WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 19, 679-687. Research Article ISSN 2277– 7105

CARROT JUICE PROTECTS CADMIUM INDUCED GENETIC DAMAGE IN GERM CELLS OF MICE

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Article Received on
03 October 2018,

Revised on 23 October 2018,
Accepted on 13 Nov. 2018

DOI: 10.20959/wjpr201819-7514

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ABSTRACT

In the present study, modulating effect of carrot juice against cadmium chloride induced clastogenicity in germ cells of mice. Has been investigated. When animals administered with various doses of carrot juice 20, 40, 80 ml/kg for 30 day and meiotic preparations were made and the prepared were screened for the presence of various types of sperm head abnormalities in germ cells of mice., The obtained results showed non mutagenic nature of plant extract. There was a significant increase in the percentage of aberrant sperms in germ cells of mice at all dose levels However when animals were co-administered seven days prior to the priming experiment with carrot juice, the frequency of aberrant sperms decreased significantly. Thus the results clearly indicate protective nature of carrot juice in cadmium chloride induced cytogenetic damage in germ cells of mice.

KEYWORDS: Cadmium chloride, chromosomal aberrations, carrot juice.

INTRODUCTION

Cadmium is one of most potent hazardous heavy metals in our environment[1] which exhibits toxic teratogenic, mutagenic and carcinogenic effects[2-4] 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.[5-8] 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.[9,10] 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.[11] Induction of of chromosomal aberrations showed a decrease after the treatment of carrot juice. Micronuclei and sperm abnormalities has been reported in somatic and germ cells of mice.[12,13]

Plant clear fact that they possess antioxidant activity and the reason for decreasing oxidation potential of lipids in food stuffs, ten of them have been used as a natural defense against diseases and infections[14], *Daucus carota* commonly known as " carrot " is one of the most important vegetables belonging to family Apiecea is an annual or biannual herb mostly confined to the temperate regions of Europe, Asia and Africa, it's active ingredients including, volatile oils, steroids, tannins, flavonoids and carotene have been reported[15], The higher serum carotenoid concentration, the lower risk of diabetes and insulin resistance can be caused by carotenoids function.[16] Carotenoid decoction has been reported to be popular remedy for jaundice in addition to its traditional uses for treating kidney, respiratory cardiovascular disorders.[17] 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 $CdCl_2$ induced genotoxicity in germ cells Hence in the present investigations we have made an attempt to evaluate the protective effects of carrot juice in $CdCl_2$ induced genotoxicity in germ cells of mice using analysis of sperm head abnormalities in germ cells of mice.

METHODOLOGY

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

2. Animals: Maintained under standard laboratory conditions at temperature 22ºC relative humidity 50±10% and 12 h photo period commercial pellet diet (Hindustan Lever India) and deionised water were provided by labium.

3. 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.

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. first experiment the dose schedule is as follows.

Group I - control animals only 0.5 ml physiological
Group II - 20 ml of CJ
Group I - 40 ml of CJ
Group V - 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
Group V - 80 ml of CJ + 3.0 mg/kg of cadmium chloride

Sperm morphology assay

All the control and treated animals were sacrificed on 35th day. This is because spermatogenesis takes about 34.5 days to complete in mice. Sperms were sampled from the caudal epididymis after the animals had been sacrificed by cervical dislocation. Sperm suspension was prepared from the caudal of each testis by mincing the caudal in physiological saline. To the suspension 2- 3 drops of 1% aqueous eosin was added and kept for about 20 min undisturbed. Smears were made on clean slides and allowed to dry air. 1000 sperm cells/mouse were assessed for morphological abnormalities according to the criteria of Wyrobek and Bruce.[18] The various types of sperm head abnormalities were observed as banana, Amorphous, hammer head shaped were observed in control and treated group of animals and data was analysed using Chi-Square test.

RESULTS

The results on the frequency of various types of sperm head abnormalities in carrot juice treated animals are presented in Table 1. The frequency (%) of aberrant sperms after

administration of 20, 40 and 80 ml/kg carrot Juice were 4.0, 4.20 and 4.60 as against 3.76 in control animals (Table 1). The differences in the number of aberrant sperm between the control and treated groups were subjected to statistical analysis and found to be insignificant at all dose levels. (P>0.05).

The results on the frequency (%) of various types of sperm head abnormalities in cadmium+ carrot juice treated animals are presented in Table 2. The frequency (%) of aberrant sperms after administration of 3.0+20, 3.0+40 and 3.0+80 cadmium +carrot juice were 8.80, 6.0 and 3.60 as against 11.10 in 3.0 mg/kg cadmium treated animals and 3.40 in control animals. The results on the frequency (%) of various types of sperm head abnormalities in mitomycin 'C' treated control group of animals were 12.36(Table 2). The differences in the number of aberrant sperm between control and treated groups were subjected to statistical analysis and found to be significant. (P<0.01).

Table 1: Frequency of sperm head abnormalities in mice treated with various doses of Carrot Juice.

Treatment	Normal sperms (%)	Aberrant sperms (%)
Control	4812	188
	(96.24)	(3.76)
20ml/kg	4800	200
	(96.00)	(4.00)
40 ml/kg	4790	210
	(95.80)	(4.20)
80 ml/kg	4770	230
	(95.40)	(4.60)

The values in parenthesis are percentages.

P>0.05.

Table 2: Frequency of sperm head abnormalities in Cadmium treated mice primed with Beta-carotene and Carrot Juice.

Treatment	Non-primed		Primed with Carrot juice(CJ)					
			20ml/kg		40 ml/kg		80 ml/kg	
	Normal sperms scored %	Abnormal sperms scored %	Normal sperms scored %	Abnormal sperms scored %	Normal sperms scored %	Abnormal sperms scored %	Normal sperms scored %	Abnormal sperms scored %
Control	4830	170	-	-	-	-	-	-
	(96.60)	(3.40)	-	-	-	-	-	-
Mitomycin C	4392	618	-	-	-	-	-	-
	(87.84)	(12.36)	-	-	-	-	-	-
3.0mg/kg	4445	555	4560	440	4700	300	4820	180
	(88.90)	(11.10)	(91.20)	(8.80)	(94.00)	(6.00)	(96.40)	(3.60)

The values in parenthesis are percentages.

*P<0.01.

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 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.[19] Beta-carotene and other carotenoids are also found in green leafy vegetables. The protective effects of carrot juice in $Cdcl_2$ induced genotoxicity in the present study indicate that dietary vegetables play an important role in inhibiting the cytotoxic damage induced by $Cdcl_2$.

Abraham et al[20] reported that when mice were primed with carrot and spinach juice, there was scavange the free radicals including the peroxy radicals and thereby, might contribute for the cytoprotective activity of *Daucuscarota* suppression of micronuclei induced by cyclophospamide. 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[21] 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. l.[22]

DCE has been reported by Straub,[23] and Olson,[24] to contain carotenes 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 withcellular injury. As reported by Burton some of the above compounds have the potential to juice in $Cdcl_2$ induced genotoxicity in the present study indicate that dietary vegetables play an important role in inhibiting the cytotoxic damage induced by $Cdcl_2$.

Earlier the protective feects of grape fruit extract against cadmium chloride induced antioxidant mechanism has been reported.[25] 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.[26]

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 0.2â€‰mg/kg CdCl(2). Other groups were treated with sulphoraphane (25â€‰Âµg/rat), vitamin E (75â€‰mg/kg), and Ficus Religiosa plant extract (100â€‰mg/kg) orally along with subcutaneous injections of 0.2â€‰mg/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.[27]

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.[28] 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 down regulating 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.[29] 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.[30-31]

CONCLUSIONS

The overall results of the present study suggest the genoprotective activity of carrot juice in $CdCl_2$ induced aberrant sperms in 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

The author (KRD) thankful to University Grant Commission for the award of UGC BSR Faculty fellowship, to Prof. Sugita Mathur, Head, Department of Zoology for providing necessary laboratory facilities.

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