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## **Toxic Effect of Potassium Dichromate on Sex Hormones and Possible Protective Effect of Rice Bran Oil in Female Albino Rats**

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### **ABSTRACT**

This study was established to investigate the toxic effects of potassium dichromate on sex hormones of female albino rats and the possible protective effects of rice bran oil. This study was carried out on thirty weanling mature female albino rats weighing between 120 and 140 g, divided randomly into 3 groups. Rats in the 1st group were used as a control (group C), the 2nd group of rats (group E) received Potassium dichromate (20 mg kg<sup>-1</sup>, I/P) for 5 months, while the 3rd group, group B, received both Potassium dichromate solution (20 mg kg<sup>-1</sup>, I/P) and rice bran oil (20 mg kg<sup>-1</sup>), with 1 h in between for 5 months. Blood samples were collected from retro orbital venous sinus in lithium heparin tubes every 30 days. Sexual hormones were determined by ELISA technique. The study showed that potassium dichromate affects the levels of sex hormones and that the rice bran oil has great effects in the prevention of the toxic effects of potassium dichromate on sex hormones.

**Key words:** Potassium dichromate, sex hormone, rice bran oil, female albino rats

### **INTRODUCTION**

Chromium (Cr) is a naturally occurring heavy metal, commonly found in the environment in two valence states: Trivalent Cr (III) and hexavalent Cr (VI). It is widely used in numerous industrial processes and as a result, is a contaminant of many environmental systems (Gumbleton and Nicholls, 1988). Commercial chromium compounds are used in industrial welding, metal finishes, leather tanning, wood preservation and is a non-negligible pollutant in the world (Wang *et al.*, 2006).

Studies involving animal models also discovered many harmful effects of Cr (VI) on mammals. Subcutaneous administration of Cr (VI) to rats caused severe progressive proteinuria, elevation of urea nitrogen and creatinine, as well as elevation in serum alanine aminotransferase activity and hepatic lipid peroxide formation (Kim and Na, 1991).

Similar studies, reported by Gumbleton and Nicholls (1988) found that Cr (VI) induced renal damage in rats when administered by single subcutaneous injections.

Bagchi *et al.* (1995), demonstrated that in rats, Cr (VI) received orally in water induced hepatic mitochondrial and microsomal lipid peroxidation, as well as enhanced excretion of urinary lipid metabolites including malondialdehyde. Moreover, some adverse health effects induced by Cr (VI) have been reported in humans. Reports of epidemiological investigations have shown that respiratory cancers were found in workers occupationally exposed to Cr (VI) compounds (Shi, 1999).

DNA strand breaks in peripheral lymphocytes and lipid peroxidation products in urine were observed in chromium exposed workers by many researches who also showed evidence of the Cr (VI)-induced toxicity to humans (O'Brien *et al.*, 2003).

Cr is a reproductive metal toxicant that can traverse the placental barrier and cause a wide range of fetal effects including ovotoxicity (Banu *et al.*, 2008).

Rice is the most important cereal product in Egypt and is an overwhelming staple food in most populations (Wadsworth, 1992). It is grown in more than 100 countries and there are around 18,000 varieties accounting for about 25% of the world's food grain production (Hernandez *et al.*, 2000). The prominent rice producing continents are Asia and America (Anonymous, 2009). The bran is the hard outer layer of rice consisting of aleurone and pericarp. Rice bran contains an array of micronutrients like oryzanols, tocopherols, tocotrienols, phytosterols, 20% oil and 15% protein, 50% carbohydrate (majorly starch), dietary fibers like  $\beta$ -glucan, pectin and gum (Jiang and Wang, 2005). Rice bran, which was earlier used primarily as animal food, is now finding major application in the form of rice bran oil (Barber *et al.*, 1974; Hammond, 1994).

India and Thailand have been the most successful countries in rice bran oil production. Rice bran oil refining industry produces residues such as wax sludge, gum sludge and soap stock that are a rich source of many nutraceuticals like oryzanols, tocopherols, tocotrienols, ferulic acid, phytic acid, lecithin, inositol and wax (Ferrari *et al.*, 1996).

Though Japan contributes to just 2% of total production of paddy in the world, it is a promising producer of nutraceuticals and other high value products from the derivatives of paddy (Patel and Naik, 2004). The rice bran obtained from different varieties of colored rice are the antioxidant compounds viz., polyphenols, carotenoids, vitamin-E and tocotrienol which help in preventing damage to body tissues and the oxidative damage of DNA (Ling *et al.*, 2001).

Ling *et al.* (2002) revealed from their study that feeding bran fractions of certain colored rice varieties to rabbits improved antioxidant status of their blood and showed significant reduction in atherosclerotic plaque formation.

Rice bran has several unique properties that render its suitability for niche markets like nutraceutical and pharmaceutical industry. One such feature is the presence of significant levels of minor-elements such as oryzanol, tocotrienol and phytosterols that have a large nutraceutical application. They are used in the development of value-added healthy products (Anonymous, 2009).

Gamma oryzanol has been found to have higher antioxidant action in comparison with tocopherol. In addition, Gamma oryzanol comprises of ferulic acid esters of sterols and triterpene alcohols. The ferulic acid esters are campesterol, stigmasterol and  $\beta$ -cytosterol and the triterpene alcohols are cycloartenol, cycloartanol, 24-methylenecycloartanol and cyclobranol (Bucci *et al.*, 2002; Piironen *et al.*, 2000).

Due to its antioxidant action, it is drawing immense interest in research world as a food additive. It has been cited as 'oxidation inhibitor' in the 'food additive list' (Bucci *et al.*, 2003).

## MATERIALS AND METHODS

**Chemicals:** Potassium dichromate ( $K_2Cr_2O_7$ ), Fluka. Sigma-Aldrich (St. Louis, MO, USA) obtained from Al-Ggomhoria pharmaceutical company. Rice bran oil Thailand origin obtained from market.

**Animals:** Thirty mature female albino rats weighing from 120-140 g were obtained from El Nile Pharmaceutical Company (Cairo, Egypt). Rats were allowed one week acclimatization period at the

animal facility of the Faculty of Medicine, Al-Azhar University (Cairo, Egypt). Animals were kept in polypropylene cages under standard conditions prescribed by the committee for the purpose of control and supervision on experiments on animals. Animals were housed in about constant temperature (22°C) and relative humidity (40-60%) vivarium on a light-dark (12 h 12 h<sup>-1</sup>) cycle. Water and food were provided ad libitum.

**Grouping of animals:** Female albino rats were divided randomly into 3 groups, 1st group (group C) as control group which were not given dichromate nor rice bran oil, 2nd group (group E) received potassium dichromate dissolved in 5% DMSO solution (20 mg kg<sup>-1</sup>, I/P, for 5 months), while the 3rd group (group B) received both potassium dichromate dissolved in 5% DMSO solution (20 mg kg<sup>-1</sup>, I/P) and rice bran oil 20 mg kg<sup>-1</sup> orally, with 1 hr in between for 5 months. Blood samples were collected from retro-orbital venous sinus in lithium heparin tubes every 30 days.

**Assay:** Sexual hormones FSH, LH, estradiol, progesterone and testosterone were determined by ELISA technique. The results had been processed by ANOVA method and student test. All assays on animals were conducted in accordance with present laws regarding animal welfare and ethics in animal experiments (Petrovici *et al.*, 2010).

## RESULTS

As it is shown in Table 1, in control group (C), FSH serum level was in physiological limits, while group (E) exposed to potassium dichromate showed significantly gradual increase of serum FSH. Group B which was given potassium dichromate and rice bran oil showed significant increase in FSH level in 1st month, which returned back gradually to physiological level starting from 2nd month to reach almost normal physiological level from 4th month.

Table 2 showed serum LH levels in physiological limits in control group, but in group E serum LH levels were markedly increased. Group (B) exposed to potassium dichromate and rice bran oil showed an increase in serum LH in 1st month which returned back to physiological level from 3rd month onwards.

Serum estradiol level was in normal limits in control group, (Table 3), while it showed significant decrease in Group (E). In group (B), levels of estradiol showed significant decrease in first month then returned to physiological level from 3rd month until 5th month.

As it is shown in Table 4, serum progesterone levels which were in physiological limits in group (C) and showed a significant decrease in dichromate exposed group (E). Group (B) exposed to potassium dichromate and rice bran oil showed significant decrease in first month which returned back gradually to physiological level starting from 2nd-5th month.

Table 1: Serum levels of FSH in female albino rats of different study groups

Period (month)	Group C (ng mL <sup>-1</sup> )		
	C	E	B
1st	2.99±0.01	8.86±0.06	6.46±0.21
2nd	2.85±0.01	10.85±0.06	4.05±0.01
3rd	2.95±0.01	15.81±0.07	3.45±0.01
4th	2.98±0.01	18.74±0.05	2.95±0.01
5th	2.99±0.01	15.93±0.02	2.70±0.01

Data was represented as Mean±SD

Table 2: Serum levels of LH in female albino rats of different study groups

Period (month)	Group C (ng mL <sup>-1</sup> )		
	C	E	B
1st	4.43±0.07	9.15±0.32	7.43±0.07
2nd	4.48±0.05	13.37±0.01	5.03±0.05
3rd	4.45±0.05	12.27±0.02	4.83±0.07
4th	4.43±0.07	13.13±0.05	4.76±0.06
5th	4.46±0.07	10.15±0.02	4.34±0.07

Data was represented as Mean±SD

Table 3: Serum levels of estradiol in female albino rats of different study groups

Period (month)	Group C (ng mL <sup>-1</sup> )		
	C	E	B
1st	48.31±0.03	20.03±0.03	29.32±0.05
2nd	47.43±0.03	18.23±0.06	39.22±0.06
3rd	48.52±0.04	17.62±0.05	45.12±0.04
4th	46.51±0.05	17.72±0.03	46.31±0.05
5th	49.21±0.03	25.32±0.02	48.32±0.07

Data was represented as Mean±SD

Table 4: Serum levels of progesterone in female albino rats of different study groups

Period (month)	Group C (ng mL <sup>-1</sup> )		
	C	E	B
1st	28.61±0.05	18.51±0.03	20.65±0.04
2nd	29.01±0.06	12.41±0.05	25.61±0.07
3rd	28.31±0.04	10.65±0.05	28.21±0.05
4th	28.65±0.05	10.64±0.05	28.66±0.07
5th	28.91±0.07	12.31±0.04	28.71±0.06

Data was represented as Mean±SD

Table 5: Serum levels of testosterone in female rats of different study groups

Period (month)	Group C (ng mL <sup>-1</sup> )		
	C	E	B
1st	87.3±4.95	157.2±4.45	127.2±5.42
2nd	98.3±5.15	287.3±3.05	100.2±2.35
3rd	78.3±3.78	307.6±4.93	95.2±4.25
4th	86.3±8.54	337.8±6.25	94.2±3.41
5th	98.3±4.88	287.3±4.14	96.2±5.45

Data was represented as Mean±SD

Serum testosterone were in normal levels in control group (group C), as shown in Table 5, while Cr exposed group (E) showed significant increase starting from 2nd month till end of study period. Group B exposed to potassium dichromate and rice bran oil showed significant increase in testosterone level in first month, followed by a decrease in 2nd month then returned back to almost physiological level from 3rd month till 5th month.

The level of serum sex hormones is known to be very useful in assessing the reproductive integrity in both animal and human subjects (Uboh *et al.*, 2007).

Generally, significant decrease in serum sex hormones level is associated with suppressed reproductive functions and exposure to several chemical agents have been reported to cause reproductive dysfunctions (Yarube *et al.*, 2009).

Chromium accumulates in the pituitary gland (Uboh *et al.*, 2005), considerably reduces cell activity, determines apoptosis (Uboh *et al.*, 2005), all irreversible effects (Uboh *et al.*, 2005).

Cr (VI) toxicity could be a potential risk to the reproductive system in developing females, it leads to decreased steroidogenesis, increased FSH and did not alter LH, cause delayed puberty, decreased follicle number and extended estrous cycle (Banu *et al.*, 2008).

Estradiol and progesterone, in normal non-pregnant and non-ovulating subjects, are produced primarily in the gonads under the influence of the pituitary FSH and LH.

This study reveals significant increase of FSH serum level and serum LH, comparative to findings in control group and significantly decreased the serum progesterone and estradiol levels in the Cr exposed female albino rats which may be due to distorted ovarian integrity. The abnormal levels of sex hormones recorded in this study also suggest a disruption of steroidogenic function in female rats exposed to potassium dichromate which is in agreement with previous findings by Ugwoke *et al.* (2004), as well as (Uboh *et al.*, 2007).

Our study is in agreement with the study of (Petrovici *et al.*, 2010) who pointed out that there were significant increase of FSH, LH and testosterone serum level directly correlated to exposure level and significant decrease of estradiol and progesterone serum level, inversely correlated with the exposure level.

The cause of serum LH and FSH level over physiological limits could also be due to the 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 (Uboh *et al.*, 2007). While other authors observed that LH remains unchanged after potassium dichromate exposure (Uboh *et al.*, 2007).

Some authors have contradictive results regarding Cr (VI) impact on progesterone level, some revealed its decrease (Uboh *et al.*, 2009) and others observed the opposite (Uboh *et al.*, 2007). The increase of serum testosterone level was in the present study concomitant with the increased level, over physiological limits, of serum LH. It is known that LH stimulates at maximum the theca androgen production. The theca cells have the enzymes for the cholesterol conversion into androgens (Banu *et al.*, 2008).

It was also found in this study that administration of rice bran oil produced an appreciable increase in the serum estradiol and progesterone levels to the range within the control level and restored the distorted integrity of the ovarian tissues to normal state. These observations therefore suggested that rice bran oil counteracted the adverse effects of potassium dichromate on the gonadal tissues in female rats.

The results obtained from this present study attempt to prove that administration of rice bran oil as supplement may be of great prophylactic and therapeutic relevance in individuals frequently exposed to potassium dichromate.

In conclusion, it is hereby documented that rice bran oil is useful in providing protection against reproductive toxicity induced by potassium dichromate in female albino rats.

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