

## MOTOR AND BEHAVIOURAL EFFECTS OF FERULIC ACID IN MICE WITH CISPLATIN-INDUCED NEPHROTOXICITY

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### ABSTRACT

**Background and Objective:** Cisplatin (CP) is commonly used to treat solid tumors. It is known to cause nephrotoxicity as well as some motor and behavioral impairments. Ferulic acid (FA) is a phenolic compound with antioxidant and anti-inflammatory properties. The present study was designed to assess the protective effects of ferulic acid on cisplatin toxicity in mice.

**Methods:** Four randomly-grouped male albino mice were treated as follows: saline (control), CP (10 mg/kg [i.p. twice]), FA (100 mg/kg, orally, 14 day) and a combination of CP and FA treatments. Motor and behavioural experiments were conducted on mice on the first, eighth and fifteenth day for the evaluation of basal motor activity (activity cage), exploration (hole-board), neuro-muscular coordination (rota rod treadmill, grip strength), nociception (hot plate, cold-water tail flick, acetic acid-induced writhing and depression-like behaviour).

**Results:** CP significantly increased the plasma creatinine and urea concentrations. It also significantly prolonged the time on hot plate test and reduced the acetic acid-induced abdominal constrictions suggesting an antinociceptive action. It significantly decreased the time on rotarod treadmill and grip strength tests, indicating impairment in neuro-muscular coordination. CP also decreased the exploratory action, and induced depression-like behaviour. FA significantly ( $p < 0.05$ ) improved the basal motor activity, neuromuscular coordination, motor activity, exploration activity and the depression-like action in CP-treated group.

**Conclusion:** FA showed an anti-depressant-like action, and improved the motor and exploratory activities in CP-treated mice.

**Keywords:** Cisplatin, ferulic acid, central nervous system, motor activity, nephrotoxicity.

### INTRODUCTION

Cisplatin (CP), also called cisplatinum, or cis-diamminedichloroplatinum (II) is a platinum compound widely used to treat solid tumours in the head, neck, lungs, ovary, cervix and testes<sup>1</sup>. It has also been reported to be active against tumours such as muscle, bones, blood vessels and soft tissues cancer.<sup>1</sup> However, CP can be toxic and cause severe side effects leading to nephrotoxicity,<sup>2</sup> hepatotoxicity,<sup>3</sup> reproductive system toxicity<sup>4</sup> and cardiotoxicity.<sup>5</sup> Acute kidney injury (AKI) is the commonest side effect that occurs in 20 – 30% of patients given this drug that causes acute renal failure (ARF), which is a condition that results in loss of kidney function as evidenced by increased serum concentration of urea and creatinine leading to loss of acid-electrolyte balance regulation inside kidney, and failure in excretion of fluids and waste products.<sup>6</sup> ARF caused by CP has also been reported to impair motor activity, cause depression-like behavior and hypoa-

gesia in mice.<sup>7</sup> Other studies have also revealed alterations in the central nervous system (CNS) related to accumulation of uremic toxins and inflammatory mediators caused by hormonal imbalances in the brains of patients affected by ARF and chronic kidney disease (CKD).<sup>8</sup>

Ferulic acid (FA) [(E)-3-(4-hydroxy-3-methoxyphenyl) prop-2-enoic acid] is a compound which is found widely in a variety of fruits (such as dates) and vegetables and in beverages (such as coffee).<sup>9</sup> Chinese medicinal herbs (e.g. *ligusticum chuangxiang*, *Cimicifuga racemosa* and *Angelica sinensis*) contain FA as one of their major components.<sup>10</sup> FA is relatively new antioxidant compound due to its ability to activate cell stress response and scavenge free radicals,<sup>9</sup> and is also an anti-inflammatory and anti-nociceptive agent.<sup>11</sup> It was also postulated that the cytoprotective action of FA is linked to its capability of down regulating cell death pathway.<sup>12</sup> The ability of FA to scavenge oxide-

tive stress products appears to depend on the radical species involved. In rat brain microtomes, the effectiveness of FA in decreasing the lipid peroxidation caused by peroxy radical has been shown.<sup>13</sup> It has also been shown that FA diminishes both hepatic toxicity and oxidation of lipid and protein.<sup>14</sup> It was also reported that FA can restore the activities of catalase (CAT) and superoxide dismutase (SOD) in hearts and pancreas in rats with streptozotocin-induced diabetes.<sup>15</sup> The anti-inflammatory action of FA and its effectiveness in binding with inflammatory proteins (e.g. pro-caspase-1, NF- $\kappa$ B, NLRP3 and PYCARD/ASC) has also been reported.<sup>11</sup> FA can also reduce several pro-inflammatory cytokines, as well as apoptosis in the pancreatic beta cells of rats.<sup>15</sup>

As the mechanism of CP-induced nephrotoxicity involves both inflammation and oxidative stress,<sup>1,6</sup> and FA has potential of antioxidant, anti-inflammatory actions, it was of interest to investigate its possible protective/ameliorative action in CP-induced nephrotoxicity. As far as we know, this has never been reported before. Here, we have assessed the possible actions of FA on CP-induced effects on behavior and motor activity in mice.

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Right running head: Ferulic acid effect in cisplatin induced behavior.

## MATERIALS AND METHODS

### Animals

Male Albino mice [CD1 strain] (n = 96) aged 4 – 5 weeks, and weighing 25 – 32 g were procured from the Small Animal House of Sultan Qaboos University (SQU), Oman. They were grouped, six to a cage to reduce stress, in polypropylene cages, and provided with standard nutritionally adequate-laboratory chow diet (Oman Flour mills, Muscat, Oman) and normal tap water ad libitum, at ambient temperature of  $22 \pm 2^\circ\text{C}$ , humidity (60%) and 12-hour light: dark cycle (light on at 6.00 AM). The animals were acclimated to their housing environment for a week prior to the start of the study. Conduction of the experiment was approved by SQU Animal Ethical Committee, and was carried out according to International laws and policies (EEC Council directives 86/609, OJL 358, 1 December 1987; NIH Guide for care and Use of Laboratory Animals, NIH Publication No. 85 – 23, 1985).

### Treatments

Mice were selected randomly and divided into four groups: control group (treated with sunflower oil as vehicle, orally), cisplatin (CP)-treated group given CP at a dose of 20 mg/kg (i.p) on the tenth and eleventh days, respectively, FA group administered with FA (100 mg/kg) in sunflower oil for 14 consecutive days, and CP + FA group given both CP and FA as in groups

two and three, respectively. Initial and final body weights of the animals were recorded on the first day and just before the end of the experiment. At the end of the experiment mice were anaesthetized by ketamine (7.5 mg/kg i.p.) and xylazine (10 mg/kg i.p.) for collection of blood. About 1.5 ml of blood was withdrawn from the abdominal aorta in heparinized tubes, and was centrifuged at 900 g for 15 minutes at  $4^\circ\text{C}$  to obtain plasma. The plasma obtained was maintained at  $-80^\circ\text{C}$  pending analyses. Then all animals were finally sacrificed by overdose of anesthesia.

### Biochemical Analyses

The concentrations of plasma creatinine and urea were measured spectrophotometrically by commercial kits, as described before.<sup>7</sup>

### Motor and Behavioural Experiments

Motor and behavioural tests were conducted on the first, eighth and 15th day of the experiment. Each set of mice used for a specific behavioural experiment was not used for another.

#### Basal Motor Activity (Activity Cage Meter)

Motor activity was measured by subjecting the animals to move in a digitalized activity meter (Ugo Basile, Comerio, VA, Italy). The vertical and horizontal movements of the mice within a duration of 15 min were recorded, but the values were excluded for the first 5 minutes from zero time after the mice had been placed within the activity cage.<sup>7</sup>

#### Thermal and Chemical Nociceptive Tests Hot Plate Test

In this test the mice were positioned in an analgesia meter (Ugo Basile, Comerio VA, Italy) having a glass cylinder with a hot metal plate set to a temperature of ( $55 \pm 0.2^\circ\text{C}$ ) for measuring analgesia.<sup>7</sup> The responding time for the reflex was recorded by measuring the time taken by the mouse to jump off the hot plate or lick its paw.<sup>16</sup> The cut-off time was 15 seconds to prevent tissue damage.

#### Cold-Water Tail Flick Test

The mouse tail was immersed 2 – 3 cm in a beaker containing cold water that retained a temperature of  $0 - 1^\circ\text{C}$ . The time between the moment the tail was immersed and its removal from the water was calculated using a stop watch and 15 seconds was considered as minimum cut off time.<sup>7</sup>

#### Abdominal Contraction (Writhing Test)

Mice were injected with acetic acid (0.6% v/v) in a volume of 20 ml/kg i.p.<sup>17</sup> Stretching of hind limbs and contraction of abdominal muscle induced by acetic acid were observed for 15 min after the administration of the acid, and the number of contractions was

totalled.

### Exploration Activity (Hole-Board Test)

A programmed hole-board apparatus (Ugo Basile, Comerio VA, Italy) was used to carry out this test. The animals were gently placed on the center of the board and the total number of dips was recorded digitally for 10 minutes.<sup>7</sup>

### Neuromuscular Coordination

#### Rota-Rod Treadmill

Mice were gently placed on the treadmill (Ugo Basile, Comerio VA, Italy) to study the neuromuscular activity. The apparatus was subdivided into five segments each by discs with a diameter of 24 cm, and the rod was 30 cm long and 3 cm in diameter. The rod was rotated at a fixed speed of 25 revolutions / minute and the duration of the animal to fall from the rotating rod was digitally recorded.<sup>7</sup>

#### Grip Strength Test

A simple apparatus was manually devised with two wooden poles of 30 cm length, connected with a smooth wire of 15 cm length, on which the animals were hung using their fore limbs. The time taken by the animal to lose its grip on the wire was recorded using a stop watch.

### Depression-like Behaviour (Forced Swimming Test [FST])

A transparent glass cylinder of 25 cm height and 19 cm in diameter was filled with water, maintained at a temperature of (25 ± 1°C), up 17cm in height. The animals were forced to swim in this cylinder for a total of six minutes. The first two minutes were considered as trial period and were excluded.<sup>7</sup> The period during which the animal stops struggling and remains immobile in water is considered as immobility time. The total immobility time was recorded and subtracted from the total duration of four minutes to get total mobility time.

### Drugs and Chemicals

Cisplatin was obtained from PCH Pharmacheme (Haarlem, Netherlands); ferulic acid from Sigma Aldrich (St. Louis, MO, USA); Urea and creatinine kits were purchased from Human GmbH (Mannheim, Germany); acetic acid from British Drug House (Dorset, U.K). The rest of the chemicals were Analytical Reagent grade.

### Statistical Analysis

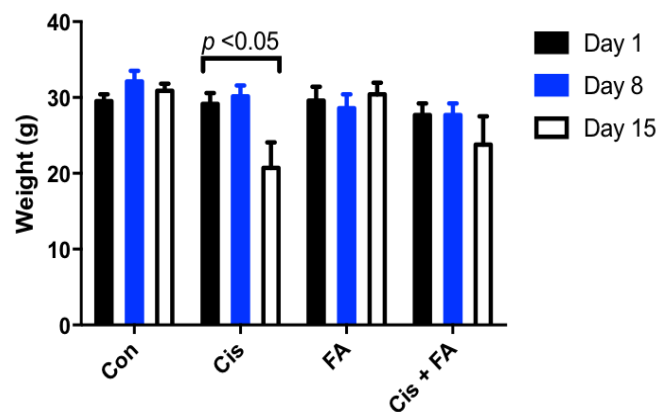
Statistical analysis and comparisons were carried out by One-way ANOVA test followed by a multiple comparison test. Student t-test was also conducted to get intra-group difference for acetic acid-writhing test. All the analyses were done using a commercially purchased statistical software package (Graph Pad Prism®,

Version 7, San Diego, CA, U.S.A). The data are presented as mean ± SEM, with n=10. A *p* value less than 0.05 was taken significant.

## RESULTS

### Effect on Body Weight

CP significantly (*p* < 0.05) decreased the body weights of treated mice compared to that of control. The other groups did not show any significant difference (Fig. 1).



**Fig. 1:** Effect of cisplatin (CP) and ferulic acid (FA) on body weights of mice compared to that of control. Cis caused significant decrease (*p* < 0.05) in bodyweight.

### Biochemical Indices

The creatinine and urea concentrations in plasma were significantly (*p* < 0.01) greater in CP-treated animals than in controls (Fig. 2a, 2b). Ferulic acid showed some statistically insignificant improvement when given concomitantly in CP-treated animals.

### Basal Motor Activity (Activity Meter)

Results showed that CP-treated group produced significant decrease in both horizontal and vertical movements compared to that of control (*p* < 0.0001 and 0.001, respectively). Also, FA showed to improve the activities in group treated with CP with a significant (*p* < 0.001) rise in the activity (Fig. 3a and 3b).

### Thermal and Chemical Nociceptive Tests

#### Hot Plate Test

The results indicated that the groups treated with CP alone and CP + FA produced significant (*p* > 0.0001) increase in time on the hot plate compared to that of the control (Fig. 4).

#### Cold-Water Tail Flick Test

No significant changes were observed between and within the groups.

#### Abdominal Contraction (Writhing Test)

By the end of the treatment, groups treated with CP

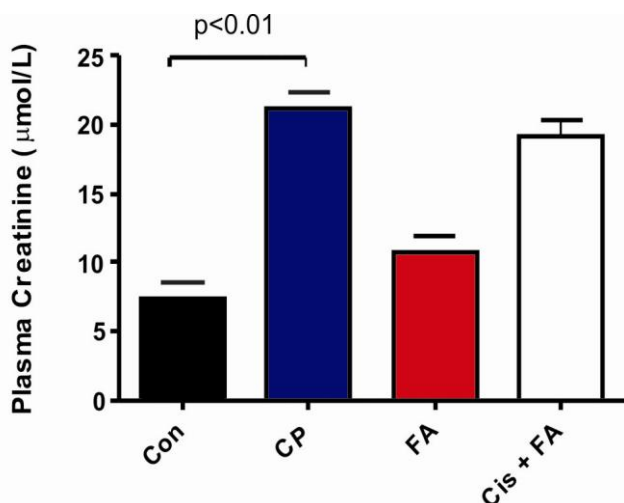


Fig. 2a

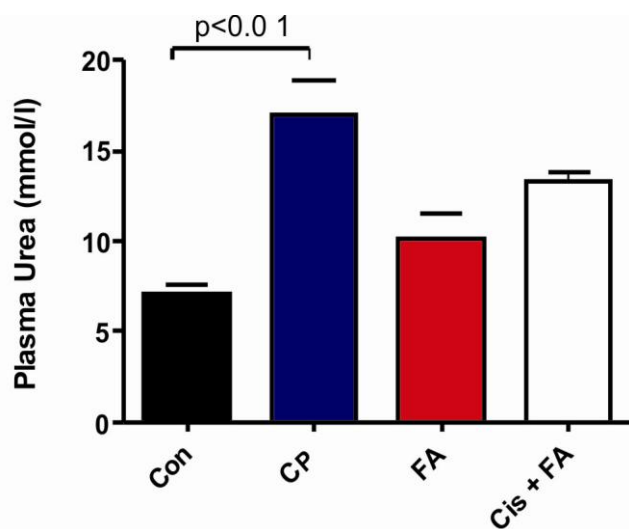


Fig 2b:

**Fig. 2a and 2b:** Some kidney function tests in mice treated with CP and FA. a) Plasma creatinine concentration b) Plasma urea concentration. Each column and vertical bar represent mean  $\pm$  SEM and  $p < 0.05$  was considered significant.

alone and CP + FA showed significant decrease ( $p < 0.001$  and  $0.05$ , respectively) in number of acetic acid-induced abdominal constrictions to that of the control (Fig. 5).

**Exploration Activity (Hole-Board Test)**

In CP-treated mice there was significant ( $p < 0.05$ ) fall in the number of head dips compared to that of control. Other groups did not produce any significant changes (Fig. 6).

**Neuromuscular Coordination Tests**

**Rota-Rod Treadmill Test**

The results showed that mice treated with CP alone

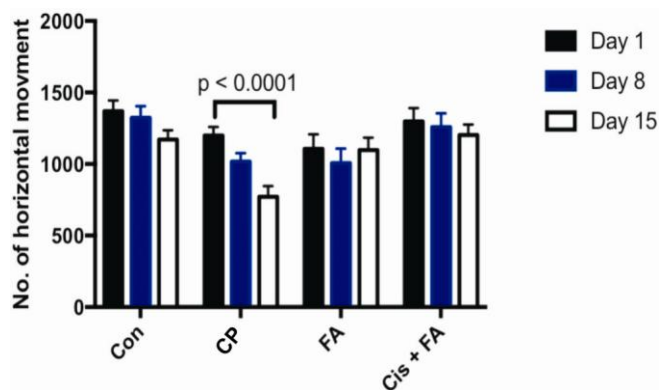


Fig. 3a:

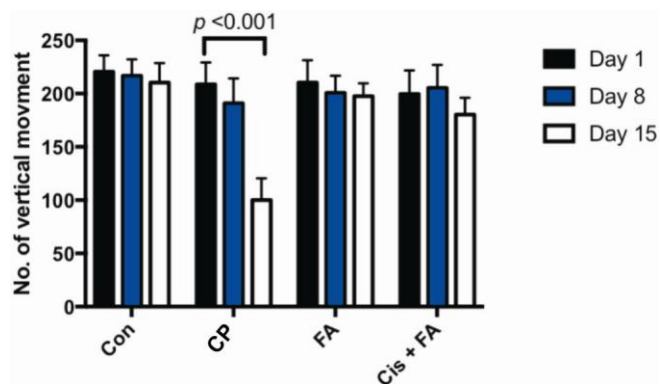
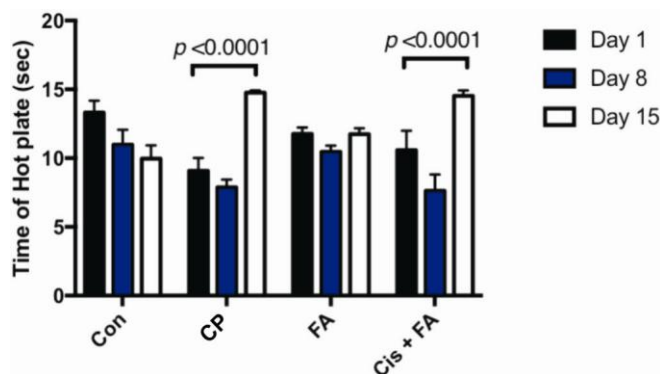


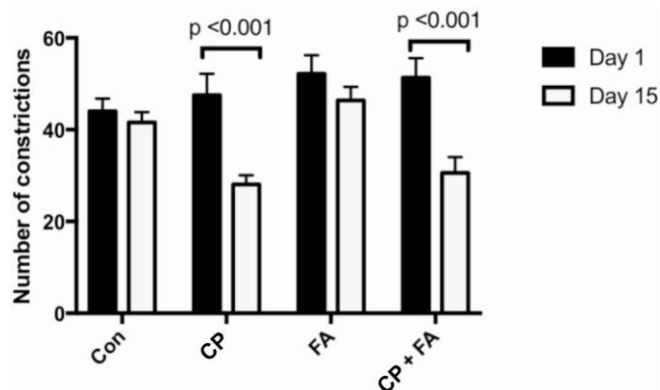
Fig. 3b:

**Fig. 3a and 3b:** Locomotion in control mice and mice treated with cisplatin (CP), ferulic acid (FA) and their combination. a) Number of horizontal movements b) Number of vertical movements. Each column and vertical bar represent mean  $\pm$  SEM. Differences were assessed by t-test and  $p < 0.05$  was considered significant.

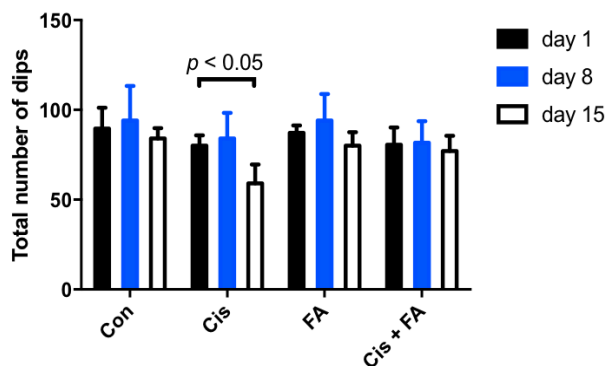


**Fig. 4:** Influence of cisplatin (CP), ferulic acid (FA) and their combination on thermal nociception of mice compared to that of control. Cis-treated group and Cis+FA –treated group was significantly different ( $p < 0.001$ ) from that of control.

produced significant ( $p > 0.001$ ) decrease in time on the treadmill compared to that of the control. When



**Fig. 5:** Changes in chemical nociception in mice treated cisplatin with (CP), ferulic acid (FA) and their combination compared to that of control. Cis-treatment showed significant decrease in chemical nociception ( $p < 0.001$ ) compared to that of control.



**Fig. 6:** Effect of cisplatin (Cis) and ferulic acid (FA) on explorative activity in mice. Treatment with cisplatin (Cis) produced significant difference with  $p < 0.05$ .

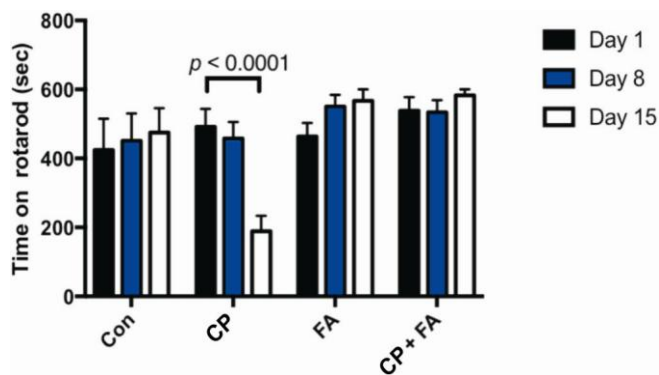
treated with FA, the CP-treated mice showed improvement and it was significantly ( $p > 0.0001$ ) different from CP-treated group (Fig 7a).

**Grip Strength Test**

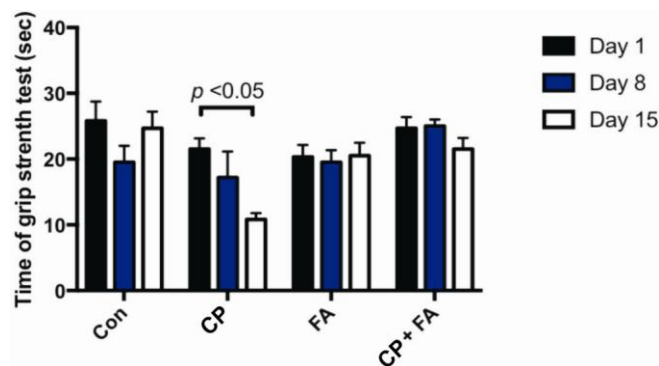
Mice treated with CP alone produced significant ( $p > 0.05$ ) decrease in time they could hang on the wire compared to that of the control. On treatment with FA this effect was reversed and was significantly ( $p > 0.001$ ) different from CP-treated group (Fig. 7b).

**Depression-like Behaviour [Forced Swimming Test (FST)]**

Results show that CP-treated group produced significant decrease in mobility time when compared with that of control ( $p < 0.05$ ). Treatment with ferulic acid showed to improve the activities in CP-treated mice with a significant ( $p < 0.001$ ) increase in the mobility (Fig. 8).

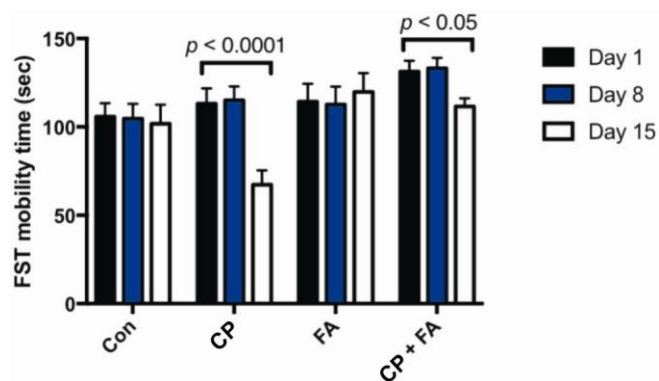


**Fig. 7a:**



**Fig. 7b:**

**Fig. 7a and 7b:** Neuro-muscular coordination influenced by cisplatin (CP) and ferulic acid (FA) and comparison to that of control. a) Time taken on rotarod treadmill and b) Time taken on grip strength rod. Each column and vertical bar represent mean  $\pm$  SEM. Differences were assessed by *t*-test and  $p < 0.05$  was considered significant.



**Fig. 8:** Effect of cisplatin (CP) and ferulic acid (FA) on depression-like behavior in mice. Each column and vertical bar represent mean  $\pm$  SEM. Differences were assessed by *t*-test and  $p < 0.05$  was considered significant.

**DISCUSSION**

Cisplatin (CP) is a common anti-cancer medication

which is also known for its serious side effects, specifically nephrotoxicity.<sup>18</sup> We have previously reported that motor and behavioural impairment in mice can be caused by CP.<sup>7</sup> In this study, we tested the possibility of FA in ameliorating the effects of CP-induced motor and behavioural changes in mice.

Significant reduction in body weight was witnessed in mice treated with CP. This could be due to the cytotoxic effect of CP that induced change in their eating behavior,<sup>19</sup> or the renal tubular damage affecting water reabsorption causing dehydration and/or inflammation.<sup>20</sup> Reduction in bodyweight was also shown by the ability of CP to escalate leptin levels causing reduction in appetite.<sup>21</sup> The biochemical indices such as plasma creatinine and urea were measured in order to assess the degree of kidney injury, and the nephrotoxicity was confirmed.

Mice that were treated with CP showed significant perpetuation in nociception time on hot plate. The reduction in number of contractions induced by acetic acid, also suggested the hypoalgesic action of CP. These results are similar to the previous studies.<sup>7,22</sup> It was demonstrated that administration of higher dose of CP produce hypoalgesic effect by affecting mechanical and thermal sensory withdrawal thresholds on the skin.<sup>22</sup> However, the result suggests no possible effect of FA in sinking the hypoalgesic effect of CP. Cold flick test did not produce any significant difference within or between the four groups. This result contradicts with that reported in previous studies. A possible reason for the inconsistent values could be the tail injury caused due to fighting. This injury may cause alteration to tail sensation in mice resulting in variation in nociception values.<sup>23</sup>

We investigated the locomotor activity in mice treated with CP. Results show that there was a significant reduction in motor activity in both horizontal and vertical activities with CP treatment. However, when FA was administered with CP, it significantly improved the immobility caused by CP suggesting the possible effect of FA in improvement of CP-induced locomotion impairment. The effect of CP, FA and their combination on neuromuscular coordination was studied in mice. It was found that CP significantly decreased the time on rota-rod treadmill and grip strength test suggesting a deterioration in neuromuscular coordination. FA administration on the other hand, produced significant improvement in neuromuscular coordination impairment caused by treatment with CP. The decrease in the locomotor activity and neuro muscular coordination specified the magnitude of excitability of the CNS,<sup>30</sup> and showed the central inhibitory action of CP.<sup>24</sup>

FST was used to determine the depression-like behaviour in mice based on previous report.<sup>32</sup> Mice treated with CP produced significant reduction in swimming time signifying depression-like state. The result

showed that FA can ameliorate the depression caused by CP treatment but it was insignificant although showing an antidepressant action. Depression and immobility are linked with each other and it has been already shown that drugs with anti-depressant activity can increase mobility.<sup>25</sup> The exploratory tendency was decreased significantly in CP treated mice suggesting a diminished interest towards novelty (neophilia). FA showed significant ability in improving the reduction in neophilia induced by CP treatment.

The current study has shown that FA has potential ameliorating effects on CP-induced side effects in mice. It also improved the locomotor activity, neuromuscular coordination, exploratory activity and reduced the depression-like behaviour. However, FA did not show any alterations against CP-induced hypoalgesic effect. Further studies are warranted to approve the use of FA in mitigating CP induced toxicity.

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#### Authors' Contribution

BHA designed the experiment and wrote the manuscript. MSA, MA and AR conducted the animal work and analyzed the data. AN participated in the conception of the work and in writing and revising the manuscript. All authors read the final version of the manuscript and approved it.

#### Disclosure Statement

The authors declare that there are no conflicts of interest.

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