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Full Length Research Paper

Anti-genotoxic effects of crude garlic extract on cisplatin induced toxicity on germ cells and morphology of sperms in *in vivo* mouse

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The present investigation was directed to study the possible anti-genotoxic activity of orally administered crude garlic extract (GE) against cisplatin-induced genotoxicity in mouse by using chromosomal aberrations (CA's) in germ cells and sperm morphology essay (SMA). Three different doses of garlic extract (125, 250, 500 mg/kg) were tested for their modulatory capacity on the mutagenecity exerted by CP (10 mg/kg, i.p.). GE alone did not induce any significant variation in the incidence of CA's and frequency of aberrant sperms. Pretreatment of mice with GE for 7 days and simultaneously with a single dose of Cisplatin significantly reduced the frequency of CA's and frequency of aberrant sperms, suggesting that the garlic extract modulates the CP induced genotoxicity in a dose dependent manner.

Key words: Garlic, chromosomal aberrations, germ cells.

INTRODUCTION

cis-Dichlorodiamminoplatinum-II (CP) is an established anticancer drug used for the treatment of a variety of animal tumors and human cancers (Calabresi and Parks, 1985; Greene, 1992) such as ovarian, cervical, germ cell tumors and testicular teratoma (Muggia, 1984; Magdy, 2003; William and Christopher, 2009). The majority of antineoplastic drugs besides their generic growth property display genotoxic effects which in turn contribute to growth inhibition. These genotoxic effects may lead to initiation of unrelated tumours years after the cessation of chemotherapy (Beretta, 1991) by generating free radicals (Weijl et al., 1998; Wozniak et al., 2004) and also responsible for secondary malignancies observed in animals and some cured patients treated with CP (Kemph and Ivankovic, 1986; Greene, 1992; Pillaire et al., 1994). Much attention has been focused to reduce the mutagenic side effects of cisplatin by administration of modulating agents, usually free radical scavenges.

Garlic exhibits various beneficial effects like antitumor, antimutagenic, antioxidant and anticancer properties (Amagase, 2006; Banerjee et al., 2003; Kyo, 2001; Khanum et al., 2004; Lau, 2001; Sato and Miyata, 2000). One of the most important biological effects observed recently with garlic is tumor inhibitory properties against various types of cancers (Belman, 1983; Sparnine et al., 1988; Fukushima et al., 1997). Hence, the present study is undertaken to evaluate the potential protective effects of orally administered garlic extract against Cisplatin-induced genotoxicity towards mouse chromosomal aberrations (CA) in germ cells and on morphology of sperms as an *in vivo* model.

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MATERIALS AND METHODS

Chemicals

Trisodium citrate (Merck), NaCl (Loba Chemie), Methanol (s.d fine chemie), acetic acid (Qualingens), and 2% Giemsa stain solution in phosphate buffer (pH 6.8) were all purchased from E. Merck, India. Cisplatin, garlic extract, ethanol, mitomycin-C and eosin were purchased from Cipla. However, all other chemicals used in the experiments were of analytical grade.

Preparation of garlic extract

Fresh garlic bulbs (*Alliumsativum L.*) purchased from the local market Dried and ground bulbs were submitted to extraction with 500 ml ethanol in a soxhlet apparatus for 72 h. After extraction, the solvent was filtered and made to evaporate by Rotavapor. The

obtained garlic alcoholic extract was stored at -20°C until use (Khalid, 2009).

Experimental animals

Eight weeks old randombred 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 and autoclaved to free from micro organisms) and kept in air-conditioned room at the temperature 25°C (± 2°C) and RH 65 ± 5% and a photo-cycle of 12:12 h light and dark periods, were fed with pelleted diet (purchased 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, D_3, K, thiamine, riboflavin, pantothenic acid, niacin, folic acid, minerals and trace elements) and water *ad libitum*.

Treatment

Garlic (125, 250 and 500 mg/kg) extractions were given in orally for 7 consecutive days and 10mg/ kg of Cisplatin was administrated on day 7 one hour after regular exposure to antimutagen as a single intraperitonial dose. This is repeated for four weeks. Control (H_2O) and positive control (0.1 ml of mitomycin-C) group of animals were also maintained simultaneously. 5 animals were used in each treatment and control group. Slides were screened with Leica CW 4000 Image analyzer.

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 (Evans et al., 1964) and stained with 2 ml of 2% Giemsa (2 ml of 2% Giemsa in 46 ml of double distilled water plus 2 ml of phosphate buffer* pH 6.8) for 7-8 min. Approximately 500 meiotic metaphases screened for numerical (Autosomal Univalents, Sex- Autosomal Univalents, euploids and aneuploids) and structural (translocations) Aberrations.

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 in air. 1000 sperm cells/mouse were assessed for morphological abnormalities according to the criteria of Wyrobek and Bruce (1975).

Statistical analysis

The significance in the difference between control and treated groups was statistically analyzed by using x^2 test. Data are expressed as mean ± SES in the Tables 1 and 2. Results were considered statistically significant at $P < 0.05$ (Bliss, 1967).

RESULTS AND DISCUSSION

Evaluation of chromosomal aberrations

The present study showed that the cisplatin significantly ($P < 0.05$) increased the chromosomal aberration frequencies from germ cells. These results are similar to the results observed by Adler and el-Tarras (1990), Choudhury et al. (2000) indicating the genotoxicity of this drug for germ cells. The results obtained in the present study showed that GE did not induce any increase in the incidence of CA's as compared to the control value. These findings clearly indicates that GE did not have genotoxic effect at the doses tested (125, 250 as well as 500 mg/kg of GE for 7 consecutive days). These observations obtained are agreeable with that of al-Bekairi et al. (1990) who observed the increase in the weight of seminal vesicles and epididymides of male animals after the administration of *Allium sativum* in drinking water (100 mg/kg/day) for three months. Pretreatment of mice with GE for 7 consecutive days after cisplatin treatment significantly decreased the percentage of abnormal metaphases. These results are comparable with that of Kikelomo et al. (2008).

Evaluation of sperm morphology essay (SMA)

The results obtained from the Sperm morphology essay are presented in Table 2. Treatment of mice with GE did not affect the parameter studied as compared to the control value. The percentage of sperm head abnormalities were increased after the administration of 10 mg/kg of Cisplatin. The increase was significant at the dose tested ($P < 0.01$). These findings agreed with those of Nersesyan et al. (2004) and Misra and Choudhury (2006). But a significant dose dependent reduction in the sperm head abnormalities was observed in GE primed animals.

Many fruits and vegetables are known to prevent chromosomal and DNA damage in animals (Ito et al., 1986; Miyata et al., 2004). It is due to many biologically active compounds which can trap the aggressive metabolites of mutagens. Several studies in the recent years have shown the antigenotoxic and antimutagenic effects of garlic for various drugs and chemicals (Shukla and Taneja, 2002; Bhuvaneswari et al., 2004; Siddique and Afzal, 2005a; Belloir et al., 2006). Studies of the anticarcinogenic effects of garlic on several carcinogens were found to be effective in different ways such as antioxidant property (Khanum et al., 2004; Lampe, 2003),

Table 1. Effect of different concentrations of garlic extract on CA's induced by CP in germ cells of mice.

Treatment	Normal metaphases scored (%)	Abnormal metaphases scored (%)
Control	485 (97.00)	15 (3.00)
Mitomycin C	426 (85.20)	74 (14.80)
10 mg/kg Cisplatin	419 (83.80)	81* (16.20)
	Garlic extract	
125 mg/kg	482 (96.40)	18 (3.60)
250 mg/kg	479 (95.80)	21 (4.20)
500 mg/kg	476 (95.20)	24 (4.80)
	Garlic + CP	
10 mg/kg+125 mg/kg	443 (88.60)	57* (11.40)
10 mg/kg+250 mg/kg	464 (92.80)	36* (7.20)
10 mg/kg+500 mg/kg	483 (96.60)	17* (3.40)

*P<0.05

Table 2. Frequency of sperm head abnormalities recorded in cisplatin treated mice primed with garlic extraction.

Treatment	Normal sperms scored (%)	Aberrant sperms scored (%)
Control	4870±0.32 (97.40)	130±0.03 (2.60)
Mitomycin C	4685±0.03 (93.70)	315±0.09 (6.30)
10 mg/kg Cisplatin	4670±0.02 (93.40)	330*±0.1 (6.60)
	Garlic extract	
125 mg/kg	4860±0.03 (97.20)	140±0.1 (2.80)
250 mg/kg	4850±0.02 (97.00)	150±0.1 (3.00)
500 mg/kg	4840±0.02 (96.80)	160±0.08 (3.20)
	Garlic + CP	
10 mg/kg+125 mg/kg	4760±0.03 (95.20)	240*±0.09 (4.80)
10 mg/kg+250 mg/kg	4790±0.03 (95.80)	210*±0.08 (4.20)
10 mg/kg+500 mg/kg	4810±0.02 (96.20)	190*±0.07 (3.80)

*P<0.05.

ability to scavenge free radicals, boosting the cellular antioxidants such as glutathione that prevent drug toxicity (Wei and Lau, 1998), increasing glutathione levels and blocking carcinogen binding to DNA (Amagase and Milner, 1993). Garlic is also a rich source of water- and lipid-soluble organosulfur compounds. Laboratory investigations have shown that both water- and lipid-soluble sulfur compounds from garlic provide its anticarcinogenic benefits.

In the present study, the frequency of CA's in germ cells and sperm head abnormalities of mice treated with GE against Cisplatin showed the significant reduction. This reduction nearly reached the normal levels after treatment. The results obtained clearly indicate that the garlic extract modulates the CP induced genotoxicity in a dose dependent manner. Our results are in accordance with that of Assayed et al. (2008) in their study after administration of garlic extract for 5 consecutive days altered the abnormal spermatozoa parameters induced by cypermethrin in male white rats. Garlic also attenuated the adverse effects of testicular damage and spermiotoxicity induced by cadmium in rats (Kikelomo et al., 2008).

These findings proved that the consumption of antioxidants enriched diet can modulate the DNA damage caused by anti tumor agents which may be imparted to the ability of dietary antioxidants to trap free radicals generated by drugs. It is concluded that GE reduces the genotoxic action of CP in mice germ cells and sperm head abnormalities. Also in this study, all the selected doses of GE were found to be sufficient for the

modulatory effects. However, more detailed investigations *in vivo* are necessary before using garlic supplementation in chemotherapy.

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