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Teratogenicity of Hexavalent Chromium in Rats and the Beneficial Role of Ginseng

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Chromium in traces is known to be essential for growth. In higher dietary concentrations it becomes very toxic to human beings and animals. The chief source of environmental pollution with chromium arises from human activities. It is commonly utilized in many fields of modern industries as leather tanning, metal finishing and manufacturing of cement, textiles, ceramics and pigments. Chromium is emitted into the environment in high dangerous concentrations through the discharged effluents of these industries. Highly levels of chromium salts have been reported in agricultural and animal products near to chromate industry (WHO 1988). In our country, Saad, (1992) found that the effluents obtained from the Egyptian Company for Leather Tanning (EL-Basateen, Cairo) are heavily contaminated with chromium. The routine treatment carried out by the company didn't completely remove these salts from its effluents. Plants absorb chromium from the soil as well as from the air (Khasim et al 1989). Plants grown in the vicinity of chromium emitting industries or those fertilized or irrigated by sewage sludge are exposed to substantial amounts of chromium. These plants ultimately reach the animals through its food chain. Hexavalent chromium is more toxic than other forms due to the high permeability through biological membranes. It enters the cell via the general anion channel protein and subsequently interacts with intracellular proteins and nucleic acids. Many studies have indicated that hexavalent chromium causes anemia and impairs liver and kidney functions (Kumar and Barthwal 1991). It enhances carcinogenesis and causes various forms of genetic damage even at low doses (Domingo 1996). Hexavalent chromium has pronounced toxic effect upon embryonic and fetal development and is responsible for certain malformations in mammalian and chick embryos (Trivedi et al 1989).

Ginseng is the active principle of *Panax ginseng*, a medicinal plant grown in South Korea. It is recently used for medical purposes in Egypt. It is known to repair the damaged tissues of kidney, liver, brain and endothelial cells of myocardium; also it reduces pulmonary damage induced by free radicals (Li et al 1990; Okamura et al 1994). Therefore the aim of this study is to estimate the teratogenic effects of hexavalent chromium in rats. It is also an attempt to evaluate the beneficial role of ginseng for minimizing this effect.

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MATERIALS AND METHODS

Healthy, three months old female white rats, Wistar strain, (170±27g, body weight) were mated with healthy mature males. Zero day of pregnancy was confirmed by the presence of sperms at the next morning in their vaginae. Pregnant females were randomly divided into four groups ten of each. They were housed individually in separate cages, provided with feed and water *ad libitum.* Females in the 1st group received drinking water contained 50ppm chromium as potassium chromate K_2CrO_4. This treatment was assigned from the 6th to the 15th day of gestation. Females in the 2nd group supplemented with the same dose of chromium in addition they were orally intubated with 20mg/ kg body weight ginseng daily throughout the gestation period. Third group was daily dosed with ginseng only, while the last group served as a control, supplemented with tap water.

Chromium dosage was selected on the basis of our preliminary study, that 100ppm caused complete pre-implantation loss and on the fact that this dose was approximately equivalent to the 1/30 of the oral LD50 for female rats (Hertel 1982). On the base of water consumption the selected dose was equivalent to 5mg/kg body weight (Harkness and Wagner 1995). Selection of ginseng dose was based on the dose of Reynolds (1991).

Signs of toxicity on pregnant females were assessed daily, while their body weights were recorded periodically every week. One day before delivery date dames were anaesthetized and sacrificed. A heparinized blood sample from each female was collected (3ml). Both ovaries from each female were examined and the number of corpora lutea was recorded. Total numbers of uterine implantation, pre and post implantation losses, resorbed, dead and live feti per litter were recorded. Dead and live feti were weighed and examined for external gross abnormalities. One third of both dead and live feti were fixed in Boun's solution for visceral examination. The others were fixed in ethanol, eviscerated and stained by Alizarin red stain for skeletal examination (Manson et al 1982). One fetus with its placenta was taken from each female for metal analysis. Total chromium contents of maternal blood, placenta and fetal tissues were determined by Atomic Absorption Spectroscopy (Perkin Elmer 2380 model). Chromium was measured following digestion of the samples in a hot HNO_3: HCl (1:3) mixture and oxidizing the clear extract by potassium permanganate (AOAC 1984). Histopathological examination was performed on placental tissue (Drury et al 1976). Data of fetotoxicity and chromium estimation were analyzed by one way ANOVA (Gad and Weil 1982).

RESULTS AND DISCUSSION

Dams of all groups were active and did not show any signs of toxicity or any notable changes in their behavior. No significant differences in food intake and water consumption were recorded among control and treated groups during the gestation period. Significant reduction in gestational weight gain was observed on the dams of the 1st and 2nd groups when compared to their respective control and

3rd groups (table 1). However this reduction was much greater in the 1st group than in the 2nd one. Reduction in body weight gain is mainly attributed to the fetal retarded growth and resorption recorded in these groups. Similar reduction in gestational weight gain has been recorded in rats (Saxena et al 1990).

Embryo and fetotoxicity data following maternal exposure to hexavalent chromium are tabulated in table (1). No significant differences in the number of corpora lutea were observed among all groups. Mothers of the 1st group had significant increases in the numbers of pre and post implantation losses. Intrauterine hemorrhage is recorded in some females (Fig 1). Complete absence of implantation in uterine horns in one dam was observed while corpora lutea were present (Fig 2). Significant decrease in fetal weight (Fig 3) as well as in the number of live feti were recorded in this group when compared with control or other treated groups. Visceral and skeletal examination of the obtained feti revealed a significant increase in the numbers of renal pelvis dilatation (Fig 4) and incomplete ossification of the skull bone (Fig 5). The incidences of fetal abnormalities were higher in the 1st group than that of the 2nd group. This finding provided a possible suggestion to the protective ability of ginseng against chromium. Kim et al (1993) and Salikhova et al (1994) stated that ginseng enhances DNA repair and has an antimutagenic property. Therefore ginseng could reduce the mutagenic and teratogenic effects of chromium resulting in lower incidence of fetal losses and malformations. There is no published data establish the direct causal role of ginseng in the etiology of such effects. However it enhances superoxide dismutase (SOD) activity and scavenges cytotoxic oxygen free radicals. Further research for clarifying these mechanisms must be conducted. Similar and other teratogenic lesions have reported in rats, mice and golden hamster due to chromium exposure during various gestational periods at various doses, orally or intravenously (Gale 1982; Trivedi et al 1989; Junaid et al 1995; 1996). Women working at a plant producing dichromate exhibited a high incidence of obstetric pathology; hemorrhages during the gestation period and impaired gestational development on offspring but no teratogenic effects were revealed (Shmitova 1980; Hemminki and Vainio 1984). The teratogenic effect of chromium may be attributed to its direct action on fetal tissue or impairment of placental physiology. In the present study, chromium induced histopathological lesions in the placenta (Figs 6&7). Moreover it passed the placental barrier and accumulated in the fetal tissues in a level high enough for direct effect on the embryonic structure to be likely (table 2). Danielsson et al (1982) stated that fetal retention of chromium is presumed to produce resorption in mice. Mutagenicity and related studies have convincingly shown that hexavalent chromium is genetically active; it causes irritation in nucleotide pools, induces single strand breaks DNA, DNA cross- links and decreases the fidelity of DNA replication. This effect is due to its oxidizing power and free radicals generation. It causes the formation of epoxyaldhde which has mutagenic potential (DeFlora et al 1990;Manning et al 1994; Kawanishi and Hiraku 1995; Sudgen and Wetterhahn 1996;Sridevi et al 1998).

Estimation of chromium content in maternal blood is used as an index for maternal bioavaliability by mothers , while its level in placenta and fetal tissue is

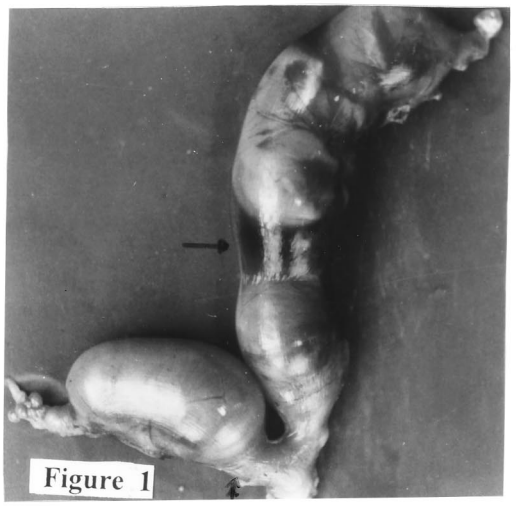
assessed to determine its ability to cross placental barrier. The present results indicated that dams exposed to chromium showed significant increase of chromium levels in their blood, placental and fetal tissues when compared with control and that group supplemented with chromium and ginseng (table2). Therefore ginseng caused a reduction in chromium concentrations. This may be due to enhanced chromium elimination through urine and feces. Reynold (1991) reported that ginseng increases the levels of glutathion, it has diuretic effect and produces light diarrhea in the morning. Wiegand et al (1984) stated that glutathion reduces the hexavalent form to a less toxic, less absorbable trivalent form. In both the 1st and 2nd groups, placental tissue had higher chromium content than in fetal tissue. However, the ratio of chromium content between fetal and placental tissue is significantly higher in the 1st group than that of the 2nd group. We could suggest that ginseng repairs or protects placental tissue, hence it restricts the passage of chromium to the fetus. This suggestion is confirmed by the histopathological lesions recorded in the chromium and ginseng group which was very mild. Similar increase in chromium blood, placenta and fetal tissues have been reported in rats, mice, hamster and pregnant women (Shmitova 1980; Danielsson et al 1982; Saxena et al 1990).

In conclusion, the use of ginseng in cases of suspected chromium toxicity or in individuals live in areas have high environmental chromium level is recommended.

Table 1. Mean values, ± S.E of embryo and feto-abnormalities in control and treated groups.

Groups / Parameters	Control	gp.I chromium 50ppm	gp.II chromium +ginseng	gp. III ginseng 20mg/kg
Gestation weight gain / mother (g)	23.6 ± 1.3^{a}	14.2 ± 1.7^{b}	23.5 ± 1.8^{c}	33.8 ± 1.8^{a}
No. of corpora lutea/litter	7 ± 0.49^{a}	7.1 ± 0.48^{a}	7 ± 0.36^{a}	6.8 ± 0.44^{a}
No. of pre-implantation loss/ litter	0^{a}	2.1 ± 0.36^{b}	1.3 ± 0.24^{c}	0^{a}
No.of postimplantation loss/litter	0^{a}	1.5 ± 0.34^{b}	0.8 ± 0.15^{c}	0^{a}
No.of resorbed feti/litter	0^{a}	1.2 ± 0.13^{b}	0.3 ± 0.15^{c}	0^{a}
No. of dead feti/litter	0.1 ± 0.099^{a}	1.2 ± 0.24^{b}	0.6 ± 0.16^{c}	0^{a}
No. of live feti/litter	6.8 ± 0.44^{a}	1.5 ± 0.29^{b}	4.2 ± 0.25^{c}	6.8 ± 0.44^{a}
Fetal weight (g)	3.9 ± 0.42^{a}	2.6 ± 0.23^{b}	3.4 ± 0.39^{c}	4.1 ± 0.35^{a}
No. of visceral anomalies/ litter	0^{a}	2.1 ± 0.39^{b}	1.3 ± 0.54^{c}	0^{a}
No.of skeletal anomalies/ litter	0^{a}	1.0 ± 0.34^{b}	0.9 ± 0.15^{b}	0^{a}

Values in each row having different superscript are significantly different at $p< 0.05$.



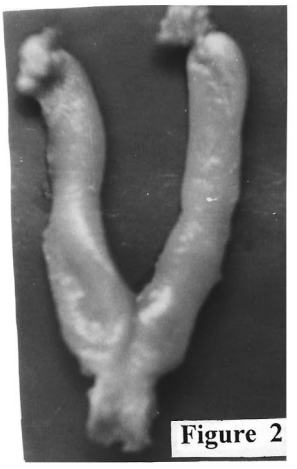


Figure 1. Uterus of female rat treated with 50ppm chromium showing intrauterine hemorrhage and post implantation losses.
Figure 2. Uterus of female rat treated with 50ppm chromium showing complete pre-implantation losses in non graved uterus contain fluid while corpora lutea were present.

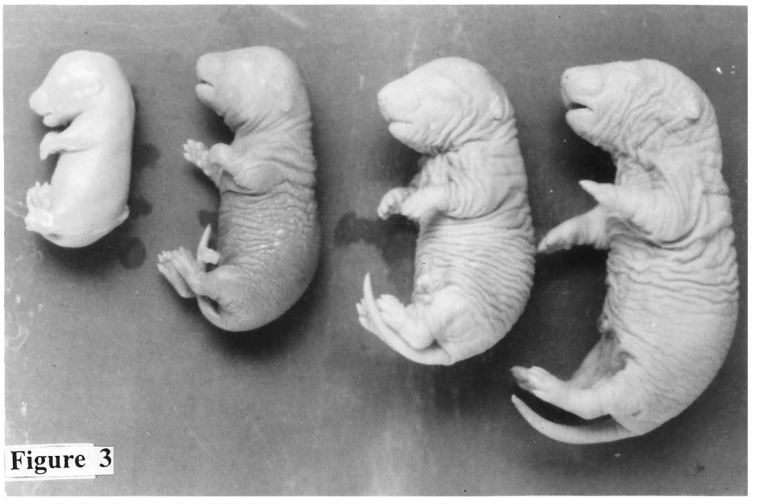
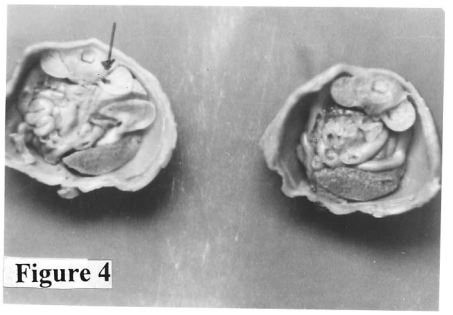


Figure 3. Feti obtained from control (the 1st from the right), treated with 50ppm chromium + ginseng(the 2nd) and treated with 50ppm chromium (the 3rd and 4th) at the 20th day of gestation showing stunted growth at different degrees.



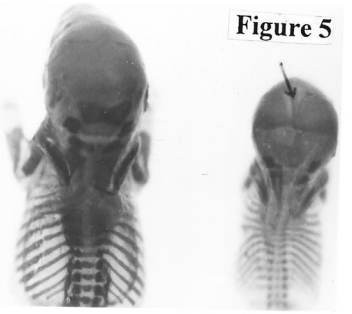
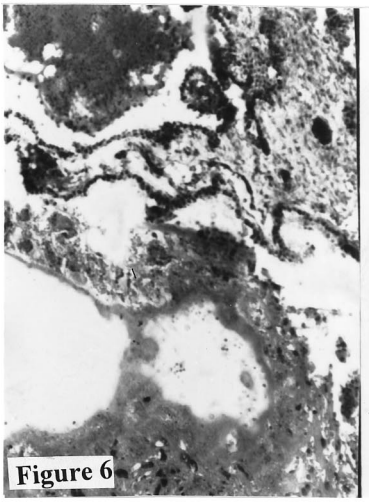


Figure 4. Cross sections in feti obtained from control (right) and treated females with 50ppm chromium (left) showing dilatation of renal pelvis.
Figure 5. Skeletons of feti obtained from control (left) and treated females with 50ppm chromium (right) showing incomplete ossification of the skull bone.



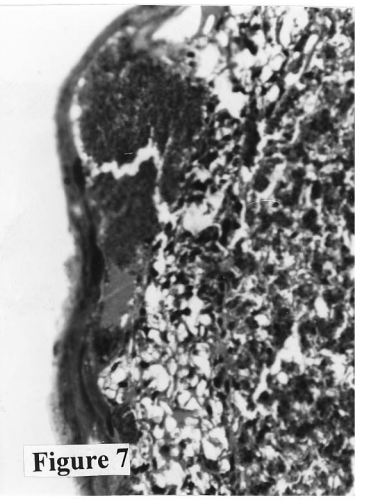


Figure 6. Placenta of female rat treated with 50ppm chromium showing necrosis in the chorionic villi of the fetal part with homogenous eosinophilic structurless had basophilic fragments of the degenerated nuclei, (X40) ·
Figure 7. Placenta of female rat treated with 50ppm chromium showing focal extravasation of red blood cells in the decidua basalis of maternal part, (X40).

Table 2. Mean values, ± S.E of chromium content(ppm, fresh weight) in maternal blood, placenta and fetal tissues of control and treated groups

Groups / Tissue	Control	gp.I chromium 50ppm	gp.II chromium +ginseng	gp. III ginseng 20mg/kg
Maternal blood	0.013±0.06 [a]	0.06±0.008 [b]	0.04±0.006 [c]	0.012±0.006 [a]
Placenta	0.04±0.003 [a]	0.2±0.019 [b]	0.16±0.02 [b]	0.033±0.005 [a]
Feti	0.015±0.003 [a]	0.13±0.03 [b]	0.07±0.012 [c]	0.012±0.003 [a]
Fetal/placental ratio	0.37±0.041 [a]	0.65±0.072 [b]	0.44±0.042 [c]	0.36±0.033 [a]

Values in each row having different superscript are significantly different at $p< 0.05$.

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