A radiographic study of embryos on hexavalent chromium toxicity in female wistar rats

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Abstract
Hexavalent chromium salts have been recognized as occupational health hazard for more than 160 years. It is a potent toxic agent. It has been found to be carcinogenic in human and animals. Hexavalent chromium is generally considered 1000 times more toxic than trivalent chromium. The experiment was conducted to study the embryonal toxicity in female wistar rats by inducing hexavalent chromium at the rate of 500 parts per million in the form of potassium dichromate in drinking water orally for three months and also to pregnant animals, with the protective and ameliorating agents like vitamin C and Emblica officinalis (E. officinalis) respectively. Foetuses were collected by sacrificing pregnant rats from all six groups for the purpose of radiographic study. The fetuses from all groups were subjected to radiographic study. The embryos from the toxin group revealed mild subdermal haemorrhages over abdomen region, ill developed fetuses when compared to the morphological features of control, protective and ameliorative group embryos.

Keywords: Chromium toxicity, embryos, amelioration, radiographic morphological study

Introduction
Chromium is a naturally occurring element found in animals, plants, rocks, soil, volcanic dust and gases (Nejla Soudani et al., 2010) [1]. Hexavalent chromium is an important reproductive and developmental toxicant as per office of environmental Health Hazard Assessment (OEHHA) and the Development and Reproductive toxicant Identification Committee (DARTIC) (Toxicology profile for chromium, United States Environmental Protection Agency, USEPA, 2001) [2]. The effect of hexavalent chromium on multiple pathways could be mitigated by Vitamin C (Sakhila et al., 2011) [3]. Plant parts of emblica officinalis showed antidiabetic, hypolipidemic, antibacterial, antioxidant, hepatoprotective and gastro protective properties (Krishna veni and Mrunalini, 2010) [4].

Materials and Methods
All rats were handled in accordance with the guidelines of Institutional Animal Ethics Committee (IAEC) (No 1/4/14, date 27.11.2014). The dams were sacrificed and the fetuses were collected to record the lesions. X-ray Radiographic studies of foetuses were carried out at Super Speciality Hospital, Narayanaguda, Hyderabad as per the standard protocol.

Experimental design

<table>
<thead>
<tr>
<th>Group</th>
<th>No of Rats</th>
<th>Type of treatment / diet</th>
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<tbody>
<tr>
<td>I (Control)</td>
<td>20</td>
<td>Basal diet</td>
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<tr>
<td>II (Toxin control)</td>
<td>20</td>
<td>Basal diet + Potassium dichromate 500 parts per million (ppm) in drinking water orally for 3 months</td>
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<tr>
<td>III (Vit C control)</td>
<td>20</td>
<td>Basal diet + vitamin C @ 100 milligram (mg/kg body weight (kg.b wt) orally for 3 months.</td>
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<tr>
<td>IV (Emblica officinalis control)</td>
<td>20</td>
<td>Emblica officinalis powder given @ 2% in feed for 3 Months</td>
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<tr>
<td>V (Chromium VI + Vitamin C control)</td>
<td>20</td>
<td>Basal diet + Potassium dichromate 500 ppm in drinking water orally for 3 months + vitamin C @ 100 mg/kg b.wt orally for 3 months.</td>
</tr>
<tr>
<td>VI (Chromium VI + Emblica officinalis)</td>
<td>20</td>
<td>Basal diet + Potassium dichromate 500 ppm in drinking water orally for 3 months + Emblica officinalis powder given @ 2% in feed for 3 months.</td>
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Results
The radiographic morphological study of fetuses/embryos through x-ray studies in the toxin group showed significantly mild sub dermal haemorrhages in the form of patches over abdomen region (Fig: 1). The fetuses were ill developed and very small in size, which is very prominent feature (Fig: 2) observed in treated embryos when compared to morphological features of other control, protective and ameliorative group embryos (III, IV, V and VI).

The toxin group fetuses were also showed abnormality in skeletal development like incomplete ossification in nasal, cranium, abdominal or caudal bones and absence of ossification in the sacral vertebrae which clearly depicted through in x-ray radiographs (Fig: 3) in comparison with the skeletal development of other control, protective and ameliorative group of embryos (Fig: 4). The morphological features and skeletal development is apparently normal in protective and ameliorative groups.

Discussion
The defects in the embryos may be due to impaired development of embryos during gestation (Kanojia et al., 1996) [3], interrupted uterine blood flow in hexavalent chromium treated rats (New and Coppola, 1977 and Garis, 1989) [6, 7], foeto toxic effects with significant reduction of implantation rate, number of fetuses, an increase in the resorption number, pre implantation and post implantation(Junaid et al., 1996a and Kanojia et al., 1996) [8, 5] and decrease in the crown rump due to embryo and foetotoxic effects (Neila Morouani et al., 2010) [9]. These adverse effects were comparatively minimum by supplementing vitamin C and emblica officinalis because of its ameliorative role. Vitamin C acts as a biological antioxidant by donating an electron to free radical, there by interrupting the radical chain reaction in biological membranes and protects from the deleterious effects like fetal abnormalities in rats (Kesinger and Stevens, 2009) [10]. Absence of ossification in the sacral vertebrae found through x-rays analysis and the same was reported by Neila Morouani et al., 2010 [9] during the organogenesis period.

Fig 1: Note the morphological features of the fetuses showing defects in the abdomen and thigh region in the toxin group (group II) rats

Fig 2: Note the apparently normal morphological features of the fetuses in the control group (Group I, III) rats, abnormal and very small sized fetuses of group II through radiographic studies (30 days)

Fig 3: Note the abnormality in skeletal development of fetuses showing incomplete ossification in nasal, cranium, abdominal or caudal bones in the toxin group (group II) rats through radiographic studies (30 days)

Fig 4: Note the very small size of the fetuses and a characteristic and pronounced ill development in toxin group compared to control group (I, III) of rats (30 days)

References
