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Histological studies on cerebral cortices in rats (*Rattus norvigicus*) exposed to methyl mercury

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Abstract
The study was conducted on 36 adult male Sprague Dawley rats which were randomly assigned into three equal groups, Group II and III were exposed to 2.5ppm and 5ppm of MeHg in the form of Methyl Mercuric Chloride in drinking water ad-libitum daily for 14 and 35 days of treatment. Motor and visual areas revealed deranged cortical cell organization with shrinkage and decrease in number of neurons in both the treatment groups at 14 days post exposure and further increased at 35 days of exposure. Perineuronal space, deranged neurofibrillar network, haemorrhages in capillaries and vacuolation in neuropil were apparent in the motor area of 5ppm group at 35 days post exposure. Astrogliosis was observed in all the treated groups but more manifested in 2.5ppm group at 35 days post exposure. Increased vascularity with hypertrophy of the capillaries in visual cortex of both the treated groups and was distinctly visible in 5ppm group at 35 days exposure compared to 14 days. Ruptured endothelial basement membrane was thrown in to capillary lumen, astrocytes invaded in to some capillaries, vacuolated external and internal pyramidal cells were more evident in 2.5ppm compared to 5ppm at 35 days than at 14 days post exposure.

Keywords: Brain, cerebral cortex, methyl mercury, rat, astrocytes

Introduction
Methyl mercury (Me Hg), an organic form of mercury, can easily cross the blood-brain barrier and placenta and is accumulated in the brain of fetuses more than in mothers [1]. MeHg was one of the potent neurotoxin and stands as the sixth most serious pollution threat to the planet [2]. The primary target organ for oral exposure to MeHg was the brain, the effects on this organ accounting for the developmental toxicity of the chemical [3-4]. Laboratory animals and human population exposed to toxic levels of MeHg during pre and post-natal life caused neurological abnormalities, cognitive impairment, and behavioral disturbance [5]. Hence the present study was conducted to make an attempt to elucidate the histological/ cellular changes in cerebral cortices by exposing to low and high doses of methyl mercuric chloride.

Materials and Methods
Present study was conducted on 36 adult male Sprague Dawley rats of 5 weeks age. Rats were procured from National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, India. After quarantine, subjects were randomly assigned into three equal groups *viz.* control group (group I), low Me Hg group (group II) and high Me Hg group (group III). The groups II and III were exposed to 2.5ppm and 5ppm of MeHg in the form of Methyl Mercuric Chloride (Sigma Aldrich 33368) in drinking water ad-libitum daily. All the three groups were fed with commercial pelleted rat diet (chow) ad-libitum daily and the rats were maintained under standard conditions of light (12/12-h light/dark cycle) and room temperature (22 ± 2ºC) as per CPCSEA norms.
The experimental animal protocol followed the ethical principles approved by the Institutional Animal Ethics Committee approved by CPCSEA (through reference 4/IAEC/NTRCVSc/GVM-2013-14 dated 10.12.13). The work was carried out in the Experimental laboratory animal house and Department of Veterinary Anatomy, N.T.R. College of Veterinary Science, Gannavaram, and Andhra Pradesh, India.
The body weights of rats were recorded twice in a week. Six animals in each group were sacrificed humanely under ether inhalation anesthesia after 14 and 35 days of exposure to study the early and prolonged post exposure effects.

Experimental Design

Treatment groups	Number of animals sacrificed	
	14 days post exposure	35 days post exposure
Control (Group I)	6	6
2.5ppm (Group II)	6	6
5.0ppm (Group III)	6	6

After each sacrifice, fresh brains were collected from the cranial cavity, morphometric details of brains were recorded and cerebral cortices were separated. The cerebral cortex from each animal was transferred to neutral buffered formalin for fixation. Paraffin sections of 4-5 µm thickness were used for histological study[6].

Results

Histological changes at 14 days post exposure

The motor area of cerebral cortex showed deranged cortical organization with reduced number of neurons in both 2.5 and 5ppm treated groups. There was increased glial cell population and cortex presented with pleomorphic pyramidal cells. Further, increased vacuolation with respect to size and number was observed reflecting the affected neurons and the neuropil in 2.5ppm than in 5ppm and control groups. Slightly increased vascularity, hypertrophied endothelial cells and dilatation in the capillaries was apparent. Few external pyramidal cells and many internal pyramidal cells showed perineuronal space without alteration in the shape of the cell. Vacuoles in the neuropil, increase in size and number of astrocytes and astrocytosis was evident in 5ppm group than in 2.5ppm and control groups. Neuronal loss and neuronal cell shrinkage was evident and some of the pyramidal cells lost nissal substance and some lost dendrites. Some had pyknotic nuclei in 5ppm than in 2.5ppm and control group (Fig 1 &2).

In the visual cortex, numbers of neurons was comparatively reduced in 5ppm group than in 2.5ppm group. Capillary dilatation was slightly more in 5ppm group than in 2.5ppm group. Perineuronal vacuolation was observed in both the treated groups. In some capillaries, endothelial cells along with basement membrane were detached and thrown in to lumen. Few astrocytes showed pyknotic nucleus and were penetrated in to capillaries in both the experimental groups but more evident in 5ppm group. Necrotic astrocytes with pyknotic nuclei were observed more in number in 2.5ppm than 5ppm group (Fig 3). Inner visual cortex showed more number of dilated capillaries, increased number of pyknotic cells and many neurons appeared irregular in shape / pleomorphic neurons. Astrocytes became pyknotic and vacuolation in the neuropil and perineuronal vacoulation was more appreciated in 5ppm than in 2.5ppm and control groups as an indication of spongiosis. External and internal pyramidal cells were hypertrophied and vacuolated in both treated groups. The size of internal pyramidal cells was greater in 5ppm than in 2.5ppm and control groups. Chromatin degeneration and nuclear vacoulation was marked more in many of the internal pyramidal cells of 2.5ppm group.

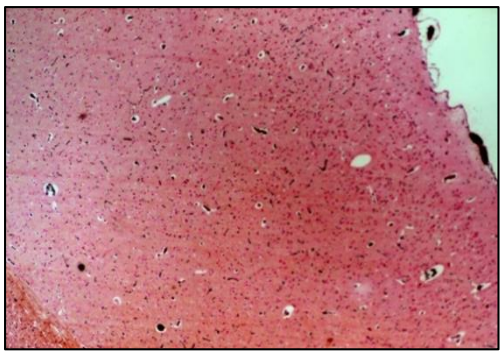

Fig 1: Photomicrograph showing motor area of cerebral cortex in control group at 14 days post exposure Silver method for Hg X100

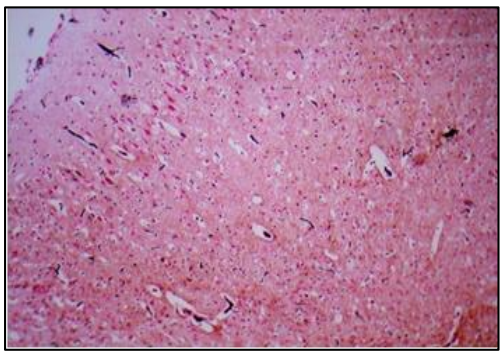

Fig 2: Photomicrograph showing motor area of cerebral cortex in 2.5 ppm group at 14 days post exposure Silver method for Hg X100

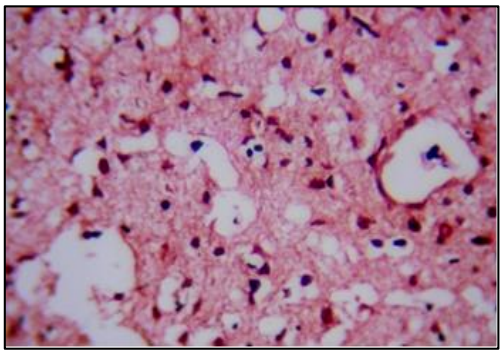

Fig 3: Photomicrograph showing inner visual area of cerebral cortex in 5 ppm group at 14 days of post exposure Silver method X400

Histological changes at 35 days post exposure

The motor cortex of cerebrum showed deranged neurofibrillar network between axons and dendrites in both the treated groups compared to control. The dendrites were detached from pyramidal cells and were thrown in the neuropil appeared predominantly in 5ppm group than in 2.5ppm group (Fig 4). Neuronal cell density was decreased in 5ppm than in 2.5ppm and control groups. The external pyramidal cells appeared to be more densely packed in 2.5ppm group than in 5ppm and control groups. The internal pyramidal cells were few and interspersed with fusiform layer cells in 2.5ppm whereas, greater number in 5ppm group. Vacuolation and

perineuronal space around pyramidal neurons and many pyramidal neurons were altered their shape in 5ppm group than in 2.5ppm group. This alteration might be the reason for disruption of pyramidal tracts on exposure of methyl mercury (Fig 5).

The size and number of astrocytes were almost twofold in 2.5ppm than in 5ppm and control groups. It may be an indication of astrocytosis in 2.5ppm group (fig 6). Vacuolation, psychotic nucleus and per neuronal space around some astrocytes in 5ppm group than in 2.5ppm group. Oligodendrocytes were slightly increased in number which might be the compensatory mechanism for myelin in both the treated groups. There was dilatation and hypertrophy of the capillaries in both the treatment groups and was markedly visible in 5ppm group at 35 days compared to 14 days post exposure. Hemorrhages in the capillaries, pronounced invasion of astrocytes and endothelial cells into capillary lumen may indicate the disruption of blood brain barrier in 5ppm which was not observed in 14 days post exposure. Loss of nissal substance was more evident in 2.5ppm than in 5ppm.

In the visual cortex, vascularity was increased remarkedly by increasing the size and number of capillaries in both the treated groups than in control and 35 days when compared to 14 days post exposure. Neuronal cell density was reduced in both the treated groups compared to control groups. Hypertrophy and hyperplasia of astrocytes was observed in 2.5ppm group than in 5ppm and control. Rupture of basement membrane, invasion of astrocytes and endothelial cells into the dilated capillaries was more evident in 2.5ppm than in 5ppm. No such evidence was noticed in control group. The visual cortex showed astrocytosis, some astrocytes were necrosed, pyknotic and perineuronal vacoulation was pronounced in 5ppm group which appeared to be spongiosis (Fig 7). Neuropil was increased in both the treated groups than in control. Astrocyte size was reduced in 2.5ppm than in 5ppm and control. It may lead to functional disability of the blood brain barrier in 2.5ppm group. Visual cortex of 2.5 and 5ppm showed homogenous fine network of fibers/ tracts which might be an indication that the sensory path was not altered at 35 days post exposure.

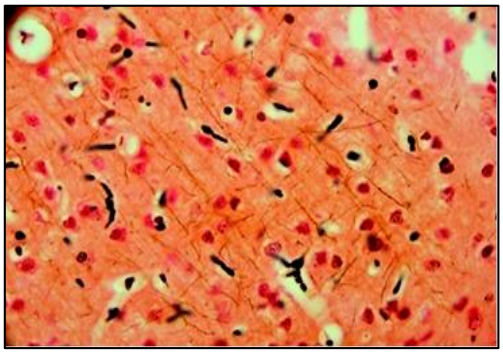

Fig4: Photomicrograph showing fibrillar network in motor area of cerebral cortex in 5ppm group at 35 days of post exposure Silver method for Hg X1000

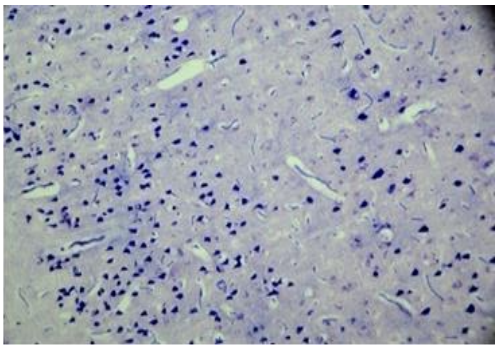

Fig 5: Photomicrograph showing pyramidal cells of cerebral cortex in 5 ppm group at 35 days of post exposure PTAH X100

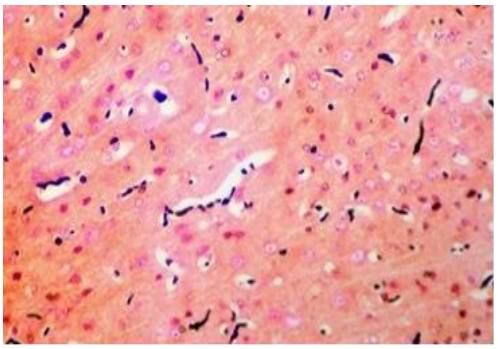

Fig 7: photomicrograph showing visual area in cerebral cortex of 5 ppm at 35 days of post exposure Silver method for Hg X1000

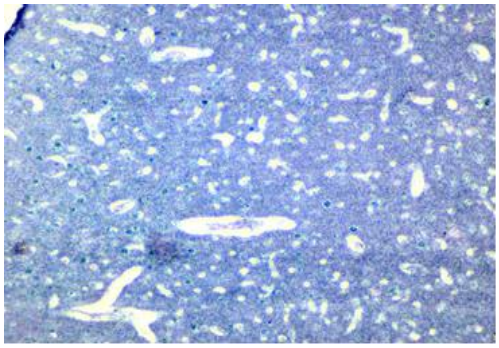

Fig 6: Photomicrograph showing astrocytes in motor area of cerebral cortex in 2.5 ppm group at 35 days of post exposure cajal's for paraffin section X400

Discussion

The neurofibrillar network between axons and dendrites in the motor area of cerebral cortex was deranged in both the treated groups at 14 days but the dendrites which were detached from pyramidal cells thrown in the neuropil appeared predominantly in 5ppm group than in 2.5ppm at 35 days post exposure. Similarly, in the cerebrum of human fetal brain [7] and in female rats [8] noticed disorientation of cortical layers within the motor cortex when exposed to 2.5 and 5ppm of methyl mercury in drinking water when compared to control.

Conversely, no senile plaques or neurofibrillary changes were observed in both cerebral hemispheres in humans exposed to the industrial MeHg compound [9]. Further, they elucidated great reduction in sub cortical white matter with no evidence

of progressive degeneration either in myelin or axis–cylinders and no fat granules in cortex and white matter. However, progressive degeneration in the neurofibrillar network was observed in the present study from 14 to 35 days with MeHg exposure.

Neuronal loss, decreased cell density and neuronal cell shrinkage was more evident in 5ppm than in 2.5ppm and control groups at 35 days methyl mercury exposure. Similar changes such as loss of nerve cells with replacement by reactive and fibrillary gliosis in cats exposed to 0.25 mg Hg/kg/day by per oral route for 12–14 weeks, either as pure methyl mercuric chloride or as methyl mercury-contaminated fish were reported [10].

Both in external and internal pyramidal layer, vacuolation in neuropil, perineuronal space around pleomorphic pyramidal neurons, detached pyramidal dendrites and dilated capillaries were observed in both the treated groups and at both treatment periods, this might lead to disruption of pyramidal tract path on exposure of methyl mercury.

Hypertrophy and hyperplasia of astrocytes was appeared only in 2.5ppm which indicated astrocytic gliosis in 2.5ppmMeHg exposure at 35 days. Perineuronal space around some astrocytes and pyknotic nucleus in some astrocytes were more evident in 5ppm group than in 2.5ppm group at 35 days post exposure. There was slight increase in the number of oligodendrocytes which might be a compensatory mechanism for myelin in both the treatment groups. Proliferation of hypertrophic astrocytes was observed in humans [11]. Gliosis was observed in the second and third layers of calcarine cortex along with atrophy of the whole cortex in methyl mercury exposed common marmosets [12]. Wide spread neuronal degeneration, neuron loss and astrocytosis was reported in methyl mercury exposed rats [13].

Dilatation and hypertrophy of the capillaries was observed in both the Me Hg treated groups and was markedly visible in 5ppm group at 35 days post exposure compared to 14 days post exposure. Haemorrhages in the capillaries, pronounced invasion of astrocytes and endothelial cells into capillary lumen was pragmatic in 5ppm group at 35 days of exposure which might be due to disruption of blood brain barrier. This was not observed in 14 days post exposure. Multiple foci of necrosis with gliosis of marked congestion of blood vessels with prominent perivascular fibrosis was noticed in Albino rats exposed to 0.330 mg/kg b.wt of mercuric chloride [14]. However, prominent perivascular fibrosis was not correlated with the present findings.

In the visual cortex, number of neuronal cells was comparatively reduced in 5ppm group than in 2.5ppm group at 14 days and 35 days of methyl mercury exposure. Capillary dilatation was slightly more in 5 ppm group than in 2.5ppm group at 14 days post exposure. Vascularity was increased remarkably with hypertrophy and proliferation of capillaries in both the treated groups. Ruptured endothelial basement membrane, thrown in to lumen was more evident in 2.5ppm compared to 5ppm at 35 days than at 14 days post exposure. Inner visual cortex showed more dilated capillaries, increased number of pyknotic cells and many pleomorphic neurons. Pyknotic and vacuolated astrocytes in the neuropil were apparent in 5ppm as an indication of spongiosis. Perineuronal Vacuolation was widely appreciated in 5ppm than in 2.5ppm and control groups at 14 and 35 days post exposure. External and internal pyramidal cells were hypertrophied and vacuolated in both the treated groups at 14 days post exposure. Concentric constriction of the visual fields, which correlated with bilateral cortical atrophy around the calcarine fissures, was recorded [15]. Female rats exposed to 0, 2.5 and 5ppm of methyl mercury in drinking water showed disorientation of cortical layers within the motor and visual cortices when compared to control group [8].

Visual cortex of 2.5 and 5ppm groups showed homogenous fine network of fibers/ tracts which may indicated that the sensory pathway was not altered at 35 days post exposure. Comparatively neuropil was increased in both the treated groups at 35 days post exposure than at 14 days of MeHg exposure. Astrocyte size was reduced in 2.5ppm than in 5ppm and control at 35 days post exposure. All these changes might lead to functional disability of the blood brain barrier in 2.5ppm group at an early exposure period. MeHg poisoning caused damage to the cerebral cortex, affected the visual, auditory and somatic sensory areas which caused a remarkable loss of neurons in these brain regions [5].

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