

EFFECT OF VARIOUS DOSES OF METHYL MERCURIC CHLORIDE ON ADENOHYPOPHYSIS OF PITUITARY GLAND IN SPRAGUE DAWLEY RATS

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Abstract: The experiment was conducted on 36 adult male Sprague Dawley rats of 6 weeks age. The subjects were randomly assigned into three groups, viz. control (group I), 2.5ppm methyl mercury exposure (group II) and 5.0ppm methyl mercury exposure (group III). Methyl mercury was fed in the form of methyl mercuric chloride in drinking water daily. The average weight, length and width of pituitary glands were increased significantly in both the treated groups compared to the control group at 14 days post exposure. However, only weight was increased in the 5.0ppm group at 35 days post exposure. Histologically in the pars distalis the size of type I acidophils was reduced and showed indistinct cell boundaries, condensation, fragmentation and margination of chromatin in the nucleus in the 2.5 and 5.0ppm groups seemingly apoptotic. Acidophils type II cells with indistinct cell boundaries, vacuolation within the anastomosing cords and hypertrophy of sub capsular capillaries of pars distalis; hypertrophy of basophils I (LH) and basophils II (FSH) cells in the pars distalis of pituitary gland was observed in the treated groups of both exposures compared to the control. Degeneration and vacuolation of the cleft cells between the two layers in the 5.0ppm group than in 2.5ppm and control groups. The diameter of the cleft was reduced in the 5.0ppm group when compared to that of 14 days and control groups.

Keywords: Pituitary gland, Methyl mercury (MeHg), Acidophil type I cell (A₁), Acidophil type II cell (A₂), Basophil type I cell (B₁), Basophil type II cell (B₂).

Introduction

The pituitary is a complex endocrine gland that produces a variety of hormones, essential to survival. These hormones have both direct and indirect effects on other endocrine glands, cell turnover, growth and metabolism. Secretion of hormones by the adenohypophysis is normally regulated by the hypothalamus and the two structures are closely linked by vascular and neural connections. Methyl mercury (MeHg) is one of the six most serious pollution threats to the planet. Fish and other aquatic species are the significant source of MeHg exposure in humans. The wide spread consumption of fish containing elevated concentrations of MeHg has prompted concern over the health effects. MeHg is transported freely throughout the body and crosses the blood-brain barrier (Verschuuren *et.al.*, 1976). The Japanese coastal city of

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Minamata became a tragic model of how heedless industrialization could taint the lives of unwitting victims. Methylmercury had been known as a potent neurotoxicant for many years (Hunter and Russell, 1940). However, very little information is available in regard to morphohistological changes of the adenohypophysis of pituitary gland in MeHg poisoning.

Materials and methods

Experimental design: The present study was conducted on 36 adult male Sprague Dawley rats of 6 weeks age. Rats were procured from National Centre for Laboratory Animal Sciences, National Institute of Nutrition, Hyderabad, India. After quarantine the rats were randomly grouped as group I (control), group II and group III (Table:1). They were housed in cages at the rate of 3 per cage and were maintained under standard conditions of light (12/12-h light/dark cycle) and room temperature ($22\pm 2^{\circ}\text{C}$). The groups II and III were exposed to 2.5 ppm and 5.0 ppm MeHg respectively in the form of MMC in drinking water given ad-libitum daily. All the three groups were fed with commercial pelleted rat diet (chow) ad-libitum daily. The animal experimental protocol followed the ethical principles as approved by the Institutional Animal Ethics Committee (through reference 4/IAEC/NTRCVSc/GVM-2013-14 dated 10.12.13).

Morphometric analysis: The results obtained among the groups were compared statistically by ANOVA using SPSS program.

Histological study: After each sacrifice, pituitary glands were collected by dissecting the skull and lifting the brains from the cranial cavity washed with normal saline. Pituitary glands were fixed in 10% neutral buffered formalin and Zenker's fluid. Tissues were processed and paraffin embedding blocks were prepared. Sections of $4\text{--}5\mu$ thickness were stained by the following methods for histological study. Haematoxylin and Eosin (H&E) for routine histological study (Bancroft and Gamble, 2003), Masson's Trichrome staining for collagen (Luna, 1968), Heath's method of staining and Monroe-Frommer method (Singh and Sulochana, 1996).

Results and Discussion

The average weight, length and width of pituitary glands were increased in both the treated groups compared to the control group and were statistically significant at 14 days post exposure. The average weight increased significantly in the 5.0ppm group only at 35 days post exposure but the average length and width were almost similar among the three groups (Table. 2). As work in this regard was not previously carried out, reports on morphometry of pituitary glands in rats after treatment with MeHg were not available for discussion.

14 days post exposure

The parenchyma of pars distalis showed degeneration with wide sinusoids in the treatment groups. It contained two types of acidophils, type I (A_1) and type II (A_2) cells. A_1 cells showed a pyknotic nucleus in the 2.5ppm group. The size of A_1 cells was reduced in both the treatment groups. Cell boundaries were indistinct. The cytoplasm appeared to be intermixed with the parenchyma of pars distalis. Some of the acidophils type I (A_1) cells showed condensation, fragmentation and margination of chromatin in the nucleus in the 2.5 and 5.0ppm MeHg groups seemingly apoptotic (Figs 1 & 2). Acidophils type II (A_2) cells appeared as scattered small sized cells with strong basophilic, eccentrically placed nucleus and a small amount of acidophilic cytoplasm surrounding the nucleus like a thin rim with indistinct cell boundaries. Similarly, Johansson *et.al.* (2006) described that when T20 pituitary cell-line was exposed to a combination of PCB's (poly chlorinated biphenyls) and MeHg at moderately toxic doses resulted in induction of necrosis and cell death. B2 cells were present in clusters at postero-lateral aspect of pars distalis (Figs 3 & 4). Vacuoles on the apical surface of nucleus in 2.5ppm group which represent the Golgi complex, indications of the secretory state of the cell. Some of the B2 cells presented a peripheral nucleus while others had a centrally placed nucleus. Nucleolus was located centrally and chromatin was distributed in a cartwheel manner. The size of the cells was increased in the 5.0ppm group. Interestingly, Moller-Madsen and Thorlacius-Ussing (1986) demonstrated the presence of intracellular accumulations of mercury (Hg) deposits in lysosomes and granules of secretory cells but Hg deposits were found only in the lysosomes of non secreting cells of adenohypophysis in rats exposed to $20\text{mg}\times 1^{-1}$ MeHg in drinking water. Apart from this, no structural damage was observed in the cells containing Hg.

35 days post exposure: Hypertrophy of sub capsular capillaries was observed at the periphery of pars distalis. Thick nerve fibres traversed the parenchyma of pars distalis from the pars nervosa up to 1/3rd of the anterior extremity of pars distalis in the 5ppm group at 35 days post exposure. The cleft was lined by simple cuboidal cells which were detached from pars distalis in the 2.5ppm group. The cleft was lined by double layered cuboidal cells on either side of pars distalis throughout its length except at the junction between distalis and nervosa where the cells were arranged in a multilayer. This may be considered as pars intermedia. The cleft cells were degenerated and formed vacuoles between the layers through which the two layers appeared to be separate and occupied the entire cleft region by leaving very narrow space in the 5.0ppm group. The size of the cleft cells was also increased

compared to the 2.5ppm and control groups. The diameter of the cleft was reduced when compared to that of 14 days and control groups. This might be due to proliferation and degeneration of the two parts of pars distalis in the 5.0ppm group. As work in this regard was not previously carried out, reports on the effect of MeHg in the cleft of pituitary glands of rats were not available for discussion. Postero-lateral aspect of pars distalis showed more stellate cells in the 2.5ppm group. Cellular density was increased in pars distalis on either side of cleft and in the centre of pars distalis. The parenchyma of pars distalis showed wide gaps between the cellular cords. Vacuolation was found within the anastomosing cords of pars distalis which might be due to degenerative changes. Vacuolation around the cells of acidophils type I was observed in 5.0ppm group compared to the 2.5ppm group. Vacuolation was not observed in the acidophils of control group. However, vacuolation in the cytoplasm was seen in acidophils type II and basophils type I in 5.0ppm group than in the 2.5ppm and control groups. Few mitotic divisions were present in the basophils of 5.0ppm group. B2 cells were located in clusters at the postero-medial and postero-lateral aspects of the pars distalis on either side of the cleft. Hypertrophy and vacuolation of B2 cells were observed in the 5ppm group whereas they were absent in the control group (Figs 5&6). In contrary to this, Kabuto (1986) assumed that the effect of MMC on pituitary-gonadal axis was inconsistent and the axis seemed to be relatively resistant to MMC in rats after single administration. Wren *et.al.* (1987) described that up on feeding of Ranch bred mink with diets containing MeHg for eight months, no observed treatment effects on thyroid, pituitary and adrenal glands were observed. There was evidence of significant placental transfer of MeHg to foetus.

Conclusion

The size of acidophils was reduced in pars distalis of pituitary gland in both the treatment groups in contrast to increased body weights. Few acidophils (type I) showed apoptotic nucleus in 2.5ppm and 5.0ppm groups. Appearance of B₂ cell clusters at postero-lateral aspect of pars distalis, vacuoles on the apical surface of nucleus might be the representation of the golgi complex in 2.5ppm group which could be the indication of secretory state of cells.

Cleft cells were detached in the 2.5ppm group. In the 5.0ppm group these cleft cells hypertrophied and vacuolated and became multilayered towards pars nervosa which might be considered as pars intermedia. However, hypertrophy of B1 and hypertrophy and vacuolation

of B2 cells appeared in both treated groups and both periods of exposures but this was more in 5.0ppm group at 35 days post exposure.

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Tables**Table 1: *Experimental design***

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

Table 2: Morphometric analysis of pituitary gland after two different treatment periods

Group	14 days treatment			35 days treatment		
	Weight (g)	Length (cm)	Width (cm)	Weight (g)	Length (cm)	Width (cm)
Control	0.045± 0.0008 ^a	0.35± 0.016 ^a	0.273± 0.007 ^a	0.048± 0.0004 ^{ab}	0.545± 0.04 ^a	0.4±0.02 ^a
2.5 ppm	0.0536± 0.0009 ^b	0.541± 0.020 ^b	0.386± 0.012 ^b	0.043± 0.002 ^a	0.55± 0.027 ^b	0.44± 0.012 ^a
5.0 ppm	0.059± 0.0011 ^c	0.555± 0.014 ^b	0.358± 0.011 ^b	0.054± 0.003 ^b	0.545± 0.014 ^a	0.44± 0.019 ^a

Values are mean±SEM (n=6) One way ANOVA (SPSS)

Means with different alphabets as superscripts differ significantly (p<0.05)

Figures

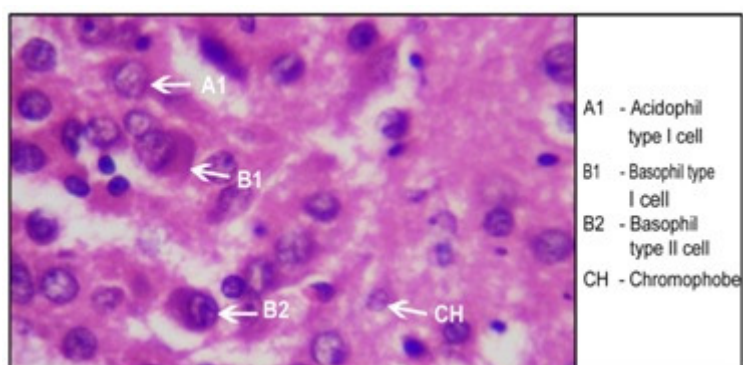


Fig. 1: Photomicrograph showing acidophils and basophils on the postero-lateral aspect of pars distalis in control group at 14 days **H&E X400**

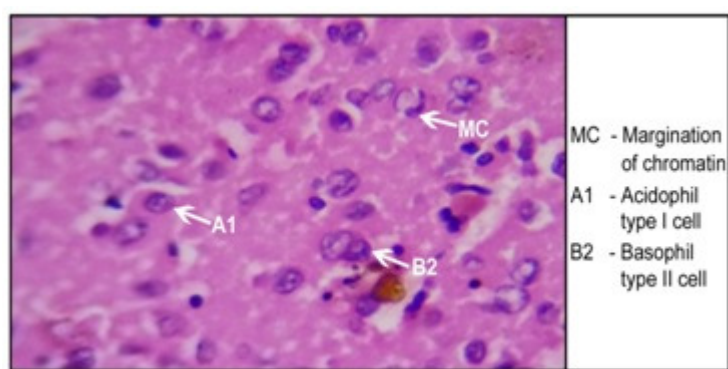


Fig. 2: Photomicrograph showing acidophils and basophils on the postero-lateral aspect of pars distalis in 5.0ppm at 14 days post exposure **H&E X400**

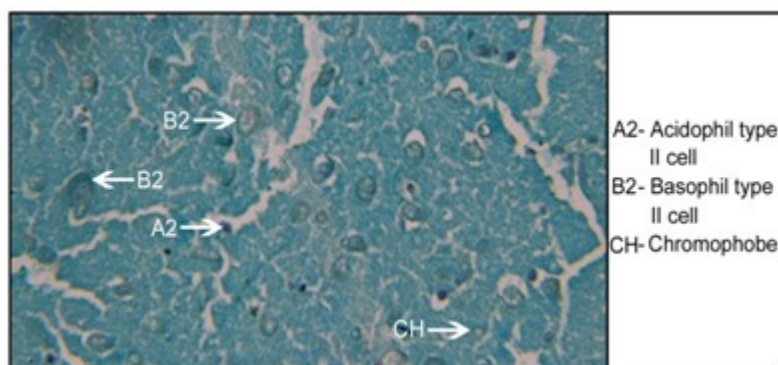


Fig. 3: Photomicrograph showing B2 and A2 cells of pars distalis in control at 14 days **Monroe-Frommer X400**

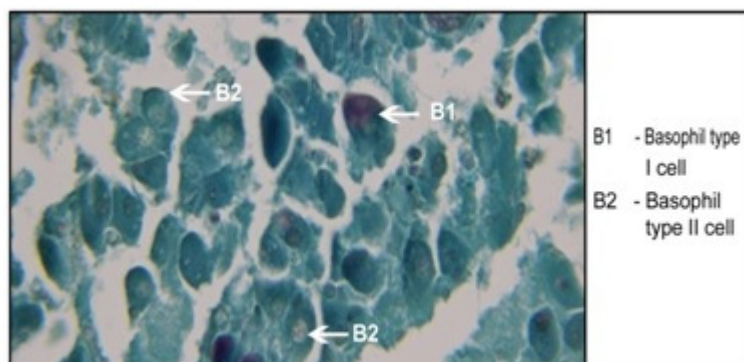


Fig. 4: Photomicrograph showing B₁ (purple colored) and B₂ (green colored) cells of pars distalis in 2.5ppm at 14 days post exposure **Monroe-FrommerX400**

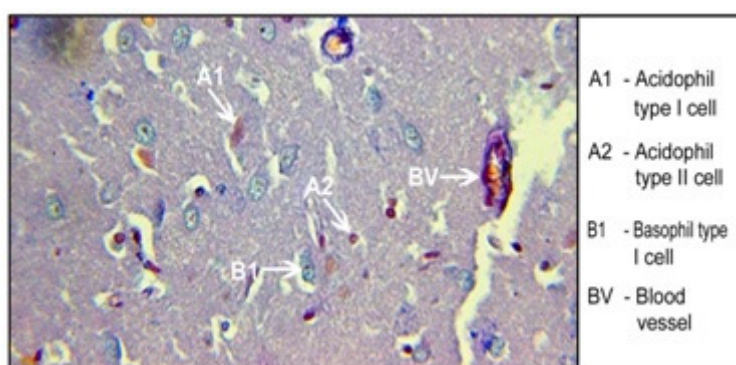


Fig. 5: Photomicrograph showing B₁ cells of pars distalis in control at 35 days **Heath X400**

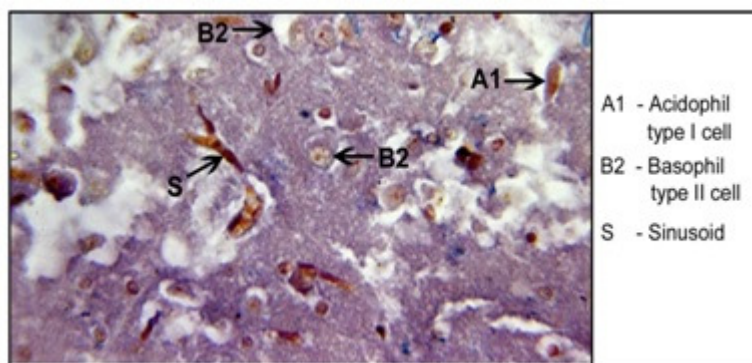


Fig. 6: Photomicrograph showing B₂ cells of pars distalis in 2.5ppm at 35 days post exposure **Heath X400**