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## Research Article

### EFFECTS OF SINGLE-DOSE HEXAVALENT CHROMIUM ON GROWTH PERFORMACE AND BIOCHEMICAL PARAMETERS OF SWISS ALBINO FEMALE MICE

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#### Abstract

Hexavalent chromium, hazardous metal, have a range of harmful consequences, including developmental toxicity, and they infiltrate the human and animal food chains due to untreated effluents emitted. Since hexavalent chromium affects both animals and humans, the aims of the current experiment were to assess the impacts of a single intraperitoneal dosage on body weight and biochemical markers (ALT, AST, and TP) as well as on hormone (progesterone) levels in female Swiss albino mice. A total 24 healthy adult mice were divided into three groups consisting of six mice per group, including control T0, and treatment groups T1 and T2 with a single dose of Cr (VI) at a dosage rate of 1 and 2 mg/kg body weight was done using an intraperitoneal administration respectively. The significance of the difference between groups was tested using a single-factor ANOVA. On the 14th day following injection of hexavalent chromium, visceral organs such as the lung, heart, liver, spleen, kidney, and blood were taken. Exposure to Cr (VI) produced a substantial decline ( $p < 0.05$ ) in weight gain coupled with considerably enhanced weight gain of the liver, kidney, and spleen ( $p < 0.05$ ). The activity of the enzymes alanine aminotransferase (ALT) and aspartate aminotransferase (AST) was statistically highly enhanced ( $p > 0.05$ ), along with the exponentially significant elevated level of total protein ( $p < 0.01$ ). No significant variation was noticed in the progesterone hormone level ( $p > 0.05$ ) among the treated groups and the control group. The outcomes of the current investigation indicated that experimental female albino mice exhibited harmful effects following injection of chromium VI.

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#### Introduction

Chromium (Cr), heavy metals and persistent environmental contaminants, which finds extensive application in various sectors including pigment manufacturing, corrosion control, plating, and nuclear weapon development, is among the deleterious environmental contaminants released into the atmosphere (Singh et al., 2016). High quantities of chromium (40 to 50,000 mg/ppm) have been detected in the effluents of these enterprises. Research indicates that this chromium pollution contaminates aquatic life, surface water, groundwater and agricultural land (Trivedi et al., 1989). At this time, it is acknowledged as a substantial environmental contaminant on account of its ecological, nutritional, and environmental toxicity (Cheung & Gu, 2007). A growing body of research on metal toxicity has demonstrated that concerns regarding metal pollution are becoming more pervasive, especially in regions where human activity is prevalent.

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Heavy metals, such as but not limited to lead, copper, chromium, mercury, cadmium and arsenic, are significant environmental hazards on account of their extreme toxicity. A "heavy metal" is any metallic element with a density greater than 4 g/cm<sup>3</sup> that has a detrimental or toxic impact even at extremely low concentrations (Nagajyoti et al., 2010). Particularly in developing countries like ours, heavy metal contamination of the environment and increased production have resulted from rapid industrialization.

Chromium levels in sediment vary between 1 and 300 mg/kg, in saline between 5 and 800 g/L, and in rivers and lakes between 26 g/L and 5.2 mg/L (Kotaś & Stasicka, 2000). Cr (III) and Cr (VI), the two most prevalent stable oxidation states of chromium in the environment, are the former and the latter, respectively. These forms' toxicity, mobility, bioavailability, and solubility differ. Cr (VI) is physiologically more hazardous than Cr (III) because it is highly soluble, stable, dominant, and rapidly permeable via the transport system of sulfate, with protein and nucleic acid interaction. Hexavalent Cr (VI) are a mutagenic, genotoxic, poisonous, and carcinogenic to animals, plants, humans, and microorganisms (Ackerley et al., 2004; Feng et al., 2003; Mount & Hockett, 2000; Turpeinen et al., 2004). Soil contamination with Cr (VI) modifies the makeup of communities of microbes and hinders their proliferation, and it also affects human physiology. If it penetrates the food chain, it can cause a significant negative health consequences including lung cancer, eardrum perforation, ulceration, skin irritation, and nasal irritation (Chandra et al., 2011; Madhavi et al., 2013; Singh et al., 2013). Cr (VI) 15 is one of the 17 contaminants recognized by the US Environmental Protection Agency (USEPA) as providing the highest risk to humans (Marsh & McInerney, 2001). Occupational exposure poses the utmost hazard, that is common in companies that use chromic acid or are engaged in the production of chromite ore, paint, or steel welding. Breathing in Cr (VI) rises the incidence and peril of lung cancer, whereas imbibing water increases the risk of liver cancer. As a result, Cr (VI) is classified as a category 1 human carcinogen by the International Agency for Research on Cancer (IARC) and ranks 17th on the Agency for Toxic Substances and Disease Registry's (ATSDR's) Hazards Priority List (Wise et al., 2022).

Numerous studies on the biological effects of chromium have been conducted, including those on postnatal development (Banu et al., 2008), embryotoxicity and fetotoxicity (Marouani et al., 2011), teratogenicity (Arshad et al., 2017), and normal glucose metabolism (Sahin et al., 2011). The hexavalent form is a potent oxidant and is frequently combined with oxygen. It is widely known to have hazardous and carcinogenic consequences in humans and animals, as well as allergic dermatitis (Kawanishi et al., 2002). The hypothesis of this study is the effects of hexavalent chromium on body weight and metabolic markers. Because published data are scarce, the current experiment was carried out to determine the effects of a single intraperitoneal dosage of hexavalent chromium on body weight as well as its impact on serum levels (ALT, AST, and TP) levels, as it affects both humans and animals.

## Materials and Methods

### *Ethical Approval*

Animals that were used in this investigation were treated carefully and thoughtfully. Throughout the investigation, it was clear that the stress and suffering of the animal were low. Before commencing the investigation, ethical consent was obtained from the Animal Experimentation Ethics Committee of Sylhet Agricultural University (Memo no: SAU/Ethical committee/AUP/22/21; Date: June 25,2022), Bangladesh.

### *Experimental Animals*

A total 24 healthy adult female Swiss albino mice aged  $12 \pm 0.5$  weeks and weighing  $30.0 \pm 5$  gm were purchased from the department of physiology at Sylhet Agricultural University, Sylhet, Bangladesh. The animals were kept in institutional animal housing in polypropylene cages under a relative humidity ( $50.5\% \pm 5\%$ ), temperature ( $25.0 \pm 2^\circ\text{C}$ ), and a constant light-dark plan (Tamta et al., 2009). Animals were fed a standardized regular meal (pellets), which comprised a 1:1 ratio of wheat and gram, as well as unlimited access to salt, sugar, milk, and water.

### *Chemicals and medications*

Potassium dichromate ( $\text{K}_2\text{Cr}_2\text{O}_7$ ) was acquired from commercial scientific Ltd. Dhaka, Bangladesh.

### Experimental Design

A total twenty-four healthy adult female mice were equally divided into three groups and each group contained eight mice, where T0, T1 and T2 were control group, treatment group 1, and treatment group T2 respectively. A dose of potassium dichromate-1 mg/kg body weight in T1; and T2 animals were administered 2 mg of potassium dichromate/kg via intraperitoneal route (Marouani et al., 2011). Adequate feed, water, and similar housing amenities were supplied in this experiment. The mice were monitored daily for physical symptoms of poisoning. Body weight was taken on the day before administering of potassium dichromate the seventh day after delivering the treatment, and the fourteenth day after giving the treatment. On the fourteenth day following the treatment of potassium dichromate, the mice were humane killed for organ weight, biochemical, and hormonal studies.

### Physical Parameters

Throughout the duration of the treatments, factors such as body weight and local damage were evaluated. During experiment any mortality that occurred in any of the groups was also reported. At the end of the treatment, biochemical and hormonal indicators were evaluated.

### Biochemical Parameters

Fresh blood was collected directly from heart into a vacutainer tube and serum was collected by centrifuging at 3000 rpm, which was subsequently frozen at -20°C for future testing. The serum was used to evaluate ALT, AST, and total protein. The estimation was carried out by the Dade Behring Dimension RxL Max/Vitros 250 Random Access Chemistry Analyzer.

### Organ Weight

The organs (heart, lung, liver, spleen, and kidney) were promptly blotted, weighed on a digital balance. This was accomplished in the laboratory of the Department of Physiology.

### Statistical Analysis

The collected data was evaluated by using IBM SPSS statistics 22 software. A single-factor ANOVA was used for different between group and 5% level of significance was considered.

## Results

After delivery of Cr (VI) by the intraperitoneal route, no significant changes in behaviour or clinical symptoms were seen in the treated group over the research period. No mice perished during the treatment with Cr (VI).

### The effect of single-dose Cr (VI) on body weight

The outcome of the impact of single-dose Cr (VI) on the body weight of Swiss albino female mice is shown in Table 1. Before giving Cr (VI), the mean body weight of female Swiss albino mice in the control group was 28.83 gm, whereas in the T1 group, the mean value was 29.49 gm, and in the T2 group, it was 27.44 gm. On the contrary, the mean value of body weight on the 7th day after giving Cr (VI) reveals a substantial reduction in body weight growth in both the T1 and T2 groups when compared to the control group. The mean body weight of T1 was 27.89 gm and T2 was 26.82 gm, whereas the control group exhibited a substantial weight gain (29.44 gm) on the 7th day after giving Cr (VI). Again, on the 14th day, the mean value of body weight revealed substantial increases in both the T1 (30.51 gm) and T2 (29.14 gm) groups, as well as in the control group (31.67 gm).

**Table 1.** The effect of single-dose Cr (VI) on body weight of Swiss albino female mice

Body Weight (gm)	Control (Mean $\pm$ SD)	Treatment (Mean $\pm$ SD)		P Value
	T0	T1	T2	
1 <sup>st</sup> Day	28.83 $\pm$ 1.12	29.49 $\pm$ 2.82	27.45 $\pm$ 1.40	0.20
5 <sup>th</sup> Day	29.45 $\pm$ 0.84	27.90 $\pm$ 2.42	26.82 $\pm$ 0.74	0.03
10 <sup>th</sup> Day	31.68 $\pm$ 1.15	30.51 $\pm$ 1.32	29.154 $\pm$ 1.93	0.03

Single factor ANOVA test; level of significance was 5%

### *The effect of Cr (VI) on the organ weight*

Table 2 illustrates the outcome of different organ weights and the influence of a single dosage of Cr (VI) on them. There was no significant increase or reduction in heart and lung weight among the three groups, and the mean value was practically comparable. However, in the case of the liver, spleen, and kidney, it displays considerable modifications. The mean value of liver weight in the control group was 1.453 gm, whereas the average value of the T1 group was 1.54 gm and the T2 group was 1.59 gm. Again, the mean value of the spleen and kidney also indicated considerably greater outcomes between treated and non-treated groups. The mean spleen weight of the treated group was 0.122 gm (T1) and 0.141 gm (T2), whereas 0.12 gm was in the non-treated group. Similarly, the mean value of the kidney weight control group was 0.348 gm, while the treatment group was 0.391 gm and 0.470 gm, respectively. So, the effect of a 2 mg intraperitoneal dosage was substantially more impressive than the effect of a 1 mg dose, and the non-treated group did not demonstrate any such alterations. The impact of single-dose Cr (VI) was significantly more evident in the liver, spleen, and kidney than in the heart and lung.

**Table 2.** The effect of single-dose Cr (VI) on organ weight of Swiss albino female mice

Organ Weight (gm)	Control (Mean ±SD)	Treatment (Mean ±SD)		P Value
	T0	T1	T2	
Heart	0.14 ± 0.003	0.15 ± 0.002	0.14 ± 0.011	0.97
Lung	0.25 ± 0.004	0.25 ± 0.02	0.25 ± 0.004	0.68
Liver	1.45 ± 0.03	1.54 ± 0.04	1.59 ± 0.02	0.01
Spleen	0.12 ± 0.006	0.12 ± 0.006	0.14± 0.004	<0.0
Kidney	0.35 ± 0.004	0.39 ± 0.01	0.47 ± 0.004	<0.01

Single factor ANOVA test; level of significance was 5%

### *The impact of Cr (VI) on biochemical parameters*

The outcome of the biochemical test and the influence of a single dosage of Cr(VI) was noted in Table 3. The mean value of alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were significant. The mean value of these two parameters was greater in the treated group than in the non-treated group. In the instance of ALT, 37.8 u/l, and 41.5 u/l were the mean values of the T1 and T2 groups that were administered 1 mg and 2 mg of Cr (VI), respectively, whereas 33.5 u/l was the mean value of the T0 group. Similarly, the mean AST values of the treated group were 254.3 u/l (T1) and 257.6 u/l (T2). On the other hand, the mean AST value of the control group was 250.3 u/l. Simultaneously, the parameter total protein (TP) also revealed a significant result of having a mean value of 6.24 g/dl for the 1mg Cr(VI) treatment group and 6.91 g/dl for the 2 mg Cr(VI) treatment group, whereas the value for the control group was 5.38 g/dl. In the case of progesterone, the significance was not that much greater, but the conclusion was meaningful. The mean value of the control group was 3.84 ng/ml, whereas T1 was 4.21 ng/ml and T2 was 4.97 ng/ml.

**Table 3.** The effect of single-dose Cr (VI) on Biochemical parameters and Progesterone hormone of Swiss albino female mice

Organ Weight (gm)	Control (Mean ±SD)	Treatment (Mean ±SD)		P Value
	T0	T1	T2	
ALT (u/l)	33.50 ± 4.03	37.8 ± 3.37	41.5 ± 4.76	0.041
AST (u/l)	250.30 ± 3.32	254.3 ± 3.50	257.6 ± 5.39	<0.001
TP (g/dl)	5.38 ± 0.19	6.25 ± 0.31	6.91 ± 0.40	<0.01
Progesterone (ng/ml)	4.19 ± 0.20	4.21 ± 0.37	4.42 ± 0.17	0.265

Single factor ANOVA test; level of significance was 5%

## Discussion

Both the control and treated dams showed no noticeable behavioral or clinical changes. Throughout the trial, no deaths were documented, which is like Marouani et al., 2011 findings. In our investigation, the effect of potassium dichromate on body weight gain and the relative weights of the liver and kidney, two key organs, indicated that there was a large reduction in body weight gain and an increase in these organs' weights. This showed that mice treated with potassium dichromate suffered a widespread adverse impact. Exposure to chromium increased the weight of the liver and kidneys while decreasing overall weight gain in female mice, indicating a general detrimental effect of such exposure (Jadhav et al., 2007). This showed that treated female mice exposed to potassium dichromate exhibited a nutritional imbalance (Ibrahim et al., 2012). Some researchers also noticed a comparable reduction in body weight increase following exposure to potassium dichromate (Chandra et al., 2010; De Lucca et al., 2009; Kim et al., 2004a; Saha et al., 2017a). There was a substantial rise in liver and spleen weight in our study, which is akin to the findings of Saha et al., 2017 but in contrast to Samuel et al., 2014 who observed a decrease in the weight of the kidney, and Kim et al., 2004b discovered no significant changes in organ weights in his findings. In the case of heart and lung weight, no significant variations were observed, that is similar to Saha et al., 2017b and Kim et al., 2004a. However, according to Samuel et al. (2014), where he observed a considerable drop in the weight of the heart and lung. The average weight of spleen was equally large, on the other hand, the Samuel et al., 2014 and Saha et al., 2017b were revealed opposite findings. The biochemical examination in this study indicated a rise in the activities of the enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total protein levels and these findings are similar to those of Saha et al., 2017b, however, he noticed a decline in the level of total protein. According to Kim et al. (2004b) also showed a decrease in total protein and ALT, which is not similar to our findings. This revealed the widespread and systemic harmful impact of heavy metals on mice (Balakrishnan et al., 2013). Moreover, a single dosage of potassium dichromate does not have any meaningful influence on progesterone levels, although Samuel et al., 2014 claimed different findings. He detected a drop in the level of progesterone following exposure to potassium dichromate. This might be due to frequent exposure or other internal variables that may influence the outcome.

## Conclusion

Hexavalent chromium, a heavy metal, has a deleterious effect on health. The outcomes of our investigation have demonstrated that it has a substantial influence on body weight. The body weight was dramatically lowered after delivering hexavalent chromium through the intraperitoneal method. In reality, the weight rise of the liver, spleen, and kidney was notably exceptional, which may have occurred owing to a biological imbalance in the body because of the exposure to hexavalent chromium, even though there was no significant change in heart or lung weight. The findings of biochemical indicators including ALT, AST, and TP also indicated a considerable increase in serum levels. Though this was merely a pilot investigation, this might allow researchers to obtain a precise conclusion.

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