Formaldehyde Intoxication and Open Field Behavior in Mice

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Abstract --- Formaldehyde (FA) is toxic over a range of doses. Even though at lower concentration, FA can cause significant alteration in the motor activity. Many animal studies have shown that FA alters behavior after repeated exposure. But little information regarding the behavioral consequences of acute low-level FA exposure is available. However, this study was conducted to investigate the effects of acute inhaled and oral exposure to FA on the explorative and locomotor behavior of mice. For this study, Swiss albino mice were divided into three groups; control, inhalation and oral group. Inhalation and Oral groups were further divided into three subgroups which were subjected to exposure of FA for 30 consecutive days. After two hours of FA exposure behavioral study for each group was performed in a confined area. We found that, FA exerts effect on locomotor and explorative behavior of mice in all treated groups in comparison to control group. Our behavioral study revealed that the number of crossing floor squares, grooming and wall climbing were decreased significantly ($p<0.05$) whereas the air and floor sniffing were increased significantly ($p<0.05$) in 2.5 ppm and 5 ppm FA exposed mice. In 10mg/kg FA exposed oral group, significant reduction were observed in the number of crossing floor squares along with increased air and floor sniffing. However, more significant alterations were recorded in inhalation groups of highest concentration than the oral groups. These findings notion that FA has toxic effects that is route specific and significantly influences the locomotor and explorative behavior of mice after exposure in a dose-related manner.

Index Terms --- Formaldehyde, locomotor activity, mice, open field behavior, toxicity.

1 INTRODUCTION

FORMALDEHYDE (FA) is the most common aldehyde in the environment. It is an easily absorbable, irritative, colorless reactive gas found in the polluted atmosphere, domestic air and also cigarette smoke etc. Therefore, one can observed that nearly all human particularly susceptible children, anatomists and medical students may be in perilous condition due to FA exposure. Despite the harmful effects from FA exposure, it is commonly used in dissection laboratories. Occupationally FA exposed histology technicians have been found to have persistent nervous system disorder and showed significantly more complaints of memory, mood, equilibrium, sleep and headache disturbances than controls [1].

The effects of FA on central nervous system have been evaluated during systemic and respiratory exposures in laboratory animals [2]. In addition, inhaled and orally exposed FA has been showed to cause behavioral and memory disorder in rats. In open field test, FA also exerts effects on behavior after single systemic or repeated inhalation exposure [3]. However, owing to FA's high reactivity, effects in the behavior following inhalation and oral exposure are more likely to be related to the route and concentration of the FA intake and that have not been clarified yet. In this study, we were aimed to investigate acute low-level FA intoxication in open field behavior test after inhalation and oral exposure and to determine whether the behavioral effects are route specific or not.

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2 MATERIALS AND METHODS

2.1 Research Animals and Their Management

The research was conducted by using Swiss albino mice (*Mus musculus*), aged 100-120 days and weighted 30±7 g. Mice with apparently good health were collected from International Center for Diarrheal Disease Research (icddr'b), Mohakhali, Dhaka. Mice were kept in the cages under normal room temperature (23-25°C) and humidity with free access of feed and water. All mice were handled according to the animal care in compliance with the Department of Anatomy and Histology under the Institutional Board Guidelines of Bangladesh Agricultural University on the care and use of laboratory animals.

2.2 Chemical Preparation

FA solution was prepared from stock paraformaldehyde powder (Merck, Darmstadt, Germany) by thermal depolymerization [4].

2.3 Research Design

The mice were randomly divided into three groups like; control, inhalation and oral groups. In control group (A) total of 5 mice were taken and inhaled distilled water. Inhalation groups further divided into three groups and exposed to 1.0 ppm (group B), 2.5 ppm (group C) and 5.0 ppm (group D) FA vapor once for two hours for 30 days. The FA concentrations used in this study were based on the concentrations that could also appear in the human work place (Kilburn, 1994). The exposure took place in cages (40×30 cm) with metal grid roof and this was covered with aluminum foil paper provided with small holes to allow fresh air exchange. During the inhalation session, FA solutions of different concentrations (0.5% for group B, 1% for group C and 2.5% for group D) were prepared from stock paraformaldehyde powder and about 5-8 ml was added per hour by means of a pipette into a flat dish which was located at the bottom of the cages to maintain the

concentrations. After FA exposure, the mice were again placed to the cages. Food and water were supplied *ad libitum* before being replaced to open field arena.

Mice of oral group were divided into three groups and exposed to 5.0 mg/kg (group B), 7.0 mg/kg (group C) and 10.0 mg/kg (group D) body weight FA once daily for 30 days. Control group (A) mice were provided distilled water.

2.4 Open Field Arena for Test

A paper made, rectangular, off white colored open field apparatus measuring 100×100×40 cm was used to perform the test. The floor was divided into 25 rectangular squares by black marker line. The behavioral parameters like ambulation (crossed squares), grooming, air and floor sniffing, wall climbing and defecation were quantitatively examined in each animal for three minutes in the open field area [3]. All behavioral parameters were registered manually by self-observation. All open field tests were carried out 2 hours after the end of the exposures. The floor of the test apparatus was thoroughly cleaned after each test.

2.5 Statistics

All behavioral parameters were taken during study period. The collected data were then analyzed by means of the Kruskal-Wallis-test followed by the two-sided Mann-Whitney test. The tests revealed the significant differences between the results of each inhalation and oral exposure group using Statistical Package for the Social Sciences (SPSS; version 11) software and disrobe the results in graphical form. The data were presented as mean±SD and considered to be statistically significant when the *P* values obtained would less than 0.05.

3 RESULTS

In the present study, there were minor fluctuations in the FA vapor concentration registered in the inhalation chamber during all exposure sessions. Animals were started to explore the new environment after placement of inhalation chamber. At the beginning of the experiment, the locomotor activity (walking, climbing) of mice was increased. There were no signs of intoxication and aggressive behavioral changes observed in the mice during the experiment. Food and water intake was decreased in all FA treated inhalation groups.

In oral groups no behavioral alterations were observed in mice of 5 mg/kg treated group in comparison to control. In 7.5 mg/kg and 10 mg/kg treated oral groups, the clinical signs including dullness, staggering gait, sitting with closed eyes and decreased response on disturbance were begun to appear after I week of experiment. At the end of experiment, a few mice in 5 mg/kg treated oral group also exhibited similar but less pronounced signs. In 10 mg/kg treated oral group, food and water intake was affected.

3.1 Open Field Performance

Open field behavior test revealed significant differences among inhalation and oral groups for the following parameters in mice like square crossing, air sniffing, floor sniffing, grooming, wall climbing and defecation.

Inhalation exposured mice of 2.5 ppm and 5 ppm treated groups, decreased grooming was observed while floor sniffing was significantly ($P < 0.05$) enhanced (Table1). Air sniffing was significantly enhanced (Table.1) only in 5 ppm treated group. In those same groups, mice crossed significantly ($P<0.05$) less number of floor quadrants when compared to control (Table.1). Wall climbing and defecation was not significantly affected.

TABLE 1
OPEN FIELD PERFORMANCE OF MICE IN INHALATION GROUPS (n=5)

Parameters	Behavior test			
	Control	1 ppm	2.5 ppm	5 ppm
Crossed quadrants	40.22±2.45	36.23 ±1.56	31.00* ±2.32	23.02*†† ±1.82
Air sniffing	12.73±1.43	23.78 ±2.12	28.16 ±1.34	25.67* ±1.74
Floor sniffing	17.12±0.03	28.44 ±1.30	33.26* ±1.46	32.54* ±1.79
Grooming	13.22±1.11	14.29 ±1.62	7.42* ±2.21	5.76*†† ±1.33
Wall climbing	7.53±0.89	6.24 ±1.59	4.26 ±0.22	6.11 ±0.71
Defecation	1.92±1.20	1.47 ±0.88	1.60 ±0.06	1.23 ±0.64

Data are mean ± SD, SD= standard deviation; significant at 5%* level in comparison to control in the same trial and also significant at 5%†† level in comparison to the results of the oral groups within the same dose group

Orally exposed mice of 10 mg/kg treated group crossed significantly ($P<0.05$) less number of floor quadrants compared to control (Table 2). Decreased grooming was also observed while air sniffing and floor sniffing was significantly ($P<0.05$) enhanced (Table. 2). Wall climbing was significantly decreased whereas the effect on defaecation was non-significant.

TABLE 2
OPEN FIELD PERFORMANCE OF MICE IN ORAL GROUPS (n=5)

Parameters	Behavior test			
	Control	5.0 mg/kg	7.5 mg/kg	10 mg/kg
Crossed quadrants	40.22 ±2.45	41.44 ±1.92	38.41 ±2.23	27.37* ±1.66
Air sniffing	12.73 ±1.43	10.00 ±0.84	12.59 ±1.78	17.22* ±1.25
Floor sniffing	17.12 ±0.03	24.45 ±0.39	36.32 ±1.26	40.12* †† ±1.83
Grooming	13.22 ±1.11	10.11 ±1.09	14.21 ±1.44	8.34* ±1.67
Wall climbing	7.53 ±0.89	5.17 ±1.97	4.33 ±1.66	4.13* ±1.01
Defecation	1.92 ±1.20	1.00± 0.92	1.12± 0.85	1.67± 1.06

Data are mean ± SD, SD= standard deviation; significant at 5%* level in comparison to control in the same trial and also significant at 5%†† level in comparison to the results of the inhalation groups within the same dose group

5 ppm treated inhalation group crossed significantly (P<0.005) less number of floor quadrants when compared to the oral groups (Table.1). Frequency of floor sniffing in 10 mg/kg treated oral group was significantly (P<0.005) increased (Table.2), while grooming was significantly (P<0.005) depressed in the 5 ppm treated inhalation group (Table.1) when comparison was done with the oral exposure groups.

4 DISCUSSION

Toxicity produced by FA through inhalation and oral exposure has demonstrated behavioral impairment in mice. In the present study, depression, staggering gait and decreased water intake were observed in mice exposed to highest concentration of FA both in oral as well as inhaled groups and similar signs were found by other researchers [5], [6]. FA exposed mice also showed lethargy and loss of appetite which is consistent with the observation of other studies [7], [8], [9], [10]. Maronpot et al [11] suggested that no obvious gross signs of neurotoxicity were found in mice with low-level FA exposure, but mice in the 20 ppm group exhibited dyspnea, listlessness and hunched posture. Other investigators found that, low-level FA exposure also resulted in statistically significant decreased motor activity [12], [13] and restless behavior [14] within 15 minutes of initial exposure.

The study reported that exploratory and locomotor activity of mice in the open field test was affected by single acute oral and inhaled FA intoxication which is solely depended on concentrations. This finding was also consistent with Malek et al [3] in case of inhaled FA exposure. However, impact of chronic FA exposure in 10mg/kg orally fed rats was also evident, that is; 60% settled in conditioned avoidance response in Cook's apparatus [15].

After FA inhalation, it is metabolized to formic acid and supposed to responsible for the deleterious effects of FA [16]. Cerebral hypoxia is induced by inhalation of formic acid vapor [17] which has effects on locomotor and exploratory activity in the mice also observed by other workers under open field behavior test [18]. Thus, in the present study it has been suggested that not only FA but also likely formic acid has the effects on behavior of mice.

The effects of inhalation exposure to FA in Wister rats were examined. Uncoordinated movement and wall-climbing were noted after initial 30minutes of exposure in the 20 ppm group, the rats in the lower dose groups did not exhibit abnormal behavior [19]. Extensive neurobehavioral impairments were observed after FA exposure including malaise, headache, indigestion, balance dysfunctions, sleep disorders as well as mental and memorial disorders [20], [21]. A pronounced impairment was found in rat after FA exposure in open field, maize trail performance and CNS function [3], [20], [22]. FA exposed rats exhibited an influence on food finding abilities including a decrease in overall success, increase in food finding time and increase in mistakes [22]. FA inhalation at the rate of 11 ppm for 7 days caused an increase in cocaine-induced locomotor activity and a conditioned fear response to odor [23] were attributed to the effects of present study and presaging that chemical encephalopathy may be caused by FA.

The results demonstrate clearly the impact of a single FA inhalation on the behavior of mice in the open field arena. But there are less significant effects of FA after oral exposure on the behavior of mice.

5 CONCLUSION

FA intoxication effects on behavior are dependent upon route and concentration rather than exposure duration. The behavioral effects produced by FA become more pronounced with increases in concentration. Though studies on gender related differences in open field behavior after FA intoxication suggested some alterations, we did not do any research to support these results. There were also a very few data available from other studies which established the toxic effects of FA on behavior of mice progeny. Further studies are needed to better establish the possible gender specific

correlation between FA exposure and behavioral impairment on mice and also their progeny in accordance to route and dose related manner.

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