INTRODUCTION
INTRODUCTION

In the present scenario of industrial development man is environmentally exposed to various chemical agents. These chemical agents may be cytotoxic in nature and cause various anomalies in the cells including chromosomal aberrations. Also, these agents may cause abnormalities in germinal cells leading to the formation of defected sperms/ova and hence causing sterility.

Exposure of human population to such cytotoxic agents including clastogens, mutagens and carcinogens is not limited to occupational settings since these agents have been found in airborne particles (Chrisp and Fisher, 1980; Hughes et al. 1980; Verma et al. 2004), diesel engine emission (Pepelko et al. 1980), cigarette smoke (De Marini, 1983; Whong et al. 1985), beverages and food (Ames, 1983), pesticides (Georgian, 1975; Trzos et al. 1978; Rani et al. 1980; Grover and Malhi, 1988; Grover et al. 1989; Nagpal et al. 1998; Amer and Ibrahim, 2000; Verma and Bhardwaj, 2003). Food is possibly the most chemically complex substance to which humans are exposed, undergoing various effects during production, storage, processing and cooking, often to form genotoxic compounds (Fishbein, 1979; Shelby and Matsushima, 1981; Dhir, 1989). Various pesticides and insecticides are used in agriculture and horticulture to protect plants from different pests. Cooking of proteinaceous foodstuffs leads to the generation of amino acid pyrolysates and quinoline compounds, which have been shown to be mutagenic in bacterial assays (Sugimura and Sato, 1983) and carcinogenic in experimental animals (Sugimura, 1985). Nutritionally essential metals too can induce diverse genotoxic effects at higher doses (Sharma and Talukder, 1987).

Cytotoxicity, carcinogenicity, mutagenicity as well as clastogenicity by these above said cytotoxic agents is caused by free radicals (e.g., reactive oxygen and nitrogen species), which are generated in vivo and cause damage to DNA, proteins, lipids and other biomolecules. Endogenous antioxidant defenses (e.g., superoxide desmutases H₂O₂ - removing enzymes, metal binding proteins) are inadequate to prevent their damages completely. So, diet-derived antioxidants are important. The evidences for a key role of vitamin E and C in free radical scavenging are strong, but for that of carotenoids and related plant
pigments are weaker. Interest is also growing in the role of plant phenolics, especially, flavonoids. Some antioxidants can exert pro-oxidant effects in vitro, but their physiological relevance is uncertain. Experimental approaches to the optimization of antioxidant nutrients intake are being proposed.

There are many reports in the literature of inhibitory effects of antioxidants and other compounds towards the clastogenicity or mutagenicity of a variety of chemical compounds (Calle and Sullivan, 1982; Ramel et al. 1986; Giri et al. 1988; Ghaskabdi and Vaidya, 1989; Brockman et al. 1992; Edenharder et al. 1999). Some compounds exert inhibitory effects on more than one clastogen. Whereas other have only been tested with a single clastogen. Now, stress is being given to look for the natural sources of anticlastogens, antimutagens and anticarcinogens. Various workers have given detailed reports on the anticlastogenic/desmutagenic activity of extracts from a number of plant and vegetable constituents suppressing the clastogenic/ mutagenic activity of several chemicals as well as various amino acid pyrolysates (Kada et al. 1978; Morita et al. 1978; Wattenberg and Loub, 1978; Yamada et al. 1979; Lai et al. 1980; Ames, 1983; Selvam et al. 1995; Ganasoundari et al. 1997,1998; Mukhopadhyay et al. 1998; Alekperov et al.1999; Biswas et al. 1999; Devi et al. 2000 ; Shishu et al. 2002). Epidemiological studies have also pointed out that fresh fruits and vegetables are low risk foods for gastric, colon and rectal cancers (Graham et al. 1972; Haenszel et al. 1972; Bjelke, 1974; Haenszel et al. 1976). Interest in the ingestion of and exposure to clastogens, mutagens and carcinogens, their mode of action and possible elimination of their toxic action through dietary anticalstogens, antimutagens and anticarcinogens has since been increasing.

Epidemiological surveys and laboratory investigations have yielded substantial evidence to establish that dietary agents can play an important role in inhibiting genotoxicity and carcinogenicity (Ames, 1983; Graham, 1983; Block et al.1992; Ferguson, 1994). Antigenotoxic and anticarcinogenic compounds are known to be present in commonly consumed vegetables, fruits, spices, cereals, nuts, vegetable oils, tea and coffee (Wattenberg, 1985; Abraham et al.1986; Abraham, 1989; Starvic,1994; Abraham et al.1998; Surh et al.1998). As a result, the intake of food and beverages with chemopreventive constituents has been recommended as an effective strategy for strengthening our defense against the
deleterious effects of genotoxins and carcinogens in the environment (Ramel et al. 1986; Morse and Stoner, 1993; Rogers et al. 1993). In this context, it would be of interest if more agents with antigenotoxic and anticarcinogenic properties are identified and assessed for their efficacy.

In the present investigations, an attempt has been made to study the cytoprotective effects of five medicinal plant extracts against genotoxicity of potassium dichromate ($K_2Cr_2O_7$) and ethyl methanesulphonate (EMS) in mice (Mus musculus) in vivo. The five medicinal plants undertaken for the study were jujube (Ziziphus jujuba), turmeric (Curcuma longa), ginger (Zingiber officinale), basil (Ocimum sanctum) and tea (Camellia sinensis). These medicinal plants were selected on the basis of their medicinal values and certain antioxidants present in them. Two cytotoxic agents i.e., $K_2Cr_2O_7$ and EMS were selected on the basis of their potent genotoxic nature as cited in the literature (Bigaliev et al. 1976; Venier et al. 1982; Munoz and Barnett, 1987; Gol'dina et al. 1989; Kondo and Ozawa, 1992; Sarkar et al. 1993; Itoh and Shimada, 1997; Kaur and Parshad, 1997; Mukherjee et al. 1997; Wise et al. 2002; Rizki et al. 2002; Goncharova et al. 2002; Benova et al. 2002; Wise et al. 2004). Two standard cytogenetic protocols i.e., assays of chromosome aberrations and sperm anomaly were adopted in the present study to determine the genotoxic effects of $K_2Cr_2O_7$ and EMS.

**Potassium dichromate ($K_2Cr_2O_7$) Exposure**

Potassium dichromate is a hexavalent chromium compound ($Cr_6^+$). Its molecular wt. is 294.19. It appears as orange red crystals. It is soluble in water and insoluble in ethyl alcohol. Potassium dichromate, as well as other chromates and dichromates, have various applications in the oxidation of organic and inorganic materials, e.g., the oxidation of anthracene to produce anthraquinone. They are used in the purification processes of chemicals, preparation of catalysts and production of pigments. Chromates are used to prevent rust and corrosion, e.g., in diesel engines. Chromium compounds are used in tanning chemicals, fungicides and wood preservatives. Production of light-sensitive dichromate celloids for lithography in the printing industry is also an outlet for chromium compounds. Welding of stainless steel releases hexavalent chromium
compounds in to the breathing zone of the welder. Welders are estimated to make up more than 1% of the workforce in the industrialized countries. Hazardous air contaminants related to manual welding, including aerosols and dusts containing chromium, are a significant source of occupational exposure.

**Ethyl methane sulphonate (EMS) Exposure**

EMS (CH$_3$SO$_3$C$_2$H$_5$) is generally used as a mutagen in research experiments. It is a colourless liquid with molecular wt. 124.15. Release of ethyl methanesulphonate to the environment from anthropogenic sources is expected to be minimal since this compound is available only as a research chemical. If released to water, EMS will hydrolyze (half-life 96 h at 20°C). Direct photolysis reaction with alkylperoxy radicals and singlet oxygen, bioaccumulation in aquatic organisms and adsorption to suspended solids and sediments in water are not expected to be important fate processes. If released to moist soil, EMS is expected to hydrolyze as fast, if not faster than in water. Mobility is expected to be extremely limited. If released to dry soil, this compound is expected to volatilize fairly rapidly. If released to atmosphere, EMS is expected to exist almost entirely in the vapour phase. This compound may be removed from the atmosphere by reaction with photochemically generated hydroxyl radicals (estimated half-life 30 days) or by wet deposition. Human exposure to EMS is expected to be limited to workers involved with research on this chemical. Dermal contact and inhalation of contaminated air are potential routes of exposure.
Medicinal Plants Undertaken for Present Study:

1.) *Curcuma longa* L. “Turmeric” (V. Haldi)

Family: Zingiberaceae

**Distribution:**

Turmeric is native to Asia and India. It is cultivated in almost all states in India.

**Important constituents:**

Rhizome contains curcumin, 4- hydroxycinnamoyl methane, bis-(4- hydroxycinnamoyl) methane, 2-(hydroxymethyl)- anthraquinone and ß- turmerone.

**Medicinal Uses:**

Turmeric is stomachic, carminative, blood purifier, vermicide, antiseptic and tonic. It is prescribed as an anti-periodic alternative in case of diabetes and leprosy. Taken with warm milk relieves pain. Its inhalation from boiling water relieves sore throat and common cold.

2.) *Camellia sinensis* L. “Tea” (V. Chai)

Family: Theaceae

**Distribution:**

Tea is native to southeast Asia, from Sri Lanka, India and China, tea has been planted widely in tropical and subtropical areas. In India tea has been planted in Assam, Darjeeling and Jalpaiguri districts of W Bengal. Other tea growing areas are Ranchi, Dehradun, Kangra and Kumaon districts. Also, grown in hilly regions of Western Ghats.

**Important constituents:**

Leaves contain caffeine, the phylline and tannins.

**Medicinal Uses:**

Decoction of leaves, used as folk bevarage, have anti-inflammatory, antihypertensive and sedative activities.
3.) *Ocimum sanctum* L. “Basil” (V. Tulsi)

Family: Labiateae

**Distribution:**

Basil is found all over India especially in the garden of each Indian family.

**Important constituents:**

Leaf oil contains cadinene, 1, 8-cineole, eugenol, limonene and methylchavicol. Flower oil have cadinene, β -caryophyllene, eugenol and humulene.

**Medicinal Uses:**

The infusion of juice of leaves is used to treat digestive complaints, bronchitis and cataract. To cure common cold, decoction of leaves is prescribed. A decoction of root is used in malarial fever.

4.) *Zingiber officinale* Rosc. “Ginger” (V. Adrak)

Family: Zingiberaceae

**Distribution:**

Widely cultivated in India.

**Important Constituents:**

Dry ginger contains proteins, fats, fibre, carbohydrates, minerals, Vitamin A, B and C. On steam distillation it yields 1.0 to 3.0% aromatic oil. The essential oil contains sesquiterpene, zinziberene, terpene, camphene and phellandrene. Also, constitutes linalool, geraniol and cineol. Ginger olcoresin contains gingerol, zingerone, shagaol, volatile oil, resins and phenols.

**Medicinal Uses:**

Ginger has carminative and stimulative properties and used in mild diarrhea, colic and dyspepsia.

5.) *Ziziphus jujuba* L. “Jujube” (V. Beri, Ber)

Family: Rhamnaceae
Distribution:

The Indian jujube is native from the province of Yunnan in southern China to Afghanistan, Malaysia and Queensland, Australia. It is indigenous and naturalized throughout India.

Important constituents:

Fruits are rich in vitamin C and contain many rare minerals. Bark contain tannin and a number of alkaloids viz- sativanine D,E,F,G,H and K. All of these act as antioxidants.

Medicinal Uses:

Bark of plant is used in healing ulcers and wounds. In soar throat leaf decoction is used as gargle. For dysentery and diarrhea, decoction of root and bark is prescribed.
Objectives of the study

The present study is aimed to investigate:

1. Cytotoxicity of $K_2Cr_2O_7$ and EMS on mice somatic and germinal cell chromosomes.

2. Effect of $K_2Cr_2O_7$ and EMS on mice sperms so as to know their effectiveness in causing sterility.

3. Comparative cytoprotective effects of the extracts of medicinal plants jujube ($Ziziphus jujuba$), turmeric ($Curcuma longa$), ginger ($Zingiber officinale$), basil ($Ocimum sanctum$) and tea ($Camellia sinensis$) at pre, concurrent and post treatment levels so as to find the effective dose against the above cited cytotoxicity.