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PROTECTIVE EFFECTS OF CURCUMIN IN CYCLOPHOSPHAMIDE INDUCED SPERM HEAD ABNORMALITIES IN MALE MICE.

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ABSTRACT

Natural dietary antioxidants are extensively studied for their ability to protect cells from damage to DNA induced by heavy metals. Two experiments were conducted to evaluate the protective effects of curcumin against cyclophosphamide induced cytogenetic damage in swiss albino mice, in the first experiment the antigenotoxic effects of curcumin were studied in germ cells of mice. Three concentrations were tested 10, 15 & 20mg/kg of the curcumin the prepared slides were observed for various types of sperm head abnormalities. There was no increase in the percentage of abnormal sperms in curcumin treated groups when compared with control values. In the second experiment when animals were treated with highest concentration of cyclophosphamide, significant increase in the percentage of abnormal sperms was noted .However when animals were primed with curcumin prior to cyclophosphamide treatment, the cells showed inhibition in the percentage of abnormal sperms. Hence present results indicate that the curcumin afforded much protection against drug induced genotoxicity in germ cells of mice.

KEYWORDS: Curcumin, genotoxicity, cyclophosphamide, germcells, mice

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INTRODUCTION

Cyclophosphamide is an anti cancerous alkylating agent. The metabolites of this compound can alkylate nucleophilic sites in DNA, RNA and protein (Heminiki, 1985; Benson, et. al. 1988). It induces DNA single strand breaks at molecular level in rat embryos (Pillams, et. al., 1989; Little and Mirkes, 1987) in testicular cells (Skare and Schrotel, 1984). Further cyclophosphamide is capable of inducing structural chromosomal aberrations in Chinese Hamster cells, in human chorionic villae and various stages of spermatogenesis in germ cells. Further cyclophosphamide exposure produced adverse effects on progeny outcome by altering sperm nuclear components. The morphometric analysis of head region of spermatozoa with chronic doses of cyclophosphamide showed a significant increase over controls (Jianping, et. al., 1995). Curcumin (Dieteruloylmethane) in a yellow pigment derived from rhizome of the plant *Curcuma Longa*. The powdered schizome of this plant called turmeric is commonly used in curries preparation. It has preservative, flavoring and coloring properties of the diet, turmeric has been used in Asian medicine for generations for the treatment of many disorders, inflammation skin wounds, hepatic and biliary disorders cough, as well as certain tumors [Sharma et., al., 2005, Chandra and Gupta, 1972]. It has been shown to have a wide spectrum of biological actions; these include anticarcinogenic, antimutagenic and antibacterial properties [Egan et al., 2004, Singh et. al., 2007, Sandhya Rani and Rudrama Devi, 2001]. Curcumin showed modulatory effects of on the levels of benzo (a) pyrene induced DNA addicts in the rat liver [Thresiamma et al., 1998]. Pretreatment with curcumin gave protection against radiation induced cellular damage. However, no concrete evidence is available to indicate the protective effect of curcumin on cyclophosphamide induced genotoxicity. Hence in the present investigation an effort has been made to test the efficiency of curcumin against cyclophosphamide induced genotoxic damage in germ cells of mice.

MATERIALS & METHODS

Animals

Healthy male Swiss albino mice (8-10 weeks old) of *Mus Musculus* species with an average body weight off 27 gms procured from National Institute of Nutrition, Hyderabad were used for experiment. The mice were housed in polypropylene cages (29mmx 220mmx 140mm) bedded with paddy husk and were maintained at a temperature of 28ºC (± 2 ºC) and 50% humidity. The mice were fed with
standard mice pellets diet (MIS Lipton India, Bangalore) with water and cadmium.

Preparation of curcumin extract

Fresh rhizome **(***curcuma longa linn***)** were purchased from the local market and made it a coarse power with mortar and pistle. The powder (about 250g)was soaked in 500ml of ethanol for 72hrs. the solvent was runned through soxhlet apparatus and concentrated through rotavapour. Final extract was lyophilized to powder and stored at 4 ^{0}c until use. (Mara Etal 2003)

Sperm morphology assay

In this assay Two experiments were conducted. In the first experiment animals were fed with 10, 15 and 20 mg/kg of curcumin in split doses for five consecutive days. For priming experiment the animals were fed with 50mg/kg cyclophosphamide intrapertionmeally twice + three doses of curcumin simultaneously. Control group of mice were treated with 1 ml of physiological saline, five equal sub divisions of each dose were injected successively at intervals of 24h and animals were sacrificed 35 days after the first injection. Sperm collection was done from the cauda epididymis in physiological saline and stained in 0.1% aqueous Eosin. 5000 sperms were scored per animal and sperm head abnormalities were

categorized according to the procedure of Wryobek and Bruce, (1975). The data was analyzed using Chi-square test.

RESULTS & DISCUSSION

Cytogenetic methods for clastrogenic activity of environmental chemicals are an essential part of routine testing programs. The morphology of sperms serves as an important and sensitive indicator in assuming reproductive toxicology. They can be used to evaluate the spermatogenic damage, fertility and heritable genetic changes. In the present studies sperm morphology were analyzed according to the criteria of (Wyrobek and Bruce 1978). According to (Topham 1980) the characteristics controlling the sperm head shape are carried on autosomes and sperm abnormality test identified those agents which cause small alteration to the testis DNA. As several kinds of mutations can lead to abnormal sperm morphology, this test is considered more sensitive in detecting germ cell mutagens than other germinal mutagenicity assays (Wyrobek, et. al., 1983). Sperm morphology assay is also said to provide a quantitative method for locating genetic damage in male germ line cells. In our laboratory several drugs have been tested for the induction of sperm head abnormalities and published elsewhere (Rudrama Devi and Reddy, 1985). In the present investigation the higher incidence of sperm abnormality induced by clophosphamide is a measure of genetic damage caused at the spermatogonial stage of the mouse germ cells The results on the frequency of sperm head abnormalities in curcumin treated animals are depitled in Table 1. There is an increase in the percentage of abnormal sperms 2.80% in controls to 3.20, 3.40, 3.60 in 10, 15 and 20 mg/kg curcumin treated animals. The differences in the frequency of sperm abnormalities were found to be statistically insignificant when compound between control and treated groups. The results on the percentage of sperm abnormalities in cyclophasmide + cur cumin treated mice are presented in Table 2. These is significant decrease in the frequency of abnormalities n pretreated curcumin + cyclophosphamide treated group (P<0.05)

Table: 1

Frequency of sperm head abnormalities in mice administered with various doses of curcumin

Treatment	Normal sperms (%)	Abnormal sperms (%)
Control	4860 (98.20)	140 (2.80)
10mg/kg	4840 (97.80)	160 (3.20)*
15mg/kg	4830 (97.60)	170 (3.40)*
20mg/kg	4820 (96.40)	180 (3.60)*

The values in parentheses are percentages. *p>0.05

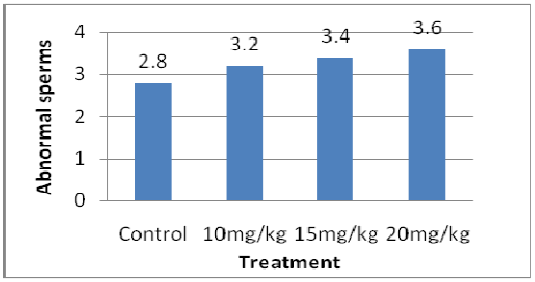
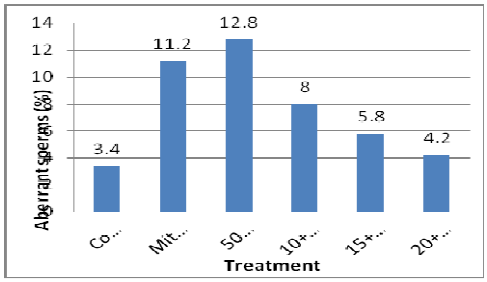


Table: 2
Frequency of sperm head abnormalities in cyclophosphamide treated mice primed with curcumin

Treatment	Normal sperms (%)	Aberrant sperms (%)
Control	4838 (97.60)	170 (3.40)*
Mitonusios	4435 (98.80)	565 (11.2)*
50 mg/kg	4360 (98.20)	640 (12.80)*
10+50 mg/kg	4600 (92.00)	400 (8.00)*
15+50 mg/kg	4710 (95.20)	290 (5.80)*
20+50 mg/kg	4790 (96.80)	210 (4.20)*

The values in parentheses are percentages. *P<0.05



The present results are comparable with that of (Jianping, et. al., 1995), who reported induction of aberrations of chromosome pairing at pachytene stage of mice oocytes at 10.30 and 50 mg/kg. in cyclophosphamide treated groups. The cyclophosphamide exerts its effects by targeting specific components of sperm nuclei. The DNA damage was determined by using alkaline elution method and secondly DNA templates function. All the rats were given cyclophosphamide 6.1 mg/kg/day daily for 1 or 6 weeks. No DNA single strand breaks and cross links in spermatozoa nuclei, damage to the genome by alkylation at this stage may be emulative, resulting in the production of dysfunctional germ cells (Qiu, et. al, 1995). But the chronic low-dose cyclophosphamide exposure failed to change the morphometric analysis of head region of spermatozoa and no differences in body, testes or epididymal weights between control and treated rats sperm count was diminished in cyclophosphamide treated group.Earlier studies have shown that post meiotic germ cells are specifically sensitive to cyclophosphamide treatment (Trasler, et. a., 1985, 87). The administration of low doses of cyclophosphamide to male rats for 6 weeks produced greater than 95% post implantation loss among their progeny (Transler, et. al., 1987). This loss caused to a male rat with cyclophosphamide was characterized by early pre implantation embryonic death (Kelly et. al., 1992). Some abnormalities in progeny outcome caused by cyclophosphamide treatment persisted to a subsequent generation (Hales, et. al., 1992). Thus the effects of cyclophosphamide exposure were both specific and heritable. Further chronic low dose exposure to cyclophosphamide produced adverse effects on progeny by altering sperm nuclear components. (Jianping et. al., 1995). Curcuma longa is a mandatory food additive and an individual in his diet 1-5g/day of powdered form of Curcuma (Turmeric).Which acts as a cleaning agent renders to protect against any diseases [Sharma et. al., 2005].

Several *in vitro* and *in vivo* studies showed the therapeutic potential of curcumin and protective effects of curcumin. It is anti inflammatory, anti hepatoxic, scabies, cancer, Alzimeir's disease [Shukla et. al. 2002, Giri et. al, 1988]. However in our study we aimed to assess the protective effect of curcumin against cyclophosphamide induced genotoxic damage. The chromosomal aberrations and a decrease in mitotic index are the most sensitive of bone marrow damage [Smalinskiene et. al., 2005, Jagetia and Aggarwal, 2007]. An effort has been made in the present investigation to assess whether such toxic effects induced by cyclophosphamide IV are neutralized or counter balanced by administration of curcumin. In addition to its preservative, flavoring or coloring properties in the diet, turmeric has been used in Asian medicine for generations for the treatment of many disorders including inflammation hepatic, biliary disorder cough and certain tumors based on short term studies conducted in animals and humans that curcumin is a safe agent when administered orally [Anand et. al, 2007]. No treatment toxicity was reported in 25 patients taking curcumin at concentrations upto 8000 mg/day for a period of 3months. Curcumin has also been shown as an immunostimulant and immunorestor in *in vivo* this mechanism may also participate in cancer preventive activity [Johnson and Mukhtar et. al, 2007] antimutagenic [Mukundam, et. al, 1993, Giri et. al, 1988].

However the geno-protective nature of curcumin has not been evaluated against cyclophosphamide induced genotoxic damage and protection by curcumin. Hence it can conclude that antioxidant such as curcumin protects the body from damage to free radicals. Further curcumin, a hydrophobic polyphenol has a wide spectrum of biological and pharmacological activities. It is a bis- α, β– saturated, β- diketone (diferuloy/methane) which exhibits keto-enol *tautomerism* having a predominant keto form to acidic and neutral solutions and stable enol form in alkaline medium [Pulla and Lokesh,1994]. Due to polyphenolic structure β – diketone functional group, curcumin is able to scavenge or neutralize free radicals by interacting with oxidative cascade, quenches oxygen and by chelating some metal ions and inhibits per oxidation of membrane lipids there by maintaining membrane integrity and their function[Meghana et. al, 2007]. Curcumin has been shown strong antioxidant activity and studies have shown curcumin reduce oxidative stress. Curcumin protects islets against streptozotocin induced oxidative stress by scavenging free radicals. Many authors have proved anti carcinogenic effects of curcumin on the inhibition of tumor formation in laboratory animals [Inano and Onada, 2001, Hamss et. al, 1999]. The results are also comparable with that of (Corona Rivera et al. 2007), who showed curcumin reusing the genotoxicity induced by copper ions by micronucleus and comet assay. Oral curcumin administration has been shown to prevent the development of cancers of the skin, soft plate, stomach, duodenum, colon ,liver, lung and Breast of rodents (Rao, et. al., 1995). Topical application has been showed to inhabit the initiation and promotion stages of chemically induced skin cancer.

CONCLUSION

Animals when treated with various doses of curcumin showed non mutagenic and the percentage of sperm head abnormalities were equivalent with that of control values. In the present study cyclophosphamide an anticancer drug showed significant increase in the frequency of abnormal sperm morphology, but when animals primed with curcumin , significant inhibition of cytogenetic damage was observed in cyclophosphamide treated animals. Thus the overall results indicate the protective nature of curcumin against drug induced damage in male mice.

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