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## Cadmium Enhances Locomotor Behaviour in F1 Generation Mice Following Maternal Exposure During Lactation: Modulation by Quercetin

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### Abstract

The present study was designed to investigate the effects of Cd exposure on locomotor behaviour in F1 mice whose dams were exposed to low dose cadmium for a short duration during the lactation period and whether quercetin could modulate the effects. The locomotor behaviour and musculoskeletal activity were tested using photoactometer and rota rod test respectively. We observed that at the given dose of Cd (1.2mg/kg) for a short duration, the muscle coordination on rota rod showed significant improvement as compared to the control group. Cotreatment of Cd with quercetin (100mg/kg) further enhanced the rota rod activity. Similarly the spontaneous motor activity as tested on the photoactometer also exhibited improvement in Cd treated group though this change was not statistically significant. However, Cd when cotreated with quercetin (50 mg/kg) showed significant enhancement in photoactometer activity compared to control. Quercetin administered alone (dose 25 mg/kg) also demonstrated significantly improved rota rod and photoactometer activity as compared to control. Thus cadmium exposure to dams at dose (1.2mg/kg) during lactation may cause enhanced locomotor behaviour in their offsprings.

**Key words:** Quercetin; Cadmium; Photoactometer; Rota rod; Locomotor behaviour

### Introduction

Cadmium is one of the most toxic environmental pollutants which have increased in distribution due to industrial activities [1]. The general population is primarily exposed through food and their immediate environment [2].

Itai-Itai disease is the most severe form of chronic Cd poisoning is caused by prolonged Cd exposure and is mainly characterized by osteomalacia [3,4]. This disease was initially described in post-menopausal Japanese women who were chronically exposed to cadmium in Japan. They suffered from defective gait, leg pain and bone deformities [5]. However the effects of cadmium exposure on locomotor activity in the reproductive age-group and on their subsequent off-springs have not been much explored. The present study was designed to investigate the effects of Cd exposure in F1 mice whose dams were exposed to low dose cadmium for a short duration during the lactation period.

### Material and Methods

#### Animals

Young (8-weeks-old) Swiss albino mice weighing 20–25 g were used in the study. The animals were procured from the Central Animal House, University College of Medical Sciences, Delhi. Animals were housed in groups of four per cage with free access to pellet diet and water in a temperature-controlled facility (temperature: 22 ± 2°C, humidity: 50% to 55%, natural light/day cycle). All the experiments were performed at daytime between 09:30 and 15:30 hours. Care of animals was as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Govt. of India, New Delhi. The study was duly approved by the Institutional Animal Ethics Committee, University College of Medical Sciences, Delhi (Approval No. IAEC/2011/35).

#### Preparation of animals

Nulliparous female mice determined to be estrous by vaginal cytology were randomly divided into pairs and were placed overnight with one male mouse for mating. The onset of pregnancy was confirmed by presence of spermatozoa in

vaginal smears on the following morning. The pregnant female animals were housed individually in breeding cage. Following delivery, the next day was regarded as post natal Day 1 (PND1). On PND 1 animals were randomly distributed to treatment and control groups for different vehicle/drugs. The F1 generation produced mice were reared till 100 days age and the experiments were performed.

### Plant material

Quercetin was obtained from M/s. Sigma Aldrich (Product no. Q4952 CAS no. 117-39-5, dated 8-03-2010) in the form of yellow amorphous powder. As per the literature provided by the manufacturer, the purity was 98% as assessed by HPLC. For the purpose of the study, the quercetin was suspended in double distilled water to prepare solutions of the required doses (25 mg/kg, 50 mg/kg and 100 mg/kg). The suspension was made homogenous using an ultrasonic sonicator before dosing

### Drugs and dosing schedule

Cadmium (M/s. Fluka Analytical, product no. 20899; Lot code 1417229) was dissolved in distilled water (or vehicle) and was administered daily in dosages of 1.2 mg/kg/day intraperitoneally (i.p.). Quercetin was administered in doses of 25 mg/kg/day, 50 mg/kg/day and 100 mg/kg/day, i.p. Control groups received equal volume of vehicle used for different treatment groups. The administration of drugs/vehicles was started on the day following delivery (PND 1) and continued for 7 days. On postnatal day 21 (PND21) the litter mates were weaned, separated, housed together by sex and were grouped in same laboratory conditions as their parents. The control and treatment groups consisted of 6-8 animals each. After breeding, one pup from each litter was selected to be studied in the F1 generation for locomotor behaviour. So the group in F1 generation consisted of 6-8 animals whose dams had been exposed to the drug.

### Evaluation of muscle coordination

The rota rod method was used similar to the one described by Dunham and Miya in 1955. The speed selector was set so that the roller rod makes 15 rpm. Prior to the test, each animal was given 1 minute exposure to the moving rod. The animals were placed on the roller for 3 minutes. Latency to fall from rolling rod was observed. Increased latency indicated improved muscle coordination.

### Measurement of spontaneous motor activity

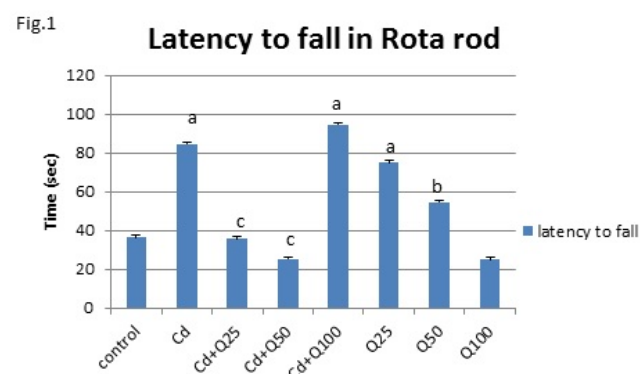
To assess spontaneous motor activity, each animal was placed in a square (30 cm) closed arena equipped with infrared light-sensitive photocells (digital photoactometer, INCO, Ambala, India) and values expressed as counts per 5 minute. The apparatus was placed in a darkened, light and sound attenuated and ventilated testing room [6,7].

### Statistical analysis

Results of the above experiments were expressed as mean  $\pm$  SEM, and the differences between means were analyzed by analysis of variance followed by Dunnett's multiple comparisons test.

## Results

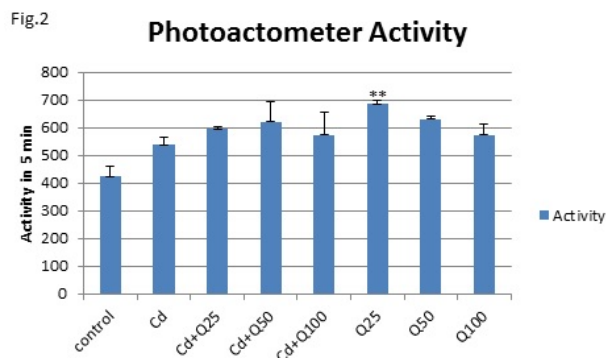
Muscle coordination on rota rod (**Figure 1**).



**Figure 1** Effect of Cadmium (Cd) and Quercetin (Q) on latency to fall on a Rota rod in F1 mice where F0 were treated in lactation period. (Q25 mg/kg, Q50 mg/kg and Q100 mg/kg are 25 mg/kg, 50 mg/kg and 100 mg/kg dose of Quercetin respectively) a  $p < 0.001$  compared to control; b  $p < 0.05$  compared to control; c  $p < 0.001$  Compared to cadmium only group.

The cadmium treated group showed significantly increased latency to fall compared to control ( $p < 0.001$ ). Cd when cotreated with quercetin (100 mg/kg) further enhanced this latency significantly ( $p < 0.001$ ). Treatment with Cd in combination with lesser doses of quercetin (25 mg/kg and 50 mg/kg) showed significantly reduced latency when compared to the Cd treated group ( $p < 0.001$ ). Treatment with quercetin (dose: 25 mg/kg and 50 mg/kg) alone significantly enhanced the latency to fall as compared to control ( $p < 0.001$  and  $p < 0.05$  respectively).

## Spontaneous motor activity



**Figure 2** Effect of Cadmium (Cd) and Quercetin (Q) on photoactometer activity on F1 mice where F0 were treated in lactation period. (Q25 mg/kg, Q50 mg/kg and Q100 mg/kg are 25 mg/kg, 50 mg/kg and 100 mg/kg dose of Quercetin respectively) \*\* $p < 0.01$  compared to control.

The cadmium treated group showed increased photoactometer activity compared to control though this difference was not statistically significant. The group treated with Cd and quercetin (50 mg/kg) however showed significantly enhanced photoactometer activity compared to control ( $p < 0.001$ ). Quercetin (dose: 25 mg/kg and 50 mg/kg) treated alone significantly improved the photoactometer activity compared to control ( $p < 0.01$  and  $p < 0.05$  respectively) (Figure 2).

## Discussion

Chronic cadmium exposure has been known to cause various motor disturbances as witnessed in Itai-Itai disease in Japan [3,4]. The present study was planned to investigate the effect of cadmium exposure on locomotor behaviour in F1 mice whose dams were treated with cadmium for seven days following delivery. The locomotor behaviour and musculoskeletal activity were tested using photoactometer and rota rod test respectively.

We observed that at the given dose of Cd (1.2 mg/kg) for a short duration, the muscle coordination on rota rod showed significant improvement as compared to the control group. Cotreatment of Cd with quercetin (100 mg/kg) further enhanced the rota rod activity. Similarly the spontaneous motor activity as tested on the photoactometer also exhibited improvement in Cd treated group though this change was not statistically significant. However, Cd when cotreated with quercetin (50 mg/kg) showed significant enhancement in photoactometer activity compared to control.

Previous reports state that chronic Cd exposure causes tubulopathy and osteomalacia, which manifests as gait and motor disturbances in post-menopausal women [5]. Our observation differs from the earlier reports since we have observed improvement in motor behaviour. This contrasting behaviour could be due to the short duration of Cd treatment unlike the chronic cadmium exposure observed in post-

menopausal women. In addition, we have performed our experiments in young mice where female mice following delivery were treated and their off-springs were tested for locomotor activity in their adulthood. Thus in the earlier studies, the various pathways causing osteomalacia may have been accelerated owing to advanced age of the subjects and increased duration of exposure. In a recent study it has been reported that acute cadmium administration dose dependently increased anxiety in rats [8].

In the present experiment the relatively lesser age of the experimental animals and exposure at low dose (1.2 mg/kg) for only one week may have caused better musculoskeletal activity. Quercetin is a bioflavonoid and dietary antioxidant, present in fruits and has many beneficial effects including free radical scavenging, anti-inflammatory, anti-cancer and cardio-protective activity [9,10]. Quercetin administered alone (25 mg/kg and 50 mg/kg) demonstrated significantly improved photoactometer activity as well as muscle coordination on rota rod as compared to control. This effect can be ascribed to its antioxidant potential. Studies on quercetin in our laboratory have exhibited improved oxidative stress parameters in F1 generation mice whose dams were exposed to Cd along with quercetin (data under publication). Hence the enhanced activity in groups treated with Cd and quercetin could be attributed to reduced oxidative stress.

In the rota rod test, quercetin at lower dose (25 mg/kg and 50 mg/kg) when co-treated with Cd, caused significantly reduced latency to fall from the rod compared to when treated with Cd alone. There could have been some interaction between Cd and quercetin at this dose resulting in attenuation in muscle coordination. More studies are needed to probe deeper into the interplay between Cd and quercetin.

Studies in adult male mice have shown that cadmium raised serum  $Ca^{2+}$ ,  $Na^+$  concentration without significant effect on  $K^+$  resulting in increased motor activity and anxiety linked behaviour [11]. Increase in motor activity was also seen in off-springs after maternal exposure to Cd during lactation [12]. Another study has reported that exposure to cadmium in periconception period showed increased anxiety-like behaviour in their off-springs [13]. Such behaviour can also be reflected as increased spontaneous activity which was observed in this study. This effect could be a consequence of developmental programming rather than direct impact of Cd on the organ system of the developing foetus. Nevertheless, the direct exposure through placental transfer [14,15] and also during lactation [16,17] cannot be completely ruled out since Cd has a long half-life [18]. In contrast, a separate study demonstrated reduced anxiety-like behavior reflected by increased open arm entries in the EPM in offspring from mothers exposed to 0.6 mg/kg body weight of Cd from gestational days 7 to 15 [19]. Thus the effect on behaviour can be said to be governed by certain factors such as dose as well as the exact period of cadmium exposure. Our observations could be different because we have administered a dose of 1.2 mg/kg Cd from 14<sup>th</sup> - 21<sup>st</sup> day of gestation.

## Conclusion

In conclusion, our study shows that Cd treatment in low dose during lactation may cause enhanced motor activity in their F1 generation in adulthood. There could be a modulation of effect if Cd is co-administered along with quercetin depending on the dose of quercetin administered. Further studies are needed to shed more light in this area.

## Conflict of Interest Statement

We declare that there is no conflict of interest.

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