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Curcumin Prevents Chromium Induced Sperm Characteristics In Mice

K Rudrama Devi*, M Mosheraju and K Dilip Reddy

Human Genetics and Molecular Biology Laboratory, Department Of Zoology, Osmania University, Hyderabad 500007
**Professor, Department of Zoology, University College of sciences, Osmania University, Hyderabad -500007, Andhra Pradesh, India.*

Abstract
Chromium was discovered in 1797 and found in all phases including air, water, and soil biota. Major uses of hexavalent chromium are metallurgical metal plating leather tanning etc. In spite its uses it has been reporting ITAR 1990 as a potent carcinogen teratogen and mutagen. The clastogenic potential of chromium has been well documented. In contrast to the activity of chromium, curcumin is a yellow colored derived from powdered rhizome *Curcuma Longa* is useful for the treatment of many disorders inflammation, skin wounds, certain tumors. Hence in the present investigation, studies were carried out to evaluate the protective role of curcumin on chromium induced gentoxicity in germ cells of male mice. In the present study four batches were of animals were maintained. Group II animals were given 60 mg/kg of chromium once in a week, whereas group III mice were given curcumin 5 and 7.5 mg /kg for five weeks. Group IV animals were pretreated with 5 and 7.5 mg/kg curcumin for 5 weeks plus chromium 60mg/kg once in a week. All the animals were sacrificed on the 35th day and cauda epidymis was processed and slides were screened for the incidence of various types of sperm head abnormalities. A significant increase was observed in chromium treated animals. However when animals pretreated with curcumin, the percentage of sperm characters decreased the significance of two dose levels. Thus the results clearly indicate the protective effect of curcumin against chromium damage in mice.

Key words: Curcumin, chromium VI, Metaphases, sperm head abnormalities.

Introduction
Elemental chromium was first discovered and characterized by a French chemist Nicolas – Louis vanquelin in Siberian red lead ore Crocoite in 1797, (Costa and Klein 2008). It is naturally elements in crustal abundance and is found virtually in all pahses including air, water, soil and biota (Losi et al., 1994).The major industries using chromium are the metallurgical, chemical and refracotyr brick industries (Losi at al., 1994). Major uses of hexavalent chromium compounds include metal plating, manufacture pigments and dyes, corrosion inhibitors chemical synthesis, refractory production leather tanning and wood preservation (Blade at al., 2007, Shanker at al., 2005)

Chromium has been studied for its potential genotoxicity in rats and mice (Wang et al., 2006, Devi 2001, Kiran et al., 2000) in chromium exposed population (Wu et al., 2000, Sarto et al., 1982, Gambelunghee at al 2003). It induced DNA damage by suppressing both DNA replication and transcription (Xu et al., 2004). It induces tumor in experimental animals and chromosomal aberrations, sister chromatid exchanges, cell transformations, gene mutations in mammalian cell cultures (Manning et al., 1994). Chromium and its intermediates interact directly with DNA to form DNA complexes that results in DNA protein and DNA amino acids crosslink (Tsouet et al., 1996).Workers occupationally exposed to chromium are considered to be an elevated risk for developing cancer through inhalation (Gibbs et al., 2000, De flora 2000).

Curcumin (Dieteruloylmethane) in a yellow pigment derived from rhizome of the plant *Curcuma Longa*. The powdered rhizome 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 (Agarwal et al., 2003, Sharma et al., 2005). It has been shown to have a wide spectrum of biological actions, these include anticarcinogenic, anti mutagenic and anti bacterial properties (Goel et al., 2000, Egan et al., 2004, Singh et al., 2007, Sandhya Rani and Rudrama Devi 2001, 2002). Curcumin showed modulatory effects of on the levels of benzo (a) pyrene induced DNA addicts in the rat liver (Mukundan 1993). Pretreatment with curcumin gave protection against radiation induced cellular damage (Thresiamma 1998). However, no concrete evidence is available to indicate the protective effect of curcumin on heavy metal chromium IV induced genotoxicity based on evaluation of sperm morphology protocol. Hence in the present investigation an effort has been made to test the efficacy of curcumin against chromium induced genotoxic damage in germ cells of mice.

Materials and Methods:

Chemicals:

Chromium VI, potassium dichromate (Molecular.Wt 291.26) molecular formula $K_2Cr_2O_7$, Curcumin crystalline $C_{21}H_{20}O_6$ was procured from Labo chem. Pvt. Ltd. The following features were noted. Molecular weight = 368.39, Minimum assay (Acidimetric 99% Melting point = 170-180^oC.

Animal Treatment:

The study was conducted after obtaining permission by Institution ethical committee On twenty four adult male Swiss albino mice 22-40 days old and weighing around 25 to 35 g were maintained in plastic cages under sterile conditions with relative humidity 50-51% and temperature 37±2^oC fed with feed and were given adlibitum access to a group 6 mice per experiment were procured and treated with potassium dichromate and curcumin. The doses were freshly prepared before treatment schedule only. The dose protocol was as follows Group 1 – control group (a) were treated only vehicle (water) for a day. Group II control group were treated with curcumin 5.0 & 7.5mg/animal/day for five weeks. Group III experimental group were administered with potassium dichromate 60mg/kg/animal for once in a week. Group IV experimental batch were pre treated with curcumin 5.0 & 7.5 mg/animal/day for 35 days and a weekly treatment of chromium IV 60mg/kg/day/animal was administered.

Sperm morphology assay:

On the 7th day, 1 hr after priming with curcumin, highest concentration of chromium was given orally to the animals on every 7th day for 5 weeks. Animals were killed on the 35th day after administration of the test chemical. The controls and treated group of animals were sacrificed by cervical dislocation. The animals were scarified on the 35th day after last treatment and sperm smears were prepared following the method recommended (Wyrobek and Bruce 1978). A total 2000 sperms per animals were scored for the incidence of different types of sperm head abnormalities such as banana, hammer head, head less, amorphous. The data was statistically analyzed using chi-square test. The protocol was approved by Institutional ethical committee of Osmania University, Hyderabad Andhra Pradesh.

RESULTS

Sperm head abnormality:

Sperm head abnormalities were classified into amorphous head, banana shaped head, without hook and hammerhead. The number of sperm head abnormalities were increased to 172-438 with in five weeks in the group of mice received 60 mg/kg of chromium (fig.3). A significant decrease in the percentage of abnormal sperm was observed in the group pretreated with the curcumin (5 and 7.5 mg/kg) from 3.5% to 3.6% in comparison with chromium treated group. $P<0.001$ (Table 1). The difference in the percentage of sperm head abnormalities between the control and group were analyzed using student t-test.

Discussion:

Cytogenetic methods for clastogenic activity environmental pollutants are routine testing programmes. The *in vivo cytogenetic* analysis of chromosomal aberrations is one of best methods to evaluate the clastogenic activity of chemicals drugs and environmental pollutions. Using analysis of sperm morphology assay in germ cells of mice is being carried out from our lab from the last two decades with heavy metals, like lead, cadmium and chromium, drugs such as cisplastin etc. (Kiran et al., 1999, Anuradha and Rudrama Devi 2009).

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 (Aggarwal et al., 2003,). 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. (Hatcher et al., 2008, Ergan et al., 2004). An effort has been made in the present investigation to assess whether such toxic effects induced by chromium 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 it humans that curcumin is a safe agent when administered orally (Agarwal 2003, Johnson 2007, Sharma 2005). 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 (Jagetia et al., 2008) and antimutagenic (Sandhya Rani and Rudram Devi 2001, Iqbal et al., 2003).

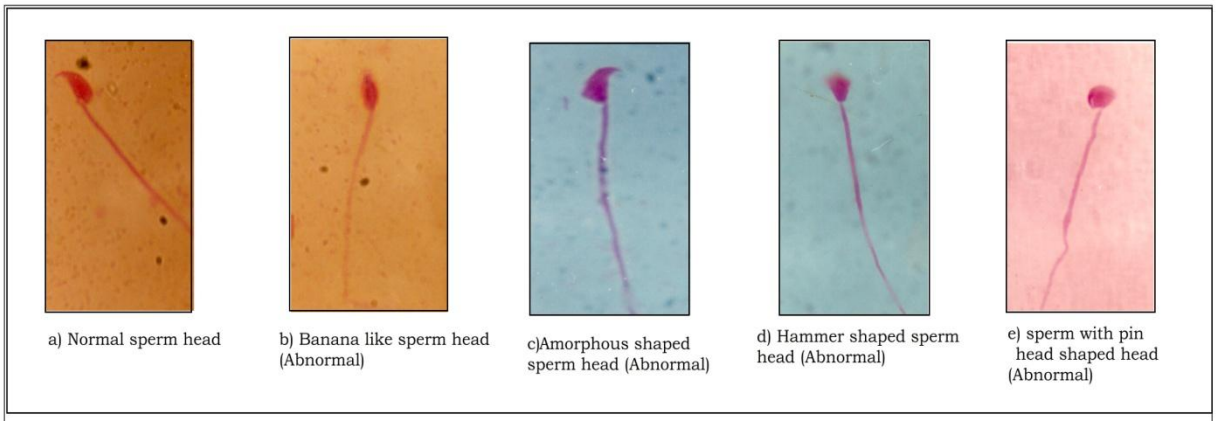

Fig: Incidence of Sperm Head Abnormalities induced by Chromium in male mice

Table: Protective effects of curcumin on chromium induced sperm alterations in mice

Group /dose	Normal sperms	Sperm head abnormalities				Abnormal sperms	
		A	B	H	HL	No.	%
I control	4828	53	41	32	46	172	344
II chromium 60mg/kg	4542	192	108	162	92	458	9.16 a*
III curcumin 50mg/kg	4830	60	36	40	34	170	3.50
7.5mg/kg	4820	64	42	44	32	180	3.60
IV curcumin + chromium 5.0+60mg/kg	4560	82	86	90	52	318	6.97*b
7.5+60mg/kg	4630	90	70	64	46	270	5.83*b

P<0.01

a- Denotes significance when compared with control group

b- Denotes significance when compared with chromium treated group

However the geno-protective nature of curcumin has not been evaluated against chromium 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 (Anand 2007), 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 (Pulla 1994). Curcumin has been shown strong antioxidant activity and studies have shown curcumin reduce oxidative stress (Iqbal et al., 2003, Cheng 2001). Curcumin protects islets against streptozotocin induced oxidative stress by scavenging free radicals (Meghana 2007). These observations are in according with our results where curcumin has been shown to render protection against chromium induced germ cell toxicity (Table.2). According to the reported studies curcumin raises the antioxidants status as wells as cause's stimulation of O^6- Methyl guanine DNA methyl transferase MGMT, a protein required for cellular protection (Niture et al., 2007, Rajeshwari 2006). Besides this curcumin induces HO^{-1} (That increases heme oxygenase activity) donates H atoms from phenolic group and inhibits iNOS induction leading to anti oxidant effect (Motterlini et al., 2000, Onoda and Inano 2000). It can be concluded that if organisms are administered with curcumin for longer period of time than perhaps toxic agents such as chromium VI might not be effective in induction of sperm head abnormalities in germ cells of mice.

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