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Effects of Potassium Dichromate on Reproduction and Fertility in Albino Female Mice

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Abstract : The acute toxicity of hexavalent chromium as potassium dichromate ($K_2Cr_2O_7$) was studied in female albino mice. Reproductive parameters in ovary and uterus and fertility rate were evaluated to investigate the possible effects of chromium intoxication. Seventy-five adult albino mice were used in this study.The mice treated orally with 500 and 1000 ppm of potassium dichromate that dissolved in tap water for 10, 20, and 30 days. The results of this study indicated that potassium dichromate produced a significant reduction ($P<0.05$) in the weights of body, ovaries, and uterus for treated female mice in comparison with control groups. Also, there were a significant decrease ($P<0.05$) in the levels of LH, FSH, and E_2 in blood serum. The findings of present study showed that potassium dichromate induced a significant decrease ($P<0.05$) in values of some reproductive parameters including diameters of ovaries and mature follicles, numbers of ovarian follicles and corpora lutea, and thickness of uterine layers. Also, the oral administration of female micewith potassium dichromate for 30 days resulted in a significant reduction ($P<0.05$) in the number of pregnant females and the number and weight of litters. Overall, the current study proved that potassium dichromate compound has a potential toxicity in female reproductive system and fertility of albino mice.
Keywords: Potassium dichromate, Reproductive parameters, Fertility, Ovary, Uterus, Mice.

Introduction

Chromium (Cr) is widely present in the crust of earth. It is found in soil, rocks, water, air and food. The compounds of hexavalent chromium are available in the environment in numerous forms and can differ in their water solubility and physical properties[1].Chromium and its salts are used in different purposes and industries for example, the manufacturing of catalysts, photographic emulsions, fungicides, pigments and paints as well as in the tanning, ceramics, cement, chrome alloy, chrome plating, welding, cooking utensils, andtextile industry. Also, some chromium compounds are involved in drugs and food industry[2].

In fact, the biological systems require minute amounts of chromium element for their normal functions. In contrast, the unsafe industrial usage for chrome can lead to pollution of the environment and to unfavorable various effects of organisms[3].The anthropogenic activities are the most important reason of widespread contamination of chromium, which affects the environment. The ingestion, breathing and skin contact are the common ways of entry of the chromium into the body[4,5]

The chromium-induced toxicity has been reviewed in several experimental and clinical reports [1,3,4,5,6,7,8,9].Moreover, data on the developmental and reproductive toxicity of chromium are summarized by OEHHA[2] and Marouani *etal*.[10]. In general, the oxidative status of chromium determines the toxicity of this element. It is well known that the trivalent chromium is an essential nutrient for human health and required in small amounts. On the other hand, the hexavalent chromium is a toxic compound and has a potential toxicity for

organisms[9]. In recent years, studying the effects of chromium compounds on reproduction and fertility in animals and humans has become an area of great interest. Therefore, the present study was designed to explore the toxic influences of potassium dichromate ($K_2Cr_2O_7$) in the reproductive efficiency and fertility of albino female mice.

Materials and Methods

Experimental animals

Seventy-five adult albino mice (*Mus musculus)* were used in this study. The present study was conducted at the animal house and animal laboratories of Faculty of Science /University of Kufa for the period from November 2015 to April 2016. Healthy mice weighing between 35-40 gm were used in this experiment. The animals were maintained in an air-conditioned room in separated plastic cages at controlled environment of 22-25 °C throughout the study. The animals received standard commercial food (pellets) and tap water *adlibitum*. The mice were left to acclimatize for two weeks before the start of the experiments.

Experimental design

The study included two stages, the first stage comprised the reproductive test and the second stage involved the fertility test. The protocol of present experiments was approved by the ethical committee of the Faculty of Science / University of Kufa.

Reproductive experiment

Forty-five mature female mice were randomly distributed into three groups (15 mice in each group). Each group was subdivided into three secondary groups (5 mice in each group) in separated cages. Tow concentrations of hexavalent chromium compound (500 ppm and 1ppm potassium dichromate) dissolved in clean tap water were investigated. The orally concentrations as drinking water of potassium dichromate were determined according to Trivedi *et al.*[11]. The control group received only drinking water. The potassium dichromate treated groups were examined for three progressive time periods (10, 20, and 30 days) from the beginning of exposure.

The vaginal smears were taken every morning by using sterile loop. The smears were transferred to glass slides and stained by methylene blue for 3-5 minutes, and then washed by distilled water and examined under microscope for recognizing the different phases estrous cycle[12]. The reproductive effects of potassium dichromate were evaluated in estrus phase.

At the end of the experimental periods (10, 20, and 30 days), all mice were anesthetized, using a mixture of ketamine and xylazine i.m, and then they were sacrificed [13]. The blood sample was obtained from animal through heart puncture by using a 3 ml disposable medical syringe.

The blood samples were placed in tubes without anticoagulant and centrifuged at 5000 rpm for 10 minutes for hormonal analysis. The blood serum was separated, transferred into Eppendorf tubes and kept in a refrigerator at -20 °C until the time of analysis [14].The levels of luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estradiol (E_2) in blood serum were measured using enzyme linked immune sorbent assay (ELISA) method, by the spectrophotometer at specific wave lengths according to the protocol of kits supplied by Monobind, USA. The concentrations of LH and FSH is expressed as ng/ml whereas the level of E_2 was expressed as pg/ml.

The recording of body weight of each animal was done initially and at the end of treatment. The relative weights were calculated according to the following formula: A/B×100%. A = organ weight, B = final body weight [15].The relative weights (%) of ovary and uterus were recorded as mg/10 gm of body weight [10].

For histopathological study, ovaries and uteri were removed and cleaned. Thereafter, these organs were fixed immediately in 10% formalin solution for later histological preparation.Ordinary histological technique was followed to prepare slides from specimens of uteri and ovaries from all animal groups to study the alterations that may be found in chromium-treated animal groups. The preparation of microscopic slides and staining techniques were performed according to Bancroft and Stevens [16].

The numbers of ovarian follicles, namely, primary, secondary, and Graafian follicles were calculated in all groups of animals under the low power of magnification (100X). At the same time, the number of corpora lutea in the cortex of ovary was also counted. The means of diameters of ovaries and Graafian follicles in each mouse were calculated by using light compound microscope under the low magnification power (40X). The thicknesses of endometrium and myometrium of uterus were measured by using the ocular micrometer through the low power of magnification (100X).

Fertility experiment

Fertility experiment was estimated in 20 mature female mice exposed to 500 and 1000 ppm of potassium dichromate for 30 days. After the completion of the treatment, they were kept for mating (2:1) with untreated adult males of the same strain in an individual cages under standard laboratory conditions. The presence of sperms in vaginal smear represents the first day of pregnancy[17]. The mice were left together for ten days, and this means elapse two estrous cycles in order to prove the mating and fertility[18].

After the removal of the males, the number of pregnant females and the number and weight of litters were recorded. The percentage of fertility rate of treated female mice with potassium dichromate were calculated from the formula: number of pregnant females/ total number of females X 100[19]. In addition, the external abnormalities in the litters were examined.

Statistical analysis

Statistical package for social sciences (SPSS, version 23) program were used to analyze the data of present study. Analysis for statistical differences of means between the animal groups was done by using one way analysis of variance (ANOVA) followed by least significant difference (LSD) test to determine the significant variances. Statistical significance was accepted at $P<0.05$ values[20].

Results and Discussion

Effect of potassium dichromate on weights of body, ovaries and uterus

Administration of potassium dichromate to mice at concentrations 500 and 1000 ppm for 20 and 30 days caused a significant decrease ($P<0.05$) in the body weight gain and relative weights of ovaries and uterus compared with normal control groups.(Table 1)

Table(1): Effect of potassium dichromate (Cr^{+6}) on body weight gain and relative weights of ovaries and uterus in albino mice.

	Control groups			500 ppm Cr^{+6}			1000 ppm Cr^{+6}		
	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days
Body weight	3.26 ± 0.06	5.60 ± 0.33	9.60 ± 0.64	3.20 ± 0.11	*2.40 ± 0.23	*1.93 ± 0.13	2.80 ± 0.20	*1.63 ± 0.14	*1.06 ± 0.03
Ovary weight	9.32 ± 0.36	9.8 ±30.47	9.26 ± 0.32	9.25 ± 0.38	*7.15 ± 0.26	*6.51 ± 0.10	8.81 ± 0.54	*6.70 ± 0.12	*6.02 ± 0.43
Uterus weight	26.0 ± 2.17	26.19 ± 1.2	27.84 ± 0.63	26.99 ± 0.34	26.34 ± 0.25	*23.83 ± 1.30	26.78 ± 1.01	2.08 ± 0.09	*21.79 ±0.61

***: Significantly different at $P<0.05$ from control groups**

This reduction in body weight gain may be explained according to fact that the accumulation of potassium dichromate leads to lose appetite in mice and then decrease the body weight.According to Samuel *et al*. [21], the failure of postnatal rats exposed to chromium(VI) through mother's milk to gain body weight may be due to decreased feed intake, malabsorption of nutrients from the gastrointestinal tract and impaired feed conversion efficiency.

Depending on Rao *et al*. [22], this reduction in weights might be due to low food consumption, hormonal imbalance and reduction in protein levels. Furthermore, chromium(VI) has also been known to induce oxidative

stress through enhanced production of ROS leading to deterioration of proteins in tissues [23].In another study, it has been proposed that the high levels of chromium in mother rats might have disturbed the maternal physiology leading to decreased internal body organ weights[21].

According to Samuel *et al.*[21,24], the chromium (VI) exposure leads to higher accumulation of chromium in uterus and ovary in lactating rats. Also, estrogens are known to regulate growth and cell division in uterus [25]. Therefore, the observed decrease in uterus weight may be due to the decreased blood level of estrogens [10].

Effect of potassium dichromate on some hormonal levels

The results showed intable (2) indicate significant increases (P<0.05) in levels of both follicle stimulating hormone (FSH) and luteinizing hormone (LH) in potassium dichromate-treated groups at concentrations 500 and 1000 ppm for 10, 20 and 30 days in comparison with control groups. In contrast, treated mice with potassium dichromate showed significant decrease (P<0.05) inestradiol level compared with control animals.

Table (2): Effect of potassium dichromate (Cr^{+6}) on FSH, LH and E_2 level in albino mice.

	Control groups			500 ppm Cr^{+6}			1000 ppm Cr^{+6}		
	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days
FSH	8.37 ± 0.85	8.44 ± 0.41	9.07 ± 0.88	*13.07 ± 0.56	*17.42 ± 0.80	*27.29 ± 1.58	*14.58 ±0.19	*24.37 ± 0.49	*37.18 ±2.05
LH	3.28 ±0.17	2.97 ± 0.26	3.30 ± 0.17	4.18 ± 0.13	*10.23 ± 0.93	*14.68 ± 0.58	*7.86 ±0.58	*12.72 ± 0.50	*18.30 ±0.69
E_2	31.02 ± 0.15	31.10 ± 2.71	30.3 ±20.09	*26.29 ± 0.79	*14.60 ± 0.20	*12.09 ± 0.09	*16.93 ±1.31	*13.0 ± 0.34	*7.87 ±0.45

***: Significantly different at P<0.05 from control groups.**

The hypothalamic–pituitary–gonadal (HPG) axis plays a critical role in the control of reproduction. Two key hormonal components of the HPG axis are gonadal steroids and gonadotropin-releasing hormones (GnRH) [26].Uboh *et al*. [27] suggested that the increases in serum LH and FSH levels may be due to decrease of the serum progesterone or the negative impact on estradiol synthesis, which is considered the hormone with the most powerful inhibitor effect on LH.

Additionally, the accumulation of chromium(VI) exposure may delay the development of follicle stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR), on granulose and thecal cells, which is expected to be assisted by inhibitory action of aromatase activity in follicles leading to elevate levels of FSH and LH. Thus, increased levels of FSH and LH could also be due to decreased estradiol levels along with the direct effect of chromium on pituitary gland [10].

In previous study, the abnormal levels of sex hormones recorded in female rats suggest a disruption of steroidogenic function exposed to potassium dichromate [28]. The chromium (VI) exposure-induced impairment of steroidogenic mechanisms might have led to decreased synthesis of steroid hormones despite increase in the FSH levels, and this may be the reason behind the delayed sexual maturation [24].According to Muthusami *et al.*[29], estrogen deficiency was shown to be associated with oxidative stress. In addition, the decreased levels of steroid hormones might be due to the chromium(VI)-induced decrease in the number of ovarian follicles [24].

Effect of potassium dichromate on numbers of ovarian follicles and corpora lutea

The results obtained from the present study showed significant decreases (P<0.05) in the mean number of ovarian follicles (primary, secondary, and Graffian follicles)in potassium dichromate-treated mice at concentration 500 and 1000 ppm for 20 and 30 days in comparison with normal groups. There was a significant decrease (P<0.05) in number of corpora lutea in groups that were treated with potassium dichromate at concentrations 500 and 1000 ppm for 30 days only compared with control animals. (Table 3).

Table (3): Effect of potassium dichromate (Cr^{+6}) on numbers of primary, secondary and Graffian follicles in albino mice.

	Control groups			500 ppm Cr^{+6}			1000 ppm Cr^{+6}		
	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days
Primary follicles	6.0 ±0.36	6.1 ±0.30	6.1 ±0.30	5.8 ±0.16	*3.5 ±0.22	*2.3 ±0.21	5.6 ±0.21	*3.0 ±0.25	*2.3 ±0.21
Secondary follicles	5.0 ±0.25	5.3 ±0.21	5.1 ±0.30	4.6 ±0.33	*4.1 ±0.16	*3.8 ±0.16	4.5 ±0.34	*4.1 ±0.16	*3.8 ±0.16
Graffian follicles	4.5 ±0.42	4.3 ±0.21	4.6 ±0.21	4.3 ±0.33	*1.6 ±0.21	*1 ±0.00	4.3 ±0.33	*1.3 ±0.21	*1 ±0.00
Corpora lutea	5 ±0.57	5.33 ±0.33	6 ±0.00	5 ±0.57	4.66 ±0.33	*4.33 ±0.33	4.66± 0.33	4.33 ±0.33	*4.00 ±0.00

***: Significantly different at P<0.05 from control groups**

According to Gorski [30], the follicular maturation impairment by chromium (VI) may be either a direct effect of the metal on the ovarian tissue or mediated by an effect on the gonadotropins, necessary for normal follicular development and ovulation. Also, Baker [31] suggested that if the surge in gonadotropins is blocked, follicles fail to form more than a single layer of granulosa cells.

The explanations above confirm the results of current experiment. The growth and development of ovarian follicles in different stages depend on the gonadotropin hormones (FSH and LH) that secreted from pituitary gland. Consequently, the marked reduction in number of ovarian follicles observed in this study may be due to a decline in the level of FSH secreted by the pituitary gland. The negative changes in FSH value and ovarian tissue were proved in the present study.

The chromium(VI) exposure resulted in accumulation of this compound in blood and ovarian tissue and is responsible for extending the estrous cycle, delaying the onset of puberty, inducing follicular atresia, impairing the growth, development and differentiation of the granulosa and theca cells of ovarian follicles, and inducing fibrosis and necrosis of primary and secondary follicles [32]. Furthermore, the decreased numbers of primary and secondary follicles indicate that chromium(VI) targets follicle populations at all stages of follicle development. This might suggest that chromium(VI) toxicity could result in permanent damage to primary follicles with a temporal delay in follicular maturation [33].

The current study has revealed that at 500 and 1000 ppm of potassium dichromate exposure for 30 days through drinking water in mice results in reduction in mean number of corpora lutea. Very little is known in recent literature about the effect of chromium(VI) on corpora lutea. Nevertheless, similar findings in short and long term exposures of potassium dichromate in rats were noted by Kanojia *et al.*[17,34].Overall, the reduced number of corpora lutea was might be correlated with the reduced number of ovarian follicles and retarded development of Graffian follicles leading to reduced number of ovulations due to chromium administration.

Effect of potassium dichromate on diameters of ovary and Graffian follicles

The present data show significant reductions (P<0.05) in diameters of ovaries and Graffian follicles (mature follicles) in all treated female mice exposed to potassium dichromate compared with control groups as shown in table (4).

Table(4): Effect of potassium dichromate (Cr^{+6}) onovary diameter and Graffian follicle diametersin albino mice.

	Control groups			500 ppm Cr^{+6}			1000 ppm Cr^{+6}		
	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days
Ovary diameter	1096 ±60.18	1096 ±60.18	1068 ±14.00	*971 ±14.33	*901 ±13.33	*832 ± 0.00	*790 ± 0.00	*687 ±36.08	*554 ±50.38
Graffian follicle diameters	350 ±14.43	333 ±30.04	304 ±29.00	*266 ±4 .33	*233 ±8.33	*225 ±0.00	*220 ±4.33	*191 ±8.33	*158 ±14.43

***: Significantly different at P<0.05 from control groups**

Our results were consistent with findings of Jeber and Tawfeek[35] who investigated the effects of potassium dichromate for 14 days on ovarian follicle diameters in immature female rats. Briefly, there is no an obvious explanation from the literature to the reasons for the decline in diameters of ovary and mature follicles. Based on findings of this study, we believe that the marked decrease in these diameters may be due to the low numbers of ovarian follicles and corpora lutea as well as the histological lesions, such as degeneration and necrosis, in the content of ovary induced by accumulation of potassium dichromate in the blood and ovarian tissue.

Effect of potassium dichromate on thickness of uteral layers

The results of this study show significant decreases (P<0.05) in thicknesses of endometrium and myometrium in potassium dichromate-treated mice compared with control groups. (Table5).

Table (5): Effect of potassium dichromate (Cr^{+6}) on thickness of endometrium and myometrium in albino mice.

	Control groups			500 ppm Cr^{+6}			1000 ppm Cr^{+6}		
	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days
Endometrium	816 ±67.26	761 ±30.89	805 ±43.38	708 ±25.66	*677 ±5.66	*644 ±22.00	*561 ±11.00	*522 ±20.10	*396 ±13.33
Myometrium	494 ±29.41	488 ±11.33	488 ±19.87	455 ± 5.33	*427 ±11.33	*344 ±5.66	*283 ±9.81	*250 ± 0.00	*227 ±11.33

***: Significantly different at P<0.05 from control groups**

Our findings agree with results of Marouani *et al.*[10] who revealed that a high dose of chromium(VI) in rats caused atrophic epithelium cells of the endometrium with decreased thickness of the myometrium. The present study revealed that potassium dichromate exposure in female mice causes deleterious effects in uteral layers. The changes in measurement of endometrium and myometrium thicknesses were seen in this study could be explained by the fact that chromium induced hormonal imbalance and obvious alteration in the histoarchitecture of uterus.

Previous studies that were conducted on rats have shown that chromium exposure increases the concentration of reactive oxygen species (ROS) and provokes oxidative damage in ovaries and uterus [24]. Administration of chromium resulted in oxidative stress in female reproductive system that was reflected by altered histoarchitecture in these organs [33].

Effect potassium dichromate on fertility rate, number of litters andlitter weights

The effect of potassium dichromate through drinking water on fertility rate, number of litters and weight of litters for 30 days are shown in table (6). The present study indicates that with increased potassium dichromate intake, the percentage offertility rate in treated female mice was decreased as compared with control group. In addition, treated mice with potassium dichromate at concentrations 500 and 1000 ppm showed significant decreases ($P<0.05$) in numbers and weights of litters in comparison with control mice.

Table (4-6): Effect of potassium dichromate for 30 days on fertility rate, number of litters and litter weights in albino mice.

	Fertility rate (%)	Number of litters	Weight of litters (gm)
Control group	100	6.0 ± 0.5	2.77 ± 0.09
500 ppm Cr^{+6}	33	*1.4 ± 1.3	*2.27 ± 0.03
1000 ppm Cr^{+6}	25	*0.7 ± 0.6	*1.86 ± 0.06

***: Significantly different at P<0.05 from control groups.**

Concerning the fertility rate, the result of this study is in agreement with earlier reports that examined the effect of chromium(VI) on fertility of female rats [17,34,36]. It may be proposed that potassium dichromate may account for the reduction in percentage of pregnancy and number and weight of litters due to decrease in FSH and LH levels, follicle development, ovulation, number of embryos and then percentage of fecundity.

According to Marouani *et al.*[10], the toxic effect of the metal on fertility rate may either be a direct influence on the ovarian tissue or mediated by an effect on the gonadotropins. Murthy *etal.* [37] suggest that complete destruction of oocytes, at any time during the reproductive life span, will lead to infertility. Furthermore, the exposure of female mice to chromium results in fewer implantations and thereby reduction of their fertility [34].

The deleterious effects of chromium on litter weights of rats have been investigated in several studies [17,34,36]. They demonstrated that the impaired fetal physiology and the significant reduction in litter sizes in treated females might be due to chromium accumulation in uterus.

In mice, Trivedi *et al.*[11] suggested that the reduction in litter size may possibly be due to the direct effect of high content in the placental and fetal tissues. It was previously reported that increase of chromium(VI) level and its transmission to the placenta impairs embryonic development [36,38].

The effects of potassium dichromate on teratogenic changes have been investigated in mice[11,39] and in rats [17]. They found that the chromium exposure increased the incidences and types of external and skeletal malformations in the fetuses. However, the lack of any obvious teratogenic alterations in the litters in present study was contradictory to these reports. This discrepancy might be due to numerous factors such as dose and duration of exposure, physiological conditions, species variation, and others. Hence, the teratogenic effects of hexavalent chromium compounds in mammals require more investigation.

In conclusion, the present study proved that potassium dichromate induced considerable changes in female reproductive system, thus, hexavalent chromium compounds may be one of the environmental factors that affects reproduction and fecundity. Additionally,the progressive effects of potassium dichromate in this study showed that it has dose and duration-dependent intoxication.

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