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UNVEILING THE NEUROTOXICITY OF METHYL MERCURY IN RATS (*RATTUS NORVIGICUS*) THROUGH MORPHOMETRIC ANALYSIS OF BRAIN AND BODY WEIGHT ASSESSMENT

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ABSTRACT
The current study aims to shed light on the neurotoxicity of methyl mercury (MeHg) in rats by the combined assessment of brain morphometry and body weight changes. To evaluate the MeHg toxicity on brain morphometry and body weight changes, 36 adult male Sprague Dawley rats (aged 6 weeks) were randomly assigned to three groups *viz*., control (group 1), low MeHg exposure group (group 2) and high MeHg exposure group (group 3). The groups II and III were exposed to 2.5 and 5 ppm of MeHg in the form of Methyl Mercuric Chloride (Sigma Aldrich 33368) in drinking water *adlibitum* daily for 14 and 35 days of treatment periods. Mean brain weight was higher in 5 ppm group at 14 days post-exposure and 2.5 ppm group at 35 days post-exposure. The average length of brain was higher in both the treated groups when compared to control at 14 days post-exposure. There was a significant increase in body weight of 5 ppm group compared to 2.5 ppm and control groups at 35 days post-exposure. The present study suggests that MeHg alters the brain morphometry evidencing the neurotoxicity.

KEY WORDS: Methyl mercury, brain morphometry, body weights, rats.

INTRODUCTION:

It has been reported earlier that mercury (Hg) is a widespread environmental and industrial pollutant, which induces severe alterations in the tissues of both animals and men (Ansar and Iqbal, 2016). Mercury exists in 2 forms *viz*., inorganic (iHg) and organic (primarily MeHg); methyl mercury is neurotoxic to wild animals and human beings and severely contaminates land, water, air and the food chain throughout coastal India (Morcillo *et al*., 2017). In the recent past, prevalence of mercury intoxication by food and environment sources has been raised globally (Hematian, 2013). Methyl mercury is ranked as one of the six most serious pollution threats to the planet (Toxics Link, 2003). Brain morphometry is a subfield of both morphometry and the brain sciences, concerned with the measurement of brain structures and changes. Quantifying the anatomical features of the brain in terms of shape, mass and volume derives more specific information, especially in developmental neurotoxicity conditions (Galler *et al*., 2002). Further, examining the association between long term pollutants exposure and brain morphometry is an important pre-requisite to reveal the potency of the particular toxin causing neurotoxicity (Bjornebekk *et al*., 2016). In addition, Subjecting to methyl mercury even at lower levels is known to affect the body weight changes at a greater extent (Takahashi *et al*., 2017). No studies were ever reported on the effect of MeHg on brain morphometry. Hence, the present study was conducted to reveal the neurotoxicity of MeHg by elucidating the brain morphometry and body weight changes in adult rats exposed to various doses of MeHg.

MATERIALS & 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 MeHg group (group II) and high MeHg group (group III). The groups II and III were exposed to 2.5 ppm and 5 ppm of MeHg in the form of Methyl Mercuric Chloride (Sigma Aldrich 33368) in drinking water *adlibitum* 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 as 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, Andhra Pradesh, India. 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 (Table 1). After each sacrifice, fresh brains were collected from the cranial cavity by dissecting the skull and transferred to 10% neutral buffered formalin for one hour due to its fragility, preceded for recording morphometric details. Morphometry (weight, length and width) of brains was done with the help of monopan balance and Vernier

callipers. The body weights of rats were recorded twice in a week using weighing balance. Data of brain weights, length, width and body weights was processed for statistical analysis by ANOVA using SPSS and Sigma plot.

TABLE 1: 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

RESULTS & DISCUSSION

The average body weight of rats was higher in the 5 ppm group both at 14 and 35 days post-exposure (Table 2). The increase at 14 days post exposure was not statistically significant, whereas a significant increase was noticed from 17 to 35 days post-exposure in both the treated groups compared to control (Fig. 1). Likewise, an increased body weight gain was observed in wistar male rats exposed to MeHg at 1 mg/kg BW; however, it caused a drastic reduction in BW gain on exposure to 3 mg/ Kg/day (Fossato da Silva *et al.*, 2011). Sakamoto *et al.* (1993) reported a more severe effect of MeHg treatment on body weight changes at 35 days (PD-35) post-exposure compared to 1 (PD -1) and 14 days (PD-14) post-exposure in rats exposed to methyl mercury chloride @ 0, 2.60, 3.64, 5.10, 7.14, and 10 mg/kg/day. The body weight loss began on Day 5 in PD-35 rats and on Day 10 in PD-14 rats treated with 10 mg/kg/day of MMC, but not in PD-1 rats under the same treatment. Further, Verschuuren *et al.* (1976) and Sukamoto *et al.* (2004) also reported a gradual reduction in body weight gain from 25 d post-exposure in male wistar rats' exposed to MMC at 2.5 ppm and 5 mg/kg/day, respectively. Although non-significant, a reduction in body weight was observed during first 30 days, which became markedly significant by 85 d post-exposure in adult male Sprague Dawley rats subjected to MMC at 0.7 mg/kg BW (Shigemastu *et al.*, 2000).

TABLE 2: Average body weights (gm) of different groups of rats after different periods of exposure

Period (Days)	Control	2.5 ppm	5.0 ppm	SEM
Pre trial	142.33[a]	137.83a	144.33[a]	10.76
3	160.33[a]	144.00[a]	170.60[a]	12.74
7	176.00[a]	160.83[a]	186.00[a]	13.65
11	189.83[a]	175.00[a]	214.00[a]	13.79
14	182.00[a]	181.50[a]	223.50[a]	14.77
17	144.00[a]	211.50[b]	219.00[b]	16.03
21	153.16[a]	223.33[b]	222.60[b]	12.51
25	159.16[a]	225.83[b]	244.16[b]	10.22
29	171.83[a]	237.33[b]	255.33[b]	11.25
33	178.33[a]	246.00[b]	264.10[b]	13.02
35	179.16[a]	250.50[b]	265.83[b]	13.35

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

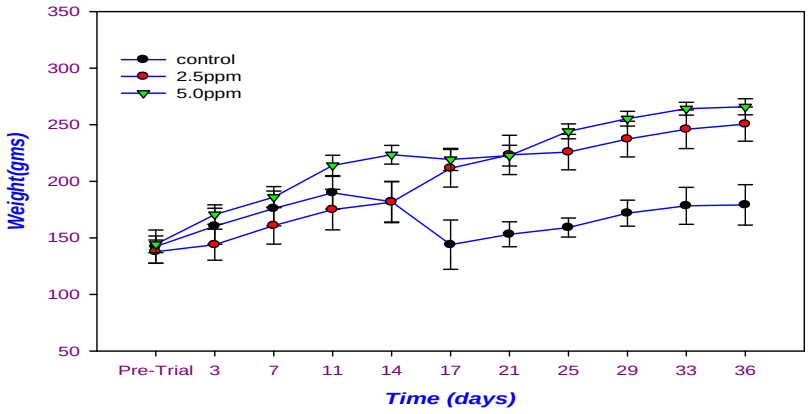

FIGURE 1: Graph showing changes in body weights of rats during 1 to 35 days post-exposure

TABLE 3: Morphometric analysis of brain after 14 days of Methyl mercury exposure

Group	Control	2.5 ppm	5.0 ppm	SEM
Weight(gm)	2.0162[a]	1.8508[b]	2.1338[c]	0.82
Length(cm)	2.2183[a]	2.7633[b]	2.7317[b]	1.05
Width(cm)	1.6517[a]	1.6050[a]	1.6000[a]	0.66

[abc]Means with different alphabets as superscripts differ significantly ($p<0.05$)

On the contrary, Dufrense and Cyr (1999) and Yasutake *et al.* (1997) did not find any significant differences in body weight of Sprague Dawley rats administered with MeHg at 25, 50, 100 or 200 micrograms/day, and 5 ppm/day, respectively. However, Hematian (2013) and Wakabayashi *et al.* (1995) reported an age dependent response in which the young rats showed weight loss on the 9th day, whereas adult rats loss their weights from 6th day after starting MMC exposure at 10 mg/kg/day.

The mean brain weights varied distinctly between the groups at 14d post-exposure (Table 3). The mean brain weights were maximum in 5 ppm group followed by control and 2.5 ppm group (Fig 2). At 35 days post-exposure, the mean weight of the brain in 2.5 ppm group was significantly greater than control and 5 ppm groups (Fig 3 & Table 4). Similarly, Verschuuren *et al.* (1976) observed an increase in relative brain weight in rats exposed to MMC at 2.5ppm in diet. However, Pan *et al.* (2005) reported a decreased brain weights, 35 days post-exposure, in rats exposed to MMC at 10 mg/kg/day. Later, Coluccia *et al.* (2007) reported unaltered brain weights in rats exposed to MMC at 0.75 mg/kg/day from post natal day 14 to 23 and control groups.

TABLE 4: Morphometric analysis of brains after 35 days of Methyl mercury exposure

Group	Control	2.5 ppm	5.0 ppm	SEM
Weight(gm)	2.0090[a]	2.0833[b]	2.0092[a]	0.83
Length(cm)	2.5400[a]	2.5617[a]	2.4467[a]	1.03
Width(cm)	1.4500[a]	1.5083[b]	1.4583[a]	0.60

[abc]Means with different alphabets as superscripts differ significantly ($p<0.05$)

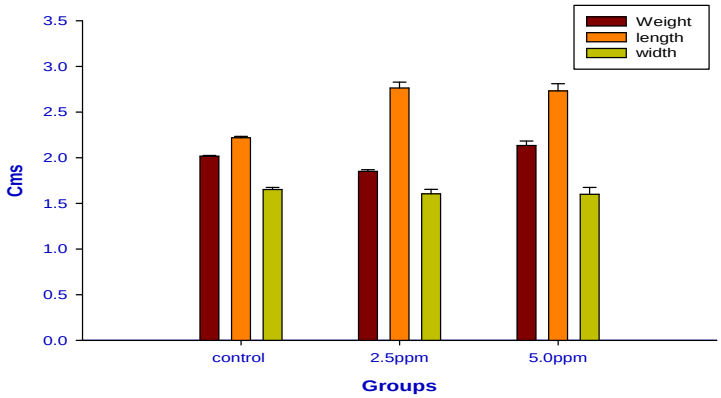

FIGURE 2: Graph showing weight, length and width of brains at 14 days post-exposure

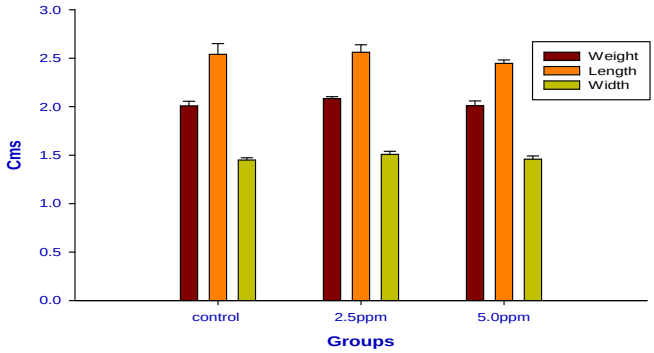

FIGURE 3: Graph showing weight, length and width of brains at 35 days post-exposure

The average length of brains was increased in both the treated groups compared to control group and there was no significant difference in mean width of brains among the groups at 14 days post-exposure (Fig 2 & 3). The length and width increased from 14- to 35-d post-exposure; no significant differences were observed in mean length among the 3 groups; however, the mean width of rats' brain was higher in both the treatment groups compared to control group. Similarly, altered brain weight, length, and width was observed during neuropoisoning cases in rats (Fang *et al*., 2011), mice (Sled et al., 2009), human beings (Filbey *et al*., 2014).

CONCLUSION

Exposure of wistar rats to Methyl mercury at 2.5 ppm and 5.0 ppm levels altered the brain morphometry and body weights, thus demonstrating the potential role of MeHg in genesis of neuro-toxicity in rat models.

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