and cell types, as well as the level of metal inside the cells and the antioxidants are the agents ameliorating these adverse effects.

The brain is particularly susceptible to the harmful effects of oxidative stress, a condition caused by an imbalance between the generation of free radicals and the cell's protective antioxidant system [63]. Free radicals have one or more unpaired electrons and thus the ability to react with crucial cell components such as proteins, DNA and lipids. Both enzymes like catalase, peroxidase and SOD, and non-enzymatic compounds, including GSH and TSH are involved in the cellular defense against free radicals. In addition to inducing lipid peroxidation (by means of MDA levels) and altering GSH concentrations, $HgCl_2$ was also reported to affect other antioxidant enzyme activities of cells. It was showed that Hg^{+2} induces H_2O_2 formation and stimulates

Table 4. Effect of Methylmercury chloride (MMC) on Brain parts, spinal cord, Lung, Heart and pancreas free sulfhydryl (GSH) activity of rats in comparison to control animals

Tissues	GSH (μmol/g tissue)		
	Control	MMC	% decrement
Cerebrum	7.78±0.16	5.35±0.11	-31.23***
Cerebellum	7.63±0.08	5.30±0.06	-30.53**
Brain Stem	7.92±0.15	5.42±0.17	-31.56***
Spinal Cord	7.89±0.08	5.41±0.16	-31.43***
Heart	6.91±0.12	5.21±0.13	-24.31*
Lung	6.99±0.11	5.26±0.16	-24.74*
Pancreas	7.45±0.13	5.27±0.10	-29.26**

Values represent Mean and SEM of ten rats per group in brain parts, spinal cord, lung, heart and pancreas. Paired samples t-test was performed to compare the parameters of control (C) and Methylmercury chloride (MMC) groups. Values: $^{***}p<0.001$; $^{**}p<0.01$; $^{*}p<0.05$ are significant. Percent decrement is MMC compared to controls.

the activities of copper-zinc SOD and xanthine oxidase in AS52 cells [64]. The present study evaluated the effects of low levels of methylmercury on antioxidant enzyme activities in different tissues of rat. Our findings demonstrated reduced levels of total (TSH) and free (GSH) sulfhydryl in MeHg-treated animals when compared with the control group. GSH is the main nonprotein thiol involved in the antioxidant cellular defense against the toxic effects of reactive oxygen species produced naturally in the organism or from the metabolism of xenobiotics [65]. It is known that the most important mechanism for Hg-induced oxidative damage is its strong reactivity with –SH components [66-67], which can lead to diminishing the antioxidant reserves, according with our study, contributing to the increase in reactive species production, damaging lipid membranes, proteins and DNA [68]. Besides, biliary excretion is the major pathway for MeHg elimination, and this mechanism appears to be dependent on GSH bioavailability [69,70], which is bound in MeHg by a direct chemical interaction, forming GS–HgCH3 complexes [70]. In a study carried out by Thompson, it was observed that exposure to high levels of MeHg throughout the gestation resulted in GSH depletion in the fetus [71]. In agreement, it was also observed reduced GSH levels in cerebellum of mice treated with high doses of MeHg (7 mg/kg per day) [72]. Besides GSH as an antioxidant, free radicals are detoxified by various antioxidants enzymes, such as CAT and SOD. We observed decreased CAT activity in our rats treated with low doses of MeHg, which could be explained by a direct inhibition of the enzyme by the MeHg [73]. Contrary to our findings, it had been observed increased CAT activity in mice exposed to inorganic mercury [74]. SOD is involved in dismutation of the superoxide anion ($O_2^{\bullet-}$) to hydrogen peroxide (H_2O_2) and oxygen and the results of the present study demonstrate a decrease in the SOD enzyme activity in rats treated with methylmercury chloride. This decrease in the activity of SOD, observed in the present study indicates either reduced synthesis of the enzyme or elevated degradation or inactivation of the enzyme during the toxic metal treatment. Catalase is inhibited by ($O_2^{\bullet-}$) [75]. Superoxide anion served to convert catalase to the ferroxy and ferryl states, which are inactive forms of the enzyme [76]. Hence, the lower activity of CAT observed in methylmercury chloride treated rats may be due to lower levels of SOD, which was similar to the reported results of the present study or may be due to inactivation of catalase owing to excess production of ROS. CAT and SOD are considered to be indispensable for the survival of the cell against deleterious effects of hydroperoxides. The combined action of SOD and CAT provide an efficient mechanism for removal of free radicals from the cell [77]. Thus their decline in the present study proves that their absence may cause cell death due to free radical damage.

In summary, oxidative stress is present during methylmercury intoxication. The concentrations of MDA in tissues were observed to increase significantly in rats treated with this metal. Present study also revealed that the peroxide-removing antioxidative system of nervous and non-nervous tissues of rats was adversely affected by low levels of methylmercury. Thus it is concluded that dietary supplementation of antioxidants might be useful in populations that are occupationally exposed to methylmercury chloride.

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