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Effect of Turmeric Oil in Reproductive Efficiency of Immature Female Rats Exposed to Oxidative Stress Induced by Potassium Dichromate

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Abstract: *This study carried out to understand the effects of Potassium Dichromate ($K_2Cr_2O_7$) and Turmeric Oil (TO) on reproductive efficiency. Twenty four weanling immature female albino rats aged 22 day divided randomly into 4 groups (6 rats in each group). 1st group received 0.1 ml dimethyle sulfoxide (DMSO) 5% solution as a control (I/P for 14 day), 2nd group received $K_2Cr_2O_7$ dissolved in distilled water (0.4 mg/kg, I/P, for 14 day), 3rd group received TO dissolved in 5% DMSO solution (20 mg/kg, I/P, for 14 day), 4th group received both $K_2Cr_2O_7$ +TO (0.4 mg/kg, I/P +20 mg/kg, I/P, ½ h in between, for 14 day). Rats treated with $K_2Cr_2O_7$ show significant increase in Malondialdehyde (MDA) level and significant decreases in uterine and ovarian weights, serum Glutathione (GSH) level, percentage of vaginal opening time and diameter of ovarian follicles in categories (101-200), (201-300) and (>400), while those treated with TO show significant increase in uterine and ovarian weights, percentage of vaginal opening time, serum GSH, and diameter of ovarian follicles in categories (1-100) and (>400), with significant decrease in MDA level, moreover treatment with $K_2Cr_2O_7$ and TO together show significant increase in uterine and ovarian weights, percentage of vaginal time opening, serum GHS level and diameter of ovarian follicles in categories (201-300) and (>400), with significant decrease in MDA level. In conclusion, $K_2Cr_2O_7$ has bad effects on reproductive efficiency of female rats and TO have improvement on some of these effects.*

Key word: *Turmeric oil, Potassium dichromate (VI), ovarian follicles, Immature rats.*

I. Introduction

Chromium (Cr) exist in a series of oxidation states from $^{-}2$ to $^{+}6$ valences, the most important stable states are elemental metal (0), trivalent (Cr III) and hexavalent (Cr VI) compounds (Barceloux, 1999; Shi *et al*., 2004; Zhitkovich, 2005; Valko *et al*., 2006). Cr (VI) is commonly used in multi-industrial processes, asbestos, cement, as well as in tobacco and food additives (Nriagu, 1988). Cr (VI) causes dermatitis, skin, lung and throat cancers, infertility, increased incidences of birth and developmental defects among children living around tanneries, leather and chrome industries (Blacksmith institute, 2007).

Women working in chrome industries and living around chromium contaminated areas experience abnormal menses (Makarov and Shimtova, 1978), postnatal hemorrhage and birth complications accompanied with high level of chromium in blood and urine (Shimtova, 1978. 1980). Cr (VI) can traverse the placental barrier in rodents (Tipton and Shafer, 1964; Barceloux, 1999), and within the pregnant uterus Cr (VI) alters early development of blastocyst (Jacquet and Draye, 1982), decreases the number of implantation sites and viable fetuses (Junaid *et al*., 1996.; Kamath *et al*., 1997; Kanojia *et al*., 1998), produces embryo-toxic and feto-toxic effects (Junaid *et al*., 1996) and increase conceptus resorption in rodents.

Chromium exposure via drinking water impairs ovarian follicular maturation and differentiation and promotes follicular atresia (Murthy *et al*., 1996), delays puberty, lengthens inter-estrous intervals and reduces numbers of ovulations in rodents (Kanojia *et al*., 1998). Chromium compounds are toxic, carcinogenic and mutagenic in humans and animals (Langard, 1990).

The toxic effects of chromium are attributed to its ability to induce oxidative stress, leading to enhanced production of reactive oxygen species (ROS), this results in a decreased cell viability, enhanced intracellular oxidized states, membrane and tissue damage, apoptotic and necrotic cell death (Bagchi *et al*., 2002).

Kawanishi et al, (1986) demonstrated that ROS including superoxide anion, singlet oxygen and hydroxyl radicals are produced by chromium (VI) during the formation of chromium (V) intermediates in cells.
Turmeric, *Curcuma longa* L. (Zingiberaceae family) rhizomes, has been widely used for centuries in indigenous medicine for the treatment of a variety of inflammatory conditions and other diseases (Ammon and Wahl, 1991). Turmeric extract consist of three different curcuminoids: Curcumin, demethoxycurcumin, and

bisdemethoxycurcumin. Curcumin (diferuloylmethane) is the most active component of turmeric, it believed that curcumin is a potent antioxidant and anti-inflammatory agent (Aggarwal *et al*., 2003).

Curcumin has been shown to possess wide range of pharmacological activities including anti-inflammatory (Srimal and Dhawan, 1973.; Satoskar *et al*., 1986), anticancer (Kuttan *et al*., 1985), antioxidant (Sharma, 1976; Toda *et al*., 1985) and antimicrobial (Negi *et al* ., 1999). Curcumin exhibits strong antioxidant activity, comparable to vitamin C and E (Toda *et al* ., 1985) and its proven anti-inflammatory and antioxidant properties has been shown to be a potent scavenger of a variety of ROS including superoxide anion radicals, hydroxyl radicals (Reddy and Lokesh, 1994) and nitrogen dioxide radicals (Unnikrishnan and Rao, 1995; Sreejayan and Rao, 1997).

It's also capable to inhibit lipid peroxidation in different animal's models (Reddy and Lokesh, 1992; Sreejayan and Rao, 1994).

The objectives of this study is to evaluate the effects of potassium dichromat (VI), an oxidizing inorganic chemical, and turmeric Oil, an antioxidant on female rats reproductive efficiency.

II. Materials And Methods

Chemicals

Potassium dichromate hexavalent ($K_2Cr_2O_7$), Fluka. AG. Buches SG. Switzerland. Turmuric Oil (TO), used in this study is extracted from turmeric rhizomes by absolute ethanol and used after purification via silica gel column. Ultraviolet and infrared scopes have done on the oil to ensure the presence of the curcuminoids.

Animals

Twenty four weanling immature female albino rats aged 22 day old divided randomly into 4 groups. Animal in 1st group received 0.1 ml (I/P for 14 day) dimethyle sulfoxide (DMSO) 5% solution as a control, the 2nd group received $K_2Cr_2O_7$ dissolved in distilled water (0.4 mg/kg, I/P, for 14 day), the 3rd group received TO dissolved in 5% DMSO solution (20 mg/kg, I/P, for 14 day), the 4th group received both $K_2Cr_2O_7$ +TO (0.4 mg/kg, I/P +20 mg/kg, I/P, ½ hr in between, for 14 day).

Body and organs weight

The animals were weighed just before sacrifices them to obtain the organs. The uterus and ovaries removed and stripped from oviduct and fat tissue, then weighed.

Vaginal opening

Vaginal opening was observed at the day of animal sacrifices.

Serum glutathione (GSH) and Malondialdehyde (MDA) concentration

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

Serum Follicles stimulating hormone (FSH) and Lutenizing hormone (LH) levels

The levels of FSH, LH estimated by using an Elisa kits provided from Monobind Inc. (Monobind Inc. Lake Forest, CA 92639, USA).

Histological examination of ovaries

To estimate the diameters of ovarian follicles, ovaries were removed from the animals and immersed in Bouin's fixative. Tissues were dehydrated, embedded in paraffin, serial sectioned (4μm) and stained with Hematoxylin and Eosin, the diameter of Oocyte containing follicles at each developmental stage were counted in every 12 section (Tawfeek, 1992).

Then the diameters of the follicles were categorized in 5 categories to facilitate the analysis of data (1-100, 101-200, 201-300, 301-400, >400 μm).

Data analysis

All data were expressed as mean ± standard error. Statistical analyses were conducted using One Way Analysis of Variance, Duncan Multiple Range Test and Fisher Freeman Halton Test. Analysis was done by SPSS (version 10.5) StatX act-3 programs.

III. Results

Generally low dose of potassium dichromate show harmful effects in treated rats particularly in reproductive performance which may lead to infertility, sterility or even cancer on high doses, in contrary turmeric oil show obvious improvement in overcoming the bad effects that have done by potassium dichromate which gives a clue that turmeric has the potential to be one of the medicinal herbs that can be used as "herb of choice" to deal with such cases of reproductive hazardous substances.

Potassium dichromate treatment causes a significant negative changes ($P<0.05$) in uterine and ovarian weights, serum GSH level (table. 1), percentage of vaginal time opening (table. 2) and diameter of ovarian follicles in categories (101-200) and (>400), (table. 3), with significant increase in serum MDA level (table. 1). Turmeric treatment and turmeric with potassium dichromate treatment show a significant increase ($P<0.05$) in uterine and ovarian weights, serum glutathione level (table. 1), percentage of vaginal time opening (table. 2) and diameter of ovarian follicles in the categories (1-100), (201-300) and (>400) (table. 3) with significant reduce ($P<0.05$) in serum MDA level (table. 1).

Table (1). Effects of potassium dichromat, turmeric oil and both on the measured parameters.

	Groups			
Parameters	Control	$K_2Cr_2O_7$	TO	$K_2Cr_2O_7$ + TO
Body weight (g)	48.08±6.13	50.94±1.4	50.02±4.7	50.76±1.52
Uterus weight (mg/100g bw)	2.37±0.19	1±0.07	4.87±0.35	3.74±0.21
Ovaries weight (mg/100g bw)	0.48±0.03	0.03±0.01	0.95±0.01	0.43±0.03
GSH µMole/Liter	0.06±0	0.03±0	0.07±0.01	0.04±0
MDA µMole/Liter	0.04±0.01	0.07±o.o1	0.02±0	0.03±0
FSH m IU/ml	1.02±0.04	1.02±0.04	1.00±0.06	1.02±0.04
LH m IU/ml	1.38±0.48	0.98±o.o4	1.02±0.15	1.40±0.7

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

Table (2). Effects of potassium dichromat, turmeric oil and both on the vaginal time opening.

	Groups							
Parameters	Control		$K_2Cr_2O_7$		TO		$K_2Cr_2O_7$ + TO	
	Exist	%	Exist	%	Exist	%	Exist	%
Exist	6	100	0	0.0	6	100	1	16.7
Not Exist	0	0.0	6	100	0	0.0	5	83.3
Total Number	6	100	6	100	6	100	6	100

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

Table (3). Effects of potassium dichromat, turmeric oil and both on ovarian follicle size.

	Groups			
Categories	Control	$K_2Cr_2O_7$	TO	$K_2Cr_2O_7$ + TO
1-100	1.83±0.47	2.16±o.30	3.33±o.21	2.50±0.50
101-200	9.33±1.89	5.16±0.87	5.33±0.91	5.83±0.16
201-300	6.83±0.60	5.50±0.50	4.00±0.57	6.50±0.56
301-400	5.66±0.76	5.16±0.40	5.16±0.79	4.00±0.63
>400	6.66±0.95	2.16±0.30	5.33±0.49	3.66±0.55

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

IV. Discussion

Banu *et al* (2008) stated "many environmental toxicants gain access to the ovary via the circulation. The risk of damage of the ovarian follicle cell population from the toxicants depends mainly on the accessibility of toxicants to ovarian follicles".

Elbetieha and AL-hamood (1997) found decrease in uterine weight and lipid peroxidation of female mice treated with chromium trichloride. Decreased sulfa-hydryle groups is an indicator of increasing oxidative stress in rat kidneys treated with potassium dichromate (Fatima and Mahmood, 2007), these findings similar with this study observation in potassium dichromate treatment group which show significant decreases in uterine and ovarian weights, serum GSH concentration, with significant increase in serum MDA concentration.

Banu *et al* (2008) found decrease in the percentage of vaginal opening time in female rats treated with potassium dichromate as an index of delay onset of puberty in female rats and decreased in number of primordial, primary and secondary follicles with no observation of antral follicles in addition trans-lactational rats exposed to potassium dichromate (VI) show reduce in the number of ovarian follicles, steroidogenic ability, delay puberty and increased the intervals between estrous cycles, which in agreement with this study findings of reducing the percentage of vaginal opening time and diameter of ovarian follicles in categories (101-200) and (>400).

Turmeric oil treatment and both potassium dichromate and turmeric oil show significant increase in the GSH concentration which may explain the increased uterine and ovarian weight and the percentage of vaginal time opening as an index for puberty; the increase in ovarian follicles diameter in category (>400) may explain the increase in the ovaries weight.

Banu *et al* (2008) show that rats treated by potassium dichromate with vitamin C in lactation period show opened vagina in normal time of puberty and it show the role of vitamin C in protecting ovaries and granulosea cells from oxidative stress induced by potassium dichromate and then rats can reach puberty in proper time and show regular estrous as rats in the control group.

The observed increase in uterine weight could be a result of elevated level of estrogen hormone which has a major role in increasing the thickness of endomaterium by increased number and height of its cells, in addition, increase in the number of straight, tubular glands of endomaterium and increasing growth, blood flow and water content (Muhialdine *et al*., 1990).

V. Conclusion

Environmental pollutants such as potassium dichromate can affect the fertility in different ways both directly and indirect which may cause temporal or permanent damage. Medicinal plants are well known for their therapeutic roles to overcome of many aliments, metabolic disorders, diseases and systemic conditions such as reproductive performance. Turmeric has been used for centuries in folk medicine and ayruvedic medicine. This study proved the negative impact of potassium dichromate on reproduction in particular and the positive overcome of these detrimental effects by using turmeric oil as antioxidant to correct the deviation in reproduction organs function as well as protects them from oxidative stress in general.

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