**Effect of Turmeric Oil on Reproductive Efficiency of Adult Male Rats Exposed to Potassium Dichromate**

Z. Kh. Jeber¹, F. Kh. Tawfeek²

¹Department of Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Selangor, Malaysia.
²Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Mosul, Iraq.

**Abstract:** This study conducted to investigate the effects of Potassium Dichromate ($K_2Cr_2O_7$) and Turmeric Oil (TO) on reproductive efficiency of adult male rats. Twenty four male rats aged 100 days divided randomly into 4 groups. G1 received 1 ml of dimethyle sulfoxide (DMSO) 5% orally as control, G2 received $K_2Cr_2O_7$ (24 mg/kg orally), G3 received TO dissolve in DMSO 5% (8.5 mg/kg orally) and G4 received both $K_2Cr_2O_7$ + TO (24 mg/kg + 8.5 mg/kg orally ½ h in between). $K_2Cr_2O_7$ treatment revealed significant decrease in body weight, total sperm count, percentage of live sperm, serum Glutathione (GSH), thickness of seminiferous epithelia and number of stage VII cells (spermatagonium, spermatocytes, spermatids) accompanied with significant increase in epididymal, seminal vesicle weight and percentage of dead and abnormal sperms and serum Malondialdehyde (MDA). TO treatment show significant increase in body, testes, epididymal, prostate weight, total sperm count, percentage of live sperms, serum GSH and Testosterone concentration, diameter and thickness of seminiferous tubules and cells number in stage VII with significant decrease in percentage of abnormal and dead sperms, serum MDA, while treatment with both $K_2Cr_2O_7$ and TO show improvement in the parameters under study. In conclusion, $K_2Cr_2O_7$ has dangerous effects on reproductive efficiency which have been improved by TO.

**Keywords:** Turmeric Oil, Potassium Dichromate (VI), Adult Male Rats.

I. Introduction

Chromium is a metallic element that exists in many oxidative states. Biologically, trivalent (Cr III) and hexavalent (Cr VI) is the most important. Hexavalent chromium compounds are generally synthetic and considered more toxic than trivalent chromium (Sugiyama, 1992). It’s widely used in refractory, pigments, stainless steel factory, leather tannery, wood processing, welding and cement manufacturing. It also has been reported to have carcinogenic, cytotoxic effects both in human and laboratory animals (Stohs et al., 2001).

High levels of chromium in blood, urine and some body tissues are found in workers occupationally exposed to chromium (Kumar et al., 2005). Workers exposed to chromium in welding industry suffered from increased risk of reduced semen quality and sperm abnormalities leading to infertility (Bond, 1993). Chromate has been shown too concentrated in the testes after intra peritoneal injection led to injuries in seminiferous epithelium in mice (Saxena et al., 1990).

Many studies demonstrated that hexavalent chromium injection causing testicular atrophy, reduced sperm count and motility in adult rats (Ernst, 1990; Ernst and Bond, 1992). Chromium compounds induce oxidative stress leading to tissue damage (Stohs et al., 2001), the formation of chromium (V) intermediates from chromium (VI) produce reactive oxygen species (ROS) including superoxide anion, singlet oxygen and hydroxyl radicals (Kawanishi et al., 1986). Its toxic effects contributed to its ability to induce oxidative stress leading to enhanced production of ROS, this result in decreased cell viability, enhanced intracellular oxidized state, membrane damage and apoptotic and necrotic cell death (Bagchi et al., 2002).

Saxena et al (1990) suggested a risk to growing testes, if rats are exposed to hexavalent chromium during the pre-pubertal stages of their development, which in turn, may disturb normal testicular physiology in adulthood. A significant higher numbers of morphologically abnormal sperm were noticed in a group occupationally exposed to chromium in compare to the unexposed persons, (Kumar et al., 2005).

Reproductive toxicity of chromium (hexavalent) have shown in male monkeys (Macaca radiata) resulting in azoospermia, disruption of spermatogenesis and premature releases of spermatocyte and round spermatids from the ad luminal compartments of the testes. The spermato- toxicity of chromium (VI) was mediated by increased free radicals and was prevented through vitamin C and E supplementation to the monkeys (Arudhias et al., 2005; Subramanian et al., 2006).

Curcumin is the major constituent of turmeric powder, which is extracted from rhizomes of plant Curcuma longa. It shows a wide range of pharmacological activities, including antioxidant and anti-inflammatory effects (Jagetia, 2007). The crude extract of turmeric rhizomes contain about 70-76% curcumin.
along with about 10% demethoxycurcumin and 8% bisdemethoxycurcumin (Arora et al., 1971). Curcumin has been used in Asian medicine for its medical properties (Ammon and wahl, 1991), it has been reported that administration of curcumin can ameliorate ischemia reperfusion injury in myocardium (Nirmal and Puvanakrishnan, 1996), liver (Shen et al., 2007), kidney (Shoskes et al., 2005), brain (Ghoneim, 2002) and other organ system.

Curcumin exhibited protective effects against oxidative damage and it’s considered as a potent cancer chemopreventive agent (Duvoix et al., 2005). Water-and fat-soluble extracts of turmeric and its curcumin compound exhibit strong antioxidant activity, comparable to vitamins C and E (Toda et al., 1985). It represented a class of anti-inflammatory, antioxidants and to be a potent inhibitor to ROS formation (Biswas et al., 2005; Venkatesan et al., 2000).

Many researches on curcumin have demonstrated a wide spectrum therapeutic effects such as anti-inflammatory (Arora et al., 1971), antibacterial (Nigi et al., 1999), antiviral (Bourne et al., 1999), antifungal (Apisaryakul et al., 1995), antitumor (Kawamori et al., 1999), antispasmodic (Itthipanichpong et al., 2003) and hepatoprotective (Park et al., 2000). Moreover its potential utility in autoimmune deficiency syndrome (AIDS) has been demonstrated (James, 1993; Mazumder et al., 1996).

The objective of this study is to assess the damages caused by K$_2$Cr$_2$O$_7$ (VI) represented by reactive oxygen species (ROS) on reproductive efficiency, and the antioxidant ability of turmeric oil (TO) to overcome the damages caused by K$_2$Cr$_2$O$_7$ (VI) in adult male rats.

II. Materials and Methods

Chemicals

Potassium dichromate Hexavalent (K$_2$Cr$_2$O$_7$), Fluka. AG. Buches SG, Switzerland. Turmucil Oil (TO), used in this study was extracted from turmeric rhizomes by absolute ethanol and used after purification via silica gel column.

Animals

Twenty four adult male albino rats aged 100 day divided randomly into four groups, each contain 6 rats. Animals in 1$^{st}$ group received 1 ml of dimethyle sulfoxide (DMSO) 5% solution orally for 60 day as negative control. The 2$^{nd}$ group received K$_2$Cr$_2$O$_7$ (24 mg/kg orally for 60 day) dissolved in distil water. The 3$^{rd}$ group received TO dissolve in DMSO 5% (8.5 mg/kg orally for 60 day). The 4$^{th}$ group received both K$_2$Cr$_2$O$_7$, TO (24 mg/kg + 8.5 mg/kg orally for 60 day $\frac{1}{2}$ hr in between).

Body and organs weights

The animals were weighed just before scarifies to obtain the organs. Testis, epididymis, prostate and seminal vesicle were cut off from body, cleaned from fat tissue and then weighed.

Total sperm count

The total sperm count estimated as described by Karmosh (2002).

Percentage of life/ dead and sperm abnormalities

The percentage calculated as described by Hemavathi and Rahiman (1993).

Serum glutathione (GSH) and malondialdehyde (MDA) concentration

Glutathione was estimated as described by Burtis and Ashwood (1999) by using Ellman’s reagent. Malondialdehyde was estimated as described by Buge and Aust (1978); Wgsock et al (1995) depending on the reaction between the MDA and TBA (thiobarbituric acid) in acidic medium.

Serum Testosterone level

Testosterone level estimated by using an Elisa kits provided from Biocheck, Inc. 323 vintage park, Dr. Foster, city, CA 94404.

Histological examination of testes

The testes were removed from the animals, immersed in Bouin’s fixative for 10 minute, then immersed in neutral buffered formalin solution. Tissues were dehydrated, embedded in paraffin, sectioned (4 µm) and stained with Hematoxylin and Eosine. Other sections were stained with Periodic Acid Schiff (PAS) to study spermatogenesis by counting the relative number of each variety of germ cells at stage VII of the seminiferous cycle (spermatogonium, spermatocyte and spermatid) as well as the diameter and thickness of seminiferous tubules by using Visopan apparatus, in 30 seminiferous tubules for each group of study (Tawfeek, 1997).
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Statistical analysis

All data are expressed as mean ± standard error. Statistical evaluation was conducted by One Way Analysis of Variance, Duncan Multiple Range Test and Fisher Freeman Halton Test. A p <0.05 was used as the criterion for statistical significance. All analysis has done by SPSS (version 10.5) StatXact-3 programs.

III. Results and Discussion

There is an auto-balance between free radicals production of body and the defense ability to eliminate these radicals in the normal situation, oxidative stress occur when the level of free radicals exceeds the antioxidant ability of the cell. Free radicals are very active, unstable and attach to lipids, to protein and to DNA leading to cell damage.

K2Cr2O7 treatment shows significant decrease in body weight (table, 1) this result comes similar with earlier observation of Chandra et al (2007), Gilbert et al (1998) both reported decreased rat body weight treated with K2Cr2O7. However, K2Cr2O7 treatment show significant increase in epididymus and seminal vesicle weights (table, 1), these results come with agreement of Pandey and Singh (2002) findings, they suggested that increase in accessory sex organs weight due to the relaxation of smooth muscles surrounding these glands leading to losing its ability to emptying its contents and increasing their weights.

K2Cr2O7 also caused a significant increase in the percentage of abnormal sperm and MDA level (table, 1). Addition K2Cr2O7 to mice feed at different doses for different periods leading to significant increase in the percentage of abnormal sperm (Zahid et al., 1990; Trivedi et al., 1989). Increasing MDA level in kidney tissue of rats treated with K2Cr2O7 reported by Fatima and Mahmood (2007). Moreover, K2Cr2O7 treatment show a significant decrease in total sperm count and the percentage of life sperm (table, 1) these results in agreements with Bonde (2002) and Zahid et al (1990) who demonstrated a significant decrease in total sperm count in mice treated with K2Cr2O7 and the percentage of life sperm in workers occupationally exposed to K2Cr2O7 respectively.

Decreased total sperm count caused by K2Cr2O7 treatment may results from increased production of ROS which damage the seminiferous tubules cells (mostly sertoli cells) which responsible for sperm maturation, leading to impairment in spermatogenesis (Hipler et al., 2000). Sikka (1996) mentioned that sperm membrane is rich in polyunsaturated fatty acids, which is very sensitive to ROS causing lipid peroxidation and decrease in sperm motility.

In addition, K2Cr2O7 group show significant decrease in GSH level (table, 1) which in agreement with Eybl et al (2006), who reported that mice treated with heavy metal (cadmium, mercury, lead) show significant decrease in liver GSH level due to decrease antioxidant enzymes (catalase, glutathione peroxidase) and this supported by Wohabe and Godin (1987) speculation that oxidative stress in animal causing oxidative catabolic effects leading to elevated lipid peroxidation in tissues accompanied with depletion tissue GSH.

K2Cr2O7 also cause significant decrease in thickness of seminiferous tubules epithelia (table, 1) this is compatible with Wei et al (2008) findings that decreased number of germinal epithelia layers of seminiferous tubules in rats exposed to testicular torsion. This study found a significant decrease in stage VII cell numbers in K2Cr2O7 treated group (table, 2) which seems in agreement with Jana et al (2006) who found that sodium arsenate treatment result in a decrease in stage VII cell numbers in rats. The changes in diameter and thickness in seminiferous tubules induced by K2Cr2O7 it believed be due to the degenerative changes and accumulation of sloughed cells in tubule lumen.

TO treatment show significant decrease in body weight (table, 1) which is similar to the findings of NTP (1996) who recorded decrease in body weight in male and female, rats and mice, normal and treated with curcumin for different periods relative to control. However, TO show improved body, testes, epididymal and prostate weights compared to K2Cr2O7.

Treatment with K2Cr2O7 and TO together (1/2 hr in between) show significant increase in testes weight, which concedes with Sharma (1976) who reports improvement in body and organs weights; this explained as result of curcumin antioxidant activity which maintain normal serum testosterone level leading to increase body and organs weights.

Nevertheless, K2Cr2O7 and TO treatment show significant increase in total sperm count, percentage of life sperm, diameter and thickness of seminiferous tubules, number of cells at stage VII and decrease in percentage of abnormal sperm, which might be due to anti-oxidative activity of TO and its prevention of damage induced by K2Cr2O7 (Chandra et al., 2007.; Li et al., 2001.; Sharma, 1976). Ashry et al (2003) reported an increase in GSH level in liver, kidney and brain of the mice treated with turmeric powder, which in concede with this study findings of the significant increase in serum GSH level and significant decrease in serum MDA level. Moreover, Wei et al (2008) reported decrease in MDA level in rats suffered from testicular torsion and then treated with curcumin.
Table 1: Effect of K₂Cr₂O₇ and TO on body, testis, epididymis, prostate, seminal vesicle weights and total sperm count, % of life/dead sperm, % of abnormal sperm, serum GSH, MDA and testosterone level, diameter and thickness of seminiferous tubules in adult male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>K₂Cr₂O₇</th>
<th>TO</th>
<th>K₂Cr₂O₇ + TO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>356.3±16.97 a</td>
<td>270±10.07 c</td>
<td>301.36±11.31 b</td>
<td>265.67±9.69 c</td>
</tr>
<tr>
<td>Testes weight (mg/100g bw)</td>
<td>0.76±0.04 b</td>
<td>0.69±0.17 b</td>
<td>0.95±0.04 a</td>
<td>0.96±0.02 a</td>
</tr>
<tr>
<td>Epididymus weight (mg/100g bw)</td>
<td>0.26±0.02 b</td>
<td>0.32±0.01 a</td>
<td>0.32±0.01 a</td>
<td>0.31±0.02 a</td>
</tr>
<tr>
<td>Prostate weight (mg/100g bw)</td>
<td>0.16±0.02 ab</td>
<td>0.15±0.01 b</td>
<td>0.18±0.01 a</td>
<td>0.17±0.01 ab</td>
</tr>
<tr>
<td>Seminal vesicle weight (mg/100g bw)</td>
<td>0.36±0.03 b</td>
<td>0.43±0.01 a</td>
<td>0.37±0.01 b</td>
<td>0.38±0.01 ab</td>
</tr>
<tr>
<td>Total sperm count ×10⁶</td>
<td>25.33±2.12 a</td>
<td>10.15±1.97 b</td>
<td>29.5±2.87 a</td>
<td>26.5±1.28 a</td>
</tr>
<tr>
<td>% life sperm</td>
<td>99.5±0.5 a</td>
<td>86.5±0.62 b</td>
<td>100±0 a</td>
<td>98.83±0.31 a</td>
</tr>
<tr>
<td>% dead sperm</td>
<td>0.5±0.5 b</td>
<td>13.5±0.62 a</td>
<td>0.0±0.0 b</td>
<td>1.17±0.31 b</td>
</tr>
<tr>
<td>% abnormal sperm</td>
<td>2.33±0.71 b</td>
<td>9.67±0.67 a</td>
<td>0.67±0.21 a</td>
<td>1.67±0.56 b</td>
</tr>
<tr>
<td>GSH (µMole/liter)</td>
<td>2.02±0.19 b</td>
<td>0.82±0.03 d</td>
<td>5.75±0.21 a</td>
<td>1.32±0.12 c</td>
</tr>
<tr>
<td>MDA (µ Mole/liter)</td>
<td>0.57±0.03 b</td>
<td>1.09±0.1 a</td>
<td>0.21±0.01 c</td>
<td>0.31±0.01 c</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>2.27±0.23 b</td>
<td>3.18±0.76 ab</td>
<td>3.62±1.29 a</td>
<td>3.42±0.69 a</td>
</tr>
<tr>
<td>Seminiferous tubules diameter (µm)</td>
<td>252.46±8.96 bc</td>
<td>246.94±11.78 c</td>
<td>261.88±5.03 ab</td>
<td>272.03±14.4 a</td>
</tr>
<tr>
<td>Thickness of seminiferous tubules</td>
<td>64.0±4.05 b</td>
<td>47.73±2.13 c</td>
<td>70.8±3.6 a</td>
<td>73.87±5.63 a</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± Standard error, P<0.05

Table 2: Effect of K₂Cr₂O₇ and TO on stage VII cell numbers in adult male rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>K₂Cr₂O₇</th>
<th>TO</th>
<th>K₂Cr₂O₇ + TO</th>
</tr>
</thead>
<tbody>
<tr>
<td>spermatogonium</td>
<td>11.0±1.71 a</td>
<td>4.47±1.18 c</td>
<td>7.8±1.01 b</td>
<td>7.2±0.82 b</td>
</tr>
<tr>
<td>spermatocyte</td>
<td>24.17±1.93 a</td>
<td>10.4±2.07 b</td>
<td>23.57±3.82 a</td>
<td>25.43±1.32 a</td>
</tr>
<tr>
<td>spermatid</td>
<td>57.4±1.98 a</td>
<td>30.7±1.03 c</td>
<td>47.27±3.11 b</td>
<td>48.67±1.79 b</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± Standard error, P<0.05
Histological photographs of Eosin and Nigrosin (a, b, c, d, e) H&E (f, g, h, i, j) stained male rat testes.

A) Sperm (660 X) from control animals, showing normal rat sperm.
B) Sperm (660 X) from K2Cr2O7 treated animals, showing abnormal (No head) rat sperm.
C) Sperm (540 X) from K2Cr2O7 treated animals, showing abnormal (short tail) rat sperm.
D) Sperm (540 X) from TO treated animals, showing abnormal (defected head) rat sperm.
E) Sperm (660 X) from both K2Cr2O7 and TO treated animals, showing abnormal (defected head) rat sperm.
F) Testes (90 X) from control animals, showing normal testes architecture, a) Normal seminiferous tubules containing sperms. b) Leydig cells.
G) Testes (370 X) from K2Cr2O7 treated animals, show a) Sever changes in seminiferous tubules, including sertoli cells and germinal epithelium degeneration. b) Interstitial edema.
H) Testes (370 X) from K2Cr2O7 treated animals, show a) Irregular, sloughed sertoli cells and germinal epithelium in the lumen of the seminiferous tubules.
I) Testes (90 X) from TO treated animals, show a) Normal testes histoarchitecture, regular seminiferous tubules containing sertoli, germinal epithelium. b) Leydig cells in the interstitial space. c) Interstitial edema. d) Congested blood vessel.
J) Testes (90 X) from both K2Cr2O7 and TO treated animals, show a) Improvement in testes histoarchitecture with regular seminiferous tubules, sertoli cells and germinal epithelium. b) Interstitial edema. Congested blood vessels.

IV. Conclusion

Current study has tested the hypothesis that potassium dichromate (VI) can cause dangerous effects on fertility and the possibility of developing sterility leading to impairment of reproduction by proving K2Cr2O7 (VI) as harmful material to biological systems and capable of producing ROS which is highly active free radicals having the ability to attach to lipid, proteins and DNA molecules causing severe damage manifested as ailments and diseases. Turmeric has been used for thousands of years in Ayurvedic Medicine for its medicinal properties which have been proved by extensive research in all fields of medicine. Therefore, turmeric has been
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used in this study for its antioxidant activities which positively overcome and correct some harm effects produced by potassium dichromate.

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