INTRODUCTION
Cadmium is one of the most potent hazardous heavy metals in our environment which exhibits toxic teratogenic, mutagenic and carcinogenic effects (Bartosiewicz et al., 2001; Wershana, K.Z. (2001; Zeng et al., 2003). Cd exposure was reported to produce various direct and indirect genotoxic effects on cells, such as cell proliferation, chromosomal aberrations, DNA strand breaks, aberrant DNA methylation, and oxidative DNA damage (Benbrahim-tallaa et al., 2007; Haldsrud & Krokje, 2009; Huang et al., 2008; Lin et al., 2007). Cd exposure inhibits DNA synthesis and cell division at concentrations above 1 μM. Increased formation of the reactive oxygen species (ROS) in presence of Cd – which can directly generate DNA damage and/or cause inhibition of DNA repair system – is generally recognized mechanism of Cd mutagenicity. Cd-induced decrease of cellular antioxidants is also considered an important mechanism of Cd contribution to oxidative stress, because it is not redox-active metal and cannot itself direct fenton reaction.

The genotoxicity of Cd in vivo and in vitro is well documented (Bertin & Averbeck, 2006; Filipic et al., 2006). Cd also shows co-genotoxic effects when combined with other mutagenic agents like UV and gamma radiation, or in presence of alkylating chemicals like methyl methanesulfonate and N-methyl-N-nitrosourea (Fatur, 2003).
Induction of chromosomal aberrations showed a decrease after the treatment of carrot juice. Micronuclei and sperm abnormalities have been reported in somatic and germ cells of mice (Namvaran et al., 2011; Rajitha & RudramaDevi, 2001; Slavicaopovic et al., 2013).

Temperate regions of Europe, Asia and Africa, it’s active ingredients including volatile oils, steroids, tannins, flavonoids and carotene have been reported (Jasicka-Misiak et al., 2005). The higher serum carotenoid concentration, the lower risk of diabetes and insulin resistance can be caused by carotenoids function (Hozawa et al., 2006). Carotenoid decoction has been reported to be popular remedy for jaundice in addition to its traditional uses for treating kidney, respiratory cardiovascular disorders (Shoba et al., 2008).

It have widely been used in foods to engender good odor, flavor, color and preservative, it is So far there are no studies on protective effects of plant extracts on CdCl₂ induced genotoxicity in somatic cells of mice. Hence in the present investigations we have made an attempt to evaluate the protective effects of carrot juice in CdCl₂ induced genotoxicity in bone marrow cells of mice using analysis of chromosomal aberrations in somatic cells of mice.

**MATERIALS AND METHODS**

**Methodology:**

1. **Chemicals:**
   Cadmium chloride from Sigma Aldrich the chemicals used in all the experiments were purchased from Hi-media analytical grade.

2. **Experimental animals**
   Eight weeks old random bred male Swiss albino mice (Mus musculus) average body weight of 25 ± 2 gms were purchased from National Institute of Nutrition, Hyderabad, were maintained in the departmental animal house under an absolute hygienic conditions as per the recommended procedures by fulfilling all the necessary ethical standards. They were housed in polypropylene shoe box type cages dimensions were 13.5" L x 7.0" W x 6.5" to 8.5"H cages, bedded with rice husk (rice husk procured locally autoclaved to free from microorganisms) and kept in AC room at the temperature 25°C (± 2°C) and RH 65 ± 5% and photo-cycle of 12:12 h light and dark periods, were fed with pelleted diet (from National Institute of Nutrition, Hyderabad) composed of 20.0% crude protein, 4.0% crude fiber, 1.0% calcium, 0.6% phosphorus, 8% fish meal, 20% ground nut cake and enriched with stabilized vitamins A, B, C, D3, K, thiamine, riboflavin, pantothenic acid, niacin, folic acid, minerals & trace elements and water.

**Plant extraction:**
   Carrots roots were purchased from a local market, identified by Prof. Prathibha Devi, Dept. of Botany, OU and the protocols used in the experiment were approved by ethical committee vide no. MR 776/410/2007/Adm -1 Dated 27-07-2007. The test material was washed and grated into smaller pieces. They were dried and pulverized into powder. The powder (20g) was soaked in distilled water overnight and filtered. The residue was allowed to dry, weighed and subtracted from the initial weight of the powder to determine the concentration of the filtrate. The filtrates were stored in the refrigerator and used for the experimental work experiment the dose schedule is as follows.

**4. Experimental design:**
   Two experiments were conducted in the first experiment animals were administered with 20, 40 and 80 ml of carrot juice (CJ) freshly prepared orally to all the test groups. Control group of animals received only 0.5 ml of physiological saline.

   - **Group I** – Control animals only 0.5 ml physiological saline
   - **Group II** – 20 ml of CJ
   - **Group III** – 40 ml of CJ
   - **Group IV** – 80 ml of CJ

   In the second experiment the dose schedule is as follows.

   - **Group I** – control animals only 0.5 ml physiological saline
   - **Group II** – 3.0 mg/kg of cadmium chloride
   - **Group III** – 20 ml of CJ + 3.0 mg/kg of cadmium chloride
   - **Group IV** – 40 ml of CJ + 3.0 mg/kg of cadmium chloride
5. Analysis of chromosomal aberrations in germ cells of mice:

Chromosome aberration analysis from germ cells

The mice were killed on 28th day, 24 h after administration of last dose of the drug. Seminiferous tubules from testis were collected in 5ml of isotonic 1.2% trisodium citrate solution and incubated at the temperature 37ºC for 45 min. The cell suspension was centrifuged in 120x17 mm conical centrifuge tubes for 10 min at 1000 rpm. To the pellet 5 ml of freshly prepared pre-chilled fixative (3:1 methanol and acetic acid) added and centrifuged. This process repeated for 4 to 5 times. The Chromosomal preparations were made by the air drying technique of Evans et al 1964 and stained with 2 ml of 2% Giemsa in 46 mlof double distilled water plus 2 ml of phosphate buffer* pH 6.8) for 7-8 min. Approximately 500 meioticmetaphases screened for numerical (Autosomal Univalents, Sex- Autosomal Univalents, euploidsandaneuploids) and structural (translocations) Aberrations.

2.1.3. Carrot Juice:
The results on the frequency of various types of chromosomal aberrations in carrot juice treated animals are presented in Table (1-2) The frequencies of various types of chromosomal aberrations recorded were as follows: the frequency (%) of autosomal univalents recorded in 3.0 mg/kg cadmium treated animals was 1.80 where as the frequencies (%) were 1.80, 2.0 and 2.20 after the administration of 20, 40 and 80ml/kg of carrot juice respectively. (Table 1). Similarly, the frequency of sex-chromosomal univalents recorded 3.0 in control animals, 6.60 in 3.0mg/kg cadmium treated animals and 5.20, 3.80 and 3.40 in 3.0+20, 3.0+40 and 3.0+80 cadmium +carrot juice treated animals as against 2.60 in control. (Table 28; Fig 10). Similarly, the frequency of sex-chromosomal univalents recorded 3.0 in control animals, 6.60 in 3.0mg/kg cadmium treated animals and 5.20, 3.80 and 3.40 in 3.0+20, 3.0+40 and 3.0+80 cadmium +carrot juice treated animals. The frequencies (%) of polyploidy were 2.20 in control animals, 6.20 in 3.0mg/kg cadmium treated animals to 4.0, 3.0 and 1.40 after the administration of 3.0+20, 3.0+40 and 3.0+80 cadmium +carrot juice. The frequencies (%) of cells with aneuploidy in 3.0mg/kg cadmium treated animals were 2.0 where as the frequencies were 1.4, 1.0 and 0.80 after the administration 3.0+20, 3.0+40 and 3.0+80 cadmium + carrot juice respectively.

Cells with translocations were 0.80 in 3.0 mg/kg cadmium treated animals. The frequencies (%) of cells with translocations were 0.60, 0.40 and 0.20 in animals administered with 3.0+20, 3.0+40 and 3.0+80
cadmium + carrot juice respectively (Table 3). The frequency of various types chromosomal aberrations recorded in mitomycin ‘C’ treated group of animals were as follows, the frequency (%) on autosomal univalents and sex-chromosomal univalents were 6.50 and 6.80 respectively. The frequency of cells with polyploids and aneuploids were 6.20 and 2.0 in mitomycin ‘C’ treated control group of animals. Translocations were also recorded in mitomycin ‘C’ treated group of control animals were 1.00% (Table 2). The percentage of total chromosomal aberrations were 15.80, 11.40 and 8.20 in animals administered with 3.0+20, 3.0+40 and 3.0+80 cadmium + carrot juice respectively as against 22.00 in 3.0 mg/kg cadmium treated animals and 9.60 in control animals. The percentage of total chromosomal was 22.50 in animals administered with mitomycin ‘C’ (Table 3).

The $x^2$ values for the differences in the incidence of chromosomal aberrations between control and treated groups were subjected to statistical analysis and found to be insignificant. ($P<0.01$).

Table 1: Frequency of chromosomal aberrations recorded in germ cells of mice after treatment with various doses of Carrot Juice (CJ)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Normal Metaphases Scored %</th>
<th>Abnormal metaphases scored %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>920 (92.00)</td>
<td>80 (8.00)</td>
</tr>
<tr>
<td>20 ml/kg</td>
<td>915 (91.50)</td>
<td>85 (8.50)</td>
</tr>
<tr>
<td>40 ml/kg</td>
<td>906 (90.60)</td>
<td>94 (9.40)</td>
</tr>
<tr>
<td>80 ml/kg</td>
<td>896 (89.60)</td>
<td>104 (10.40)</td>
</tr>
</tbody>
</table>

Table 2: Classification of various types of chromosomal aberrations recorded in germ cells of mice analysed after treatment with various doses of Carrot juice (CJ)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Changes in chromosome number</th>
<th>Structural changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose mg/kg</td>
<td>Autosomal univalents</td>
<td>Sex-chromosomal univalents</td>
</tr>
<tr>
<td>b.w.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>18 (1.80)</td>
<td>22 (2.20)</td>
</tr>
<tr>
<td>20 ml/kg</td>
<td>18 (1.80)</td>
<td>23 (2.30)</td>
</tr>
<tr>
<td>40 ml/kg</td>
<td>20 (2.0)</td>
<td>26 (2.60)</td>
</tr>
<tr>
<td>80 ml/kg</td>
<td>22 (2.20)</td>
<td>28 (2.80)</td>
</tr>
</tbody>
</table>
Table 3: Frequency of chromosomal aberrations recorded in germ cells of mice treated with Cadmium and primed with Carrot Juice (CJ)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Non-primed</th>
<th>Primed with Carrot juice</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20ml/kg</td>
<td>40 ml/kg</td>
</tr>
<tr>
<td></td>
<td>Normal metapases scored %</td>
<td>Abnormal metapases scored %</td>
</tr>
<tr>
<td>Control</td>
<td>904</td>
<td>96</td>
</tr>
<tr>
<td></td>
<td>(90.40)</td>
<td>(9.60)</td>
</tr>
<tr>
<td>Mitomycin C</td>
<td>775</td>
<td>225</td>
</tr>
<tr>
<td></td>
<td>(77.50)</td>
<td>(22.50)</td>
</tr>
<tr>
<td>3.0mg/kg</td>
<td>780</td>
<td>220</td>
</tr>
<tr>
<td></td>
<td>(78.00)</td>
<td>(22.00)</td>
</tr>
</tbody>
</table>

The values in parenthesis are percentages.

DISCUSSION

Chromosome aberrations observed in the present analysis were classified into structural numerical and other abnormalities these end points serve as indicator for evaluating the mutagenic potentials of test substances. Since these are considered as stable anomalies which continue to next generation. Further such variations in germ tissues lead to malignancy (Alldrick et al., 1986).

Various plants extract shown antimutagenic and anticarcinogenic properties. A large number of vegetable juices were also found to reduce CA in rat bone marrow cells induced b dimethylbenz(a) anthracene The effect of these vegetables juices were attributed variously to some heat resistant compound, vitamin C, β-carotene or to the interaction between different compounds. There are many as naturally occurring antimutagenic compounds which have been isolated from the edible parts of plants. Carrot is a classical example of β-carotene, which is known to be a unique antioxidant and a free-radical scavenging agent with anticarcinogenic activity. Beta-carotene and other carotenoids are also found in green leafy vegetables. The protective effects of carrot juice in CdCl₂ induced genotoxicity in the present study indicate that dietary vegetables play an important role in inhibiting the cytotoxic damage induced by CdCl₂.

Abraham et al. (1986) reported that when mice were primed with carrot and spinach juice, there was a scavenging the free radicals including the peroxo radicals and thereby, might contribute for the cytoprotective activity of Daucus carota suppression of micronuclei induced by cyclophosphamide. The role of various plant extracts as desmutagens and antimutagens are being increasingly recognized. It has been observed that the oxidative base damage was significantly reduced during the carrot juice intervention. Durnev et al. (1997) reported that chromosome damage caused by cyclophosphamide (30mg/kg) and dioxide (300mg/kg) in the bone marrow of C57BL/6 mice were significantly reduce by the food dyes E160e (beta-apo-8’ carotenal in an oil suspension) and E160a (beta-carotene in an oil suspension) at doses of 0.5, 5 and 50 mg/kg. Further protective effects of CJ in isoniazid induced hepatotoxicity has been reported by Shoba et al. (2008).

DCE has been reported by Straub (1987) and Olson (1989), to contain carotenoids including β-carotene, α-carotene, γ-carotene, lycopene, cryptoxanthin, leutein, many partly degraded carotenoids such as abscisic acid, trisporic acid, β-apo-carotenals, crocetin and many common polar carotenoids, like violaxanthin. It is well known that oxygen free radicals are strongly associated with cellular injury. As reported by Burton some of the above compounds have the potential to juice in CdCl₂ induced genotoxicity in the present study indicate that dietary vegetables play an
important role in inhibiting the cytotoxic damage induced by CdCl₂.

Earlier the protective effects of grape fruit extract against cadmium chloride induced antioxidant mechanism has been reported (Jahan et al., 2014). The aim of the present study was to investigate the effects of long-term grape juice concentrate (GJC) consumption, in two dosages, on the reproductive parameters of cadmium-exposed male rats. The effects of the concentrate on body mass gain, plasma testosterone levels, reproductive organ weights, daily sperm production, sperm morphology, testis histopathological and histomorphometrical parameters, and testicular antioxidant markers were investigated, the product was able to act as a protector of reproductive function against cadmium-induced damage (Vanessa et al., 2013).

In another study an experiment was performed to determine the effects of different antioxidants on testicular histopathology and oxidative damage induced by cadmium (Cd) in rat testis and prostate. Twenty five rats were equally divided into five days The control group was injected subcutaneously with saline while the Cd alone treated group received a subcutaneous injection of 0.2mg/kg CdCl(2). Other groups were treated with sulphoraphane (25µg/rat), vitamin E (75mg/kg), and Ficus Religiosa plant extract (100mg/kg) orally along with subcutaneous injections of 0.2mg/kg CdCl(2) for fifteen days. Histological examination of adult male rat testes showed a disruption in the arrangement of seminiferous tubules along with a reduction in the number of germ cells, Leydig cells, tunica albuginea thickness, diameter of seminiferous tubules, and height of germinal epithelium. Co-treatment with vitamin E, sulphoraphane, and Ficusreligiosa were found to be effective in reversing Cd induced toxicity Jahan et al. (2014).

Administration of black grapes extract significantly reversed activities of serum renal markers to their near-normal levels, significantly decreased lipid peroxidation, restored the antioxidant defense levels of in kidney, and produced improvement in hematological parameters when compared to cadmium-treated mice (Slavicopovic et al., 2013). Further the protective effect of quercetin on cadmium-induced oxidative toxicity was investigated in mouse testicular germ cells. After oral administration of cadmium chloride at 4 mg/kg body weight for 2 weeks, damages in spermatozoa occurred in the early stage of spermatogenesis. Cadmium treatment significantly decreased the testicular antioxidant system, including decreases in the glutathione (GSH) level, superoxide dismutase (SOD), and GSH peroxidase (GSH-Px) activities. Moreover, exposure to cadmium resulted in an increase of hydrogen peroxide production and lipid peroxidation in testes. In addition, cadmium provoked germ cell apoptosis by upregulating expression of the proapoptotic proteins Bax and caspase-3 and downregulating expression of the antiapoptotic protein Bcl-XL. However, combined administration of a common flavonoid quercetin at 75 mg/kg body weight significantly attenuated cadmium-induced germ cell apoptosis by suppressing the hydrogen peroxide production and lipid peroxidation in testicular tissue. Simultaneous supplementation of quercetin markedly restored the decrease in GSH level and SOD and GSH-Px activities elicited by cadmium treatment. Additionally, quercetin protected germ cells from cadmium-induced apoptosis by downregulating the expression of Bax and caspase-3 and upregulating Bcl-XL expression uercetin, due to its antioxidative and antiapoptotic characters, may manifest effective protective action against cadmium-induced oxidative toxicity in mouse testicular germ cells (El-Neweshy et al., 2013). Similarly protective effects of plant extracts such as phyllanthusemblica fruit extract curcumin, garlic extract against heavy metal induced genotoxicity has been reported from our laboratory (Moshe et al., 2010; RudramaDevi & Moshe, 2012).
CONCLUSIONS
The overall results of the present study suggest the genoprotective activity of carrot juice in CdCl₂ induced genotoxicity in bone marrow cells of mice. Regular consumption of carrots about 400 mg per person before mid day meal is suggested by medical doctors information is available on pubnet. So far whether boiled carrot or cooked carrot is useful is not known, but the raw red color carrots have potential antioxidant compound known as fucevital can protect infectious diseases etc.,

Acknowledgement
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REFERENCES


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