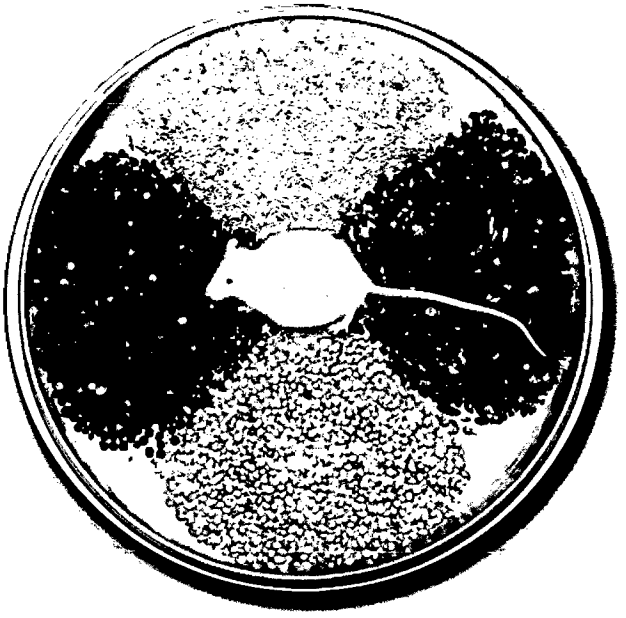
"MUTAGENIC/ANTIMUTAGENIC POTENTIAL OF SPICES OF COMMON USE IN INDIAN DIET"



Thesis Submitted to
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For the Degree of
DOCTOR OF PHILOSOPHY
IN ZOOLOGY

By
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AUGUST 2003

Dedicated to my

Parents, Brother

and Suresh

DECLARATION

I, ***Raghurama Bhat K.*** *hereby declare that this thesis entitled* ***"Mutagenic/Antimutagenic potential of spices of common use in Indian diet"*** *embodies the results of bonafide research work carried out by me under the guidance and direct supervision of* ***Dr. S.K. Shyama****, Department of Zoology, Goa University, Goa during 1998-2003. I further declare that this thesis or a part thereof has not been the basis for the award of any degree or diploma of this or any other University or Institution.*

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CERTIFICATE

This is to certify that the thesis entitled ***"Mutagenic/Antimutagenic potential of spices of common use in Indian diet"*** *is based on the results of laboratory investigation carried out by* ***Mr. Raghurama Bhat K.****, during 1998-2003 under my guidance and supervision for the degree of Doctor of Philosophy of Goa University. No part of the work has been submitted to any other University or Institute for the award of any degree or diploma.*

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CHAPTER - I

INTRODUCTION

Congenial environment, including nutritive and non-toxic food, is the utmost requisite of every man and has a telling effect on the status of his health. Although the main causes of death in developing countries are malnutrition and infectious diseases, in developed countries they are the exposure to toxic and hazardous chemicals, may be dietary components, synthetic chemicals or any other pollutants. Some of these chemicals may damage the genetic material and induce varieties of heritable diseases. After the establishment of chemicals in the environment as one of the major causes of genotoxicity and cancer, their elimination from human environment has been an immediate concern of regulatory agencies and the public. Hence the identification and elimination of such chemicals, including the components of our daily diet, from our society is the urgent need of the day.

Most of the people are consuming large quantities of plants and plant products daily in the form of food. Now-a-days consumption of vegetarian food has increased all over the world and particularly in India. The reason for this is very simple being the general belief that "vegetarian food is quiet safe and free from after effects". However the validity of this statement is at stake because of the fact that a large number of chemicals derived from plant sources have shown either mutagenic/carcinogenic properties.

Considerable interest has been evinced in the natural substances/agents capable of inactivating environmental mutagens/carcinogens. Now emphasis has been given to search, characterize and promote the use of such natural antimutagens/ anticarcinogens, which can neutralize or decrease the effects of potent mutagens/carcinogens significantly.

1.1. TOXICOLOGY

Toxicology is the science of poisons. The word toxicology is derived from two Greek words: *toxicon* = arrow (poison) and *logos* = study or discourse. In general, toxicology is the study of adverse effects of chemicals on various biological systems including humans. Scientifically, toxicology is the qualitative and quantitative study of the adverse or toxic effect of chemicals and other anthropogenic materials or xenobiotics on organisms (Gupta, 1985a; Sharma, 2000).

1.2. GENOTOXICITY

'Genetic toxicology' is the study of damages to the genes by chemical or physical agents. Damages to the genes (i.e., to DNA) if not repaired timely and correctly, change the DNA sequence and cause mutations. Mutations often result in the elimination or alteration of gene functions and if the damages are not lethal, will also lead to inheritable changes. Genotoxicity is thus customarily defined as the ability of toxic agents to damage DNA and to change DNA sequence. DNA sequence changes can be single nucleotide changes that result in point mutations or multiple nucleotide changes that result in visible chromosomal aberrations (Roy, 1986). The adverse effect of a mutation is dependent on the gene and the tissue affected. The most serious effects of mutations in somatic cells are neoplasms and in germ cells, inheritable neoplasms or birth defects (Arlett, 1990).

Mutation is any heritable change that may either be a chemical transformation of an individual gene called 'gene or point mutation' or its altered functioning or gross abnormalities in the genetic material. Mutation is a change in the genetic information, which is transmitted through mitotic or meiotic nuclear divisions (Zimmermann and Mayor, 1985). It may occur in any somatic cell or germ cell, the

results of which are usually undesirable and include fetal death (lethal mutation), congenital anomaly, genetic disease, lowered resistance to disease, decreased life span, infertility, cancer, etc. (Shaw, 1972). There is increasing evidence that mutations in somatic cells are not only involved in the carcinogenesis process but do also play a role in the pathogenesis of other chronic degenerative diseases, such as atherosclerosis and heart diseases, which are the leading causes of death in the human population (De Flora *et al.*, 1996).

Some environmental agents induce gross chromosomal abnormalities or mutations in the DNA molecule (Malling, 1972). Accordingly they are classified as mutagenic or genotoxic or clastogenic agents. Mutagens are agents that cause mutations in the exposed organisms. Chemicals, which break chromosomes and damage DNA are termed as clastogens (Shyamaprasad *et al.*, 2002; Choudhury and Sahu, 2003). A carcinogen is an agent that significantly increases the yield of neoplasms in a population. In addition, a multitude of environmental/dietary factors which by themselves are not carcinogenic, may operate to modify cancer growth resulting either in its enhancement (cancer promotion) or inhibition (anticarcinogenesis) (Kothari and Mehta, 1973).

1.3. MUTAGENICITY AND CARCINOGENICITY

Our environment contains a vast variety of agents that are detrimental to human health. Agents which are acutely toxic are readily detected because the immediate effects allow rapid identification of the source of toxicity. Tumours are not the immediate result of an exposure to carcinogenic agents. Rather, they are only detected many years after the exposure (Zimmermann and Mayor, 1985).

Genetic toxicology tests are designed to detect mutations (Natarajan and Obe, 1982; Sugimura and Nagao, 1982). Boveri's "somatic cell mutation theory of carcinogenesis" postulates that cancer can be caused by mutations. This theory supports the association of mutations to neoplasms. Accordingly, it seems reasonable to use mutagenicity tests to identify carcinogens (Gandhi *et al.*, 2003). The limitation of this is that these tests cannot detect non-genotoxic carcinogens (Choy, 1996a).

Several established carcinogenic and non-carcinogenic chemicals have been tested for their mutagenicity and shown that there exists a strong correlation between mutagens and carcinogens (Berenblum, 1972; Wilson, 1972; Loprieno, 1980). Majority of the carcinogens tested (70-80%) have been detected as mutagens (Nagabhushan, 1987).

Testing for mutagenic activity is used not only for the detection of mutagens but also carcinogens, as it has turned out that mutagenicity tests can be used as short-term carcinogenicity tests (Gupta, 1985b; Waters *et al.*, 1999). Mutation assays are shorter and less costly than classical carcinogenicity tests and thus it has become feasible to develop systems for the routine control of mutagenic/carcinogenic environmental pollution (Aeschbacher, 1980; Matter, 1980).

Each genetic toxicology test measures only mutations in a single species at a specific genetic marker and hence a battery of several genetic toxicology tests are necessary to assess the mutagenicity of a chemical (Choy, 1996b).

1.4. ANTIMUTAGENICITY AND ANTICARCINOGENICITY

'Antimutagenicity' on the other hand is the capacity to inhibit mutagenesis and several such inhibitors of mutagenesis have been demonstrated to be inhibitors of carcinogenesis (Bronzetti *et al.*, 1990). Further, antimutagenicity, which is of both

intrinsic and practical interest, has been receiving emphatic attention and is rapidly progressing in the recent years hand in hand with genotoxicity. Exposure of man, over a period, to environmental mutagens and carcinogens has a possible additive/synergic effect. Hence, contending with mutagens is of vital consequence. In this line, various antimutagens which protect genetic material from mutagens such as glutathione, antioxidant vitamins, chlorophyll, aromatic isothiocyanates and plant phenolics have been identified (Ames, 1983; Odin, 1997). More intensive and sincere effort towards identifying more efficient antimutagens is of great significance in reducing the continuously accumulating mutagenic events in tissues.

As there is no known safe concentration of threshold limit, there should be increased efforts to identify agents that can counteract or eliminate the activity of man made and naturally occurring mutagens in our environment. Such agents generally referred to as Antimutagens/Anticlastogens/Anticarcinogens, should have practical use to humans.

The term "Antimutagen" is used to describe the agents that reduce the frequency or rate of spontaneous and induced mutations by diverse mechanisms (Water *et al.*, 1996).

"Anticlastogens" are agents that can reduce the amount of chromosomal damage induced by clastogens (Gebhart, 1992). "Anticarcinogens" are agents that specifically or preferentially inhibit the development of cancer. It may act to inhibit either in the initiation or the promotion phase of the carcinogenic process. Many anticarcinogens are also antimutagens and these are also potentially important protective agents against diseases other than cancer (Ferguson, 1994). A very close correlation exists between anticlastogenic and antimutagenic action and one could also replace both terms by desclastogenic and desmutagenic respectively (Gebhart,

1992). Anticlastogenesis is an essential part of antimutagenesis and perhaps also of anticarcinogenesis. Antimutagenic substances might prevent cancer because they could destroy mutagens in or out of body cells or block mutagens which damage DNA and cause mutations in cells (Ruan, 1989).

1.5. DIET ASSOCIATED ANTIMUTAGENS/ANTICARCINOGENS

Dietary inhibitors of mutagenesis and carcinogenesis are of particular importance because they may have a role in human cancer prevention. Several inhibitors of mutagenesis have been demonstrated to be inhibitors of carcinogenesis. Majority of antimutagens have been discovered by the use of short term assays that are simple "*in vitro*" system suitable for elucidating the mechanisms of inhibition. Antimutagens may act through different mechanisms. For example, both carotene and selenium have been shown to be antimutagens, but they show no common features in either structure or the cellular processes they affect. Some antimutagens could increase the combined antimutagenic activity (synergism) e.g. Vitamin E in combination with either Vitamin C or selenium. On the other hand some substances, shown to be antimutagens and/or anticarcinogens, under certain experimental conditions may become ineffective or even genotoxic (Bronzetti *et al.*, 1990). Epidemiological studies are necessary to elucidate the correlation between inhibition of mutagenesis and cancer. Many antimutagens are widely distributed in our common food. Human diet is a complex mixture and cooperation or interaction between various components of diet is an interesting field of investigation. At present we do not have sufficient information about the behaviour of antimutagens and anticarcinogens to extrapolate data from bacteria to the effect in man. It is thus very important to establish the relationship between the antimutagenic activity of

compounds in short term assays with their capacity to prevent tumours in mammals including man.

The study of antimutagenic factors is assuming increasing importance. The increased use of antimutagens is expected to minimize the risk caused by mutagenic agents even if man cannot eliminate the exposure to some of them. Majority of the inhibitors of mutagenesis (antimutagens) have been discovered by the use of short-term assays, particularly the Ames test. The simple *in vitro* system has provided opportunities to elucidate the mechanisms of mutagenesis in prokaryotes but screening for antimutagenic activity in eukaryotes is required before one can assume anticarcinogenic activity of an agent. It is therefore necessary that antimutagens be tested in eukaryotic systems including mammals so that we can extrapolate the findings to man. According to Wattenberg (1981), chemopreventive agents can be placed in two major categories, i.e., blocking agents, which prevent carcinogens from reaching or reacting with critical target sites and suppressing agents, which prevent the evolution of the neoplastic process. Blocking agents are inhibitors of tumour initiation, while suppressing agents can be identified with inhibitors of promotion/progression. (Morse and Stoner, 1993). The ICPEMC Expert Group on Antimutagens and Desmutagens made a distinction between stage-1 inhibitors, acting extracellularly and stage-2 inhibitors, acting intracellularly (Ramel *et al.*, 1986). The first studies on antimutagens were made in Japan about 30 years ago, when Kada *et al.* (1982) classified antimutagens as:

Desmutagens: All the factors that inactivate mutagens "*in vitro*" before they reach the cells i.e., they act outside the cells. According to Jain *et al.* (1987) 'desmutagens' are substances which somehow destroy or inactivate, partially or fully

the mutagens (before mutagens can attack DNA), thereby affecting less cell population.

Bioantimutagens: All the factors that interfere with cellular functions. These factors act inside the cells (interfere with fixation of DNA damage). This classification has since been amplified as shown in the Fig.1.1.

Unfortunately the simple identification of antimutagenic substances represents a limited criterion for a rational implementation of chemopreventive measures, aimed at fortifying the host defence mechanisms. The main reason for this pessimistic view is that epidemiological investigations and the results of experimental studies reported in the literature are often contradictory. Compounds which afford beneficial effects under certain conditions may become ineffective or even deleterious e.g., at a different dose, time of administration or when associated with other chemicals. These discrepancies may also reflect the multiple biological properties of inhibitors and the complexity of the test organisms. It is therefore important to have a deeper understanding of the mechanisms of action of antimutagens.

Dietary factors seem to be one of the major causes of cancer but a number of inhibitors of mutagenesis are also components of the human diet (Bronzetti *et al.*, 1990). Diet also contains factors that can moderate the process of mutagenesis and/or carcinogenesis. Antimutagenic substances have been found in various food items including meat, eggs, cereals, fats, oils, vegetables, fruits, beverages, (tea, coffee, milk, alcohol, and so on) and in synthetic compounds such as food additives.

The antimutagenic and anticarcinogenic effects of chemicals and natural compounds are being investigated in many laboratories of the world. More than 500 chemicals have been assayed as potential cancer chemopreventive agents with representatives of at least 25 different classes showing protective effects (Ferguson,

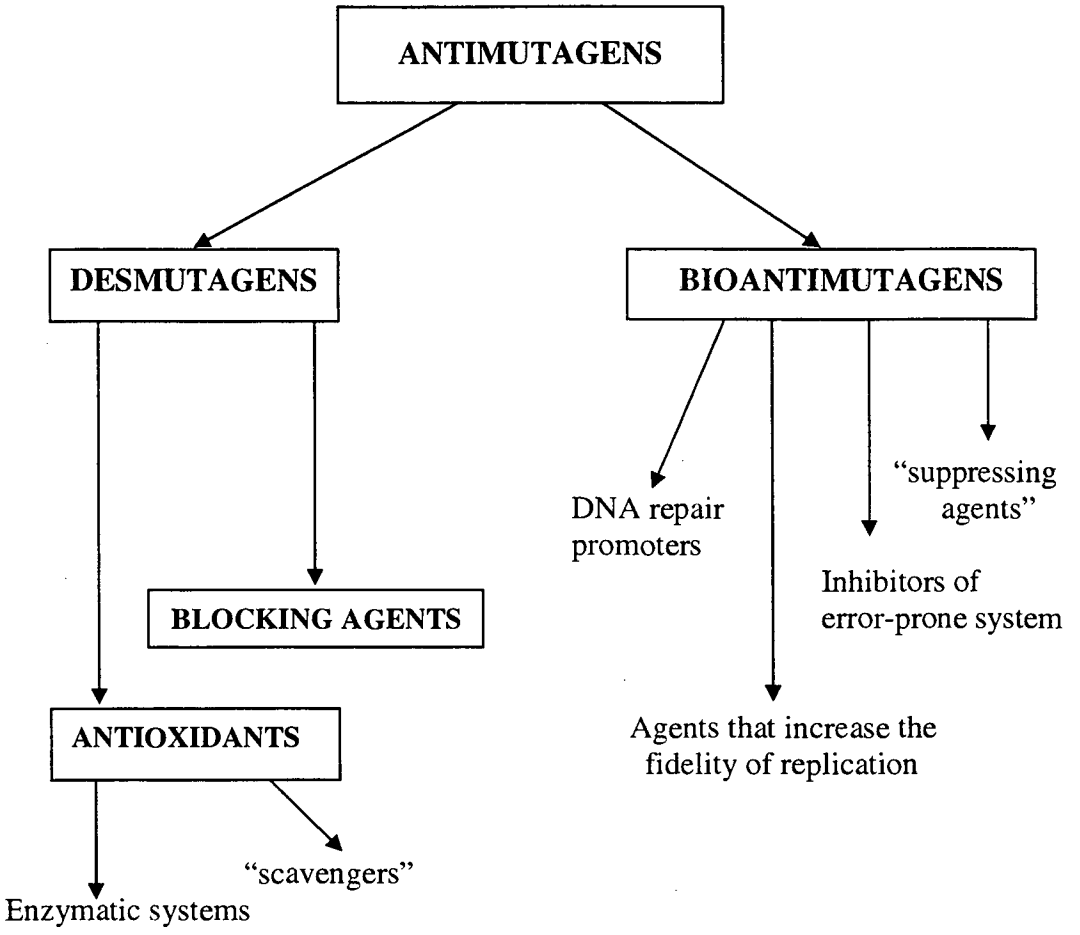


Fig. 1.1. Classification and activities of antimutagens.
(**Source**: Bronzetti *et al.*, 1990)

1994). At present there is substantial evidence to show that chemical mutagenesis and carcinogenesis can be inhibited by a large number of naturally occurring compounds of plant origin (Wattenberg, 1985; Ramel *et al.*, 1986). Interestingly many of these inhibitors are minor constituents of some of the commonly consumed vegetables, fruits, spices and beverages like tea and coffee (Stich *et al.*, 1982; Ames, 1983; Jain *et al.*, 1987; Aruna and Sivaramakrishnan, 1990; Sasaki *et al.*, 1993; Edwin *et al.*, 2002; Jankun *et al.*, 2003). In view of these findings, there is a growing awareness of the fact that it may be possible to protect humans against the genotoxicity and carcinogenicity of the environmental chemicals by manipulating the intake of dietary agents and chemicals (Ramel *et al.*, 1986).

The search for antimutagenic agents is very important since mutagenic and carcinogenic factors are omnipresent in human environment and elimination of all of them seems to be impossible. Moreover, several well known mutagenic risk factors are so closely connected with a modern lifestyle that their entire eradication may be very burdensome and even unattainable (De Flora, 1988). For these reasons the antimutagenic agents should be taken commonly and continuously. It is also well known that fruits and vegetables contain a considerable amount of antimutagenic/ anticarcinogenic compounds (Ames, 1983; Dragsted *et al.*, 1993).

1.6. AYURVEDA AND DIETARY FACTORS

India occupies even today a topmost position in the use of herbal drugs and drug extracts for the treatment of diseases. Varieties of spices are routinely used in Indian diet in order to give flavour to the delicious dishes. In Ayurveda and Homeopathy they add flavour to the life of man in keeping sound health (Chelladurai, 1991, 2003; Thomas, 2003; Vijayan *et al.*, 2003). Ayurvedic pharmacology is based

upon the concepts of Taste (Rasa), Energy or Potency (Veerya) and Post-digestive effect (Vipak). These concepts have to do with the subtle phenomena relating to taste as well as the hot (Ushna) and cold (Sheeta) effects of foods (Lad, 1994). Organic and inorganic substances create different tastes and temperature sensations when they pass through the mouth, stomach, small intestine and large intestine (Gupta, 1986).

Rasa is the first experience of taste when a substance is placed on the tongue. Further the substance is swallowed and then enters the stomach, the hot or cold experience is felt immediately or later is called veerya (potency). The veerya sensation or action, then has to do with the heating and cooling properties of substances. Food also has a pro-digestive effect that is called Vipak. For instance, most starchy foods become sweet after chewing and digestion, hence their post-digestive taste or vipak is sweet (Savnur, 1950; Dahanukar and Thatte, 1989). These three concepts directly influence three bio-energies or tridoshas (three different basic types of human constitution) i.e. Vata (=kinetic energy in the body), Kapha (=potential energy in the body) and Pitta (=controls balance of kinetic energy and potential energy) and also influence nutrition and transformation of the bodily tissues or dhatus (Sandu, 1987; Rhyner, 1998).

1.6.1. Veerya/Potency:

Veerya is the energy, potency or power of herbs, designated in Ayurveda as heating or cooling. Herbs through their taste tend to heat the body or cool it and this produces the most basic energizing effect upon the system.

Ayurvedic diagnosis of disease based on three biological humours (vata, pitta and kapha) and treatment is according to six tastes. These apply not only to herbs but also to foods and minerals. The six tastes are classified as heating or cooling to different degrees. Hottest generally is pungent, followed by sour and salty which

increase pitta (=fire or bodily heat energy). Coldest is bitter, followed by astringent and sweet which decreases pitta. Heating or cooling energy (ushna and sheeta) means that these substances contain the energies of fire or water (agni or soma) respectively (Kapoor, 1990; Svoboda, 1993).

Heating herbs said to cause dizziness, thirst, fatigue, sweating and burning sensations. Further, they promote digestion and decreases semen. Cooling herbs are refreshing, enlivening, cooling, life promoting, increases semen and promotes tissue firmness.

1.7. SPICES AND CONDIMENTS

Spices and condiments need no introduction because India is known the world over as "The Home of Spices". Spices constitute an important group of agricultural commodities that are virtually indispensable in the culinary art. Besides the consumption of huge quantities for flavouring foods, spices are also used in medicine, pharmaceuticals, perfumery, cosmetics and several other industries in our country.

According to the International Organization for Standardization (ISO), there is no clear-cut division between spices and condiments and they have been clubbed together. The term 'spices and condiments' applies to "natural plant or vegetable products or mixtures thereof, in whole or ground form, as are used for imparting flavour, aroma, piquancy and for season of foods" (cf. Pruthi, 1998).

There are over 80 varieties of spices grown in different parts of the world. India is rich in spices and about 50 species/varieties of them are grown here. Spices could be classified or grouped according to different classification, viz. according to their botanical analogies or families, economic importance (major and minor spices),

similarity in methods of cultivation, similarity in plant parts or components used (Pruthi, 1998).

There are five major spices, namely black pepper (the most important spice of India and also of the world), capsicum/chilli, ginger, turmeric and cardamom. Important minor spices grown in India includes coriander, cumin, fennel, fenugreek, garlic, onion, saffron, and vanilla. Spices may comprise different plant components or parts such as barks, berries, buds, leaves, floral parts, fruits, rhizomes, roots, seeds, etc.

Spices form a major class of natural ingredients used in most food products today. Indian food is well acclaimed the world over for its richness in taste and aroma, due to the variety of spices that form a distinguishable part of our food. In western countries and indeed in most parts of the world, both flavour and colour value of spices are utilized to a great extent.

Spice extracts serve as an alternative to whole and ground spices (Menon, 2003). Spice extracts consists of oleoresins and essential oils. Essential oils are the aromatic, volatile components present in most spices that provide the characteristic flavour and aroma to the spice and do not impart colour. Oleoresins are the viscous-to-tacky material extracted from ground spices with volatile solvents and gives more flavour than essential oil. It consists of essential oils, soluble resins and other non-volatile components responsible for the intrinsic qualities of spices such as heat components and colouring components (Sreekumar and Arumughan, 2003). Oleoresins contain natural antioxidants and are free from enzymes.

1.8. SPICES SELECTED FOR STUDY AND THEIR CHARACTERS

The dietary pattern varies from country to country and even from region to region within the country, as in India. India is well known for the use of spicy food and spices have played a crucial role in the history, economy, medicine and diet of the country. Varieties of spices such as chilli, turmeric, pepper, ginger, cardamom, clove, etc. are daily consumed in considerable amount by the Indian population.

In this context the present work was designed to study the mutagenic/antimutagenic effects of few of the spices which are being consumed by a large number of people for a very long period. Further, some of the spices that are found to be extensively used by the people of our country were selected based on economic value and potency (veerya) of spices.

As such, not many spices have been explored to any great extent for the ability to produce genotoxicity. Therefore, in this present work an attempt was made to explore scientifically the possible hidden mutagenic/antimutagenic potentials of few selected spices employing a very popular *in vivo* animal model Swiss albino mice *Mus musculus*. These spices were administered in the form as we consume them.

1.8.1. *Piper nigrum* Linn. : (Plate- Ia)

Common name: Black pepper (Sanskrit-Maricham, Hindi-Kali mirch, Konkani-Mirin)

Family: Piperaceae

Potency/Veerya: Hot (ushna)

Economic value: Major

Parts used: Fruits

Black pepper is known to everyone due to its day-to-day use as one of the most popular spices. It is rightly considered as the 'King of spices' as judged from the column of international trade and is also known as 'Black gold of India'. Pepper is the

dried, mature but unripe berries (fruit) of *P.nigrum*, a branching vine or a perennial climbing shrub. It may be also noted that white pepper is produced from the ripened berries, but are without the fleshy portion of the pericarp (Warrier *et al.*, 1995b).

Pepper constitutes an important component of culinary seasonings of universal use and is an essential ingredient of numerous commercial foodstuffs. Oil of pepper is a valuable adjunct in the flavouring of sausages, canned meats, soups, table sauces and certain beverages and liquors. One of the principle values of pepper is its ability to correct the seasoning of dishes. Just before the end of cooking, a final dash of pepper can be used effectively to adjust the flavour (Pruthi, 1998).

The ancient Aryans considered it as a powerful remedy for various disorders of the anatomical system and prescribed it as an effective cure for dyspepsia, malaria, delirium, tremors, hemorrhoids, etc. A paste of black pepper is a rubefacient and stimulant. It is locally used for boils, sore throat, piles, paralytic affections, headache, toothache, cholera, cough, gonorrhea, diarrhoea, etc. (Anonymous, 1969; Kapoor, 1990).

The principal constituents of black pepper are resin, volatile oil and piperine or piperia. It also contains a little fatty oil, starch (34.8%) and other ingredients and about 5 per cent of inorganic matter may be obtained from it by incineration (Bentley and Trimen, 2002b). The alkaloid piperine ($C_{17}H_{19}O_3N$, m.p. 129-30^0) is considered to be the major constituent (5 to 9%) responsible for the biting taste of black pepper which is absent in the leaves and stems of pepper plant. Piperine is sparingly soluble in water, readily soluble in alcohol and on hydrolysis splits into piperidine and piperic acid. Other pungent alkaloids occurring in pepper in small amounts are Chavicine, peperidine (5%) and piperetine ($C_{19}H_{12}O_3N$, m.p.146-49^0). Chavicine, a resinous isomer of piperine is said to be the most bitter ingredient of pepper and on hydrolysis

yields piperidine and isochavicinic acid (an isomer of piperic acid). The characteristic aromatic odour of pepper is due to the presence of a volatile oil (1 to 2%) in the cells of pericarp (Singh and Singh, 1996).

1.8.2. *Elettaria cardamomum* Maton.: (Plate-Ib)

Common Name: Cardamom (Sanskrit-Ela, Hindi-Chhoti elaichi, Konkani-Velchi)

Family: Zingiberaceae

Potency/Veerya: Cold (sheeta)

Economic value: Major

Parts used: Dried ripe seeds

Lesser cardamom, green cardamom or Malabar cardamom is the 'true cardamom of commerce' and is popularly known as the 'Queen of spices'. It is one of the most valued spices of the world.

A good portion of the cardamom produced in India is consumed internally, for chewing or as a masticatory, as a common ingredient of special seasonings and curry powders and for flavouring sweet meats. It is also used in pastries, cakes, other bakery products, puddings, kheer, meat curries, sausages, various kinds of foods and culinary preparations (Pruthi, 1998).

In medicine, it is used as a powerful aromatic stimulant, carminative, stomachic and diuretic. It also checks nausea and vomiting and is also reported to be a cardiac stimulant. The seeds are very useful in asthma, bronchitis, piles, stangury and diseases of the bladder (Anonymous, 1953; Warrier *et al.*, 1995a).

Cardamom seeds owe their properties essentially to the presence of a volatile oil (4 to 8%). This volatile oil has the odour and flavour of the seeds in a concentrated degree. Principal constituents of the oil are cineol, terpinene, limonene, sabinene and terpineol in the pure form or in the form of formic and acetic acids (Bentley and

Trimen, 2002b). It also contains starch (45.4%), essential oil (0.5%), vitamins and inorganic matter. Analysis of cardamom capsule shows the following: moisture (20.0%), protein (10.2%), ether extract (2.2%), mineral matter (5.4%), crude fibre (20.1%), volatile oil (7.4%) carbohydrate (42.1%), calcium (0.13%), phosphorus (0.16%) and iron (5.0 mg/100 g).

1.8.3. *Coriandrum sativum* Linn. : (Plate-Ic)

Common Name: Coriander seed (Sanskrit-Dhanyakam, Hindi-Dhania, Konkani-Kothmir)

Family: Umbelliferae

Potency/Veerya: Cold (sheeta)

Economic value: Minor

Parts used: Fruits and leaves

Coriander is one of the first spices to be used by mankind, having been known as early as 500 B.C. Coriander seed and fresh coriander leaves are well known and used almost daily in scores of curries, etc. It is actually housewife's secret of tasty dishes (Pruthi, 1998).

The fruits (seeds) are extensively employed as condiment in the preparation of curry powder, pickling spices, sausages and seasonings. They are used for flavouring pastry, cookies, buns, cakes and tobacco products.

Coriander seeds are considered to be carminative, diuretic, tonic, stomachic, antibilious, refrigerant and aphrodisiac. It is generally used as infusion or decoction in sore throats, flatulence, indigestion, vomiting and other intestinal disorders (Anonymous, 1950; Warrier *et al.*, 1995a).

Seeds contain moisture (11.2%), protein (14.1%), fat (16.1%), carbohydrate (21.6%), fibre (32.6%), mineral matter (4.4%), calcium (0.6%), phosphorus (0.37%), iron (17.9 mg/100 g) and vitamins. The aromatic odour and taste of coriander fruits is due to volatile oil (1.0%) which is pale yellow liquid. The seeds contain 19 to 21% fatty oil having dark, brownish-green colour and an odour similar to that of coriander oil. The distilled oil contains 65 to 70% of linalol (coriandrol) and pinene (Bentley and Trimen, 2002a).

1.8.4. *Cuminum cyminum* Linn. : (Plate-Id)

Common Name: Cumin seed (Sanskrit-Jeeraka, Hindi-Zira, Konkani-Jiren)

Family: Umbelliferae

Potency/Veerya: Hot (ushna)

Economic value: Minor

Parts used: Fruits/seeds

Cumin is one of the oldest spices, known since Biblical times. It comprises the dried yellowish to light greyish brown seeds. Cumin seeds have an aromatic odour that is peculiar, strong and heavy, while flavour is warm and somewhat bitter taste.

They are largely used as condiment and form an essential ingredient in all mixed spices and curry powder for flavouring soups, pickles and for seasoning breads and cakes (Pruthi, 1998).

Cumin seeds have long been considered stimulant, carminative, stomachic, astringent and useful in diarrhea, dyspepsia, and gonorrhea. It is also useful in hoarseness of voice (Anonymous, 1950; Warrier *et al.*, 1995a).

Seeds contain moisture (11.9%), protein (18.7%), ether extract (15.0%), carbohydrates (36.6%), fibre (12.0%), mineral matter (5.8%), calcium (1.1%) phosphorus (0.49%), iron (31.0 mg/100g) and vitamins. The properties of cumin fruits

both as a condiment and medicine are due to a volatile oil (2 to 4%) with an unpleasant taste. The oil is colourless or yellow when fresh. The chief constituent of the volatile oil is cumaldehyde, ($C_{10}H_{12}0$, b.p. 235^0C), which forms nearly 20 to 40 % of the oil. Besides the aldehyde, the oil contains p-cymene, pinene, dipentene, cumene, cuminic alcohol, α-phellandrene and β–terpeneol (Bentley and Trimen, 2002a).

1.9. ASSAYS SELECTED FOR STUDY

Bioassay is made up of two words viz., 'Bioas' = life and 'assay' = determination. In simple words bioassay is the determination of response of a chemical on living organisms. Finney defined bioassay as "the measurement of the potency of any stimulus which may be physical, chemical, biological, physiological or psychological, etc. by means of the reactions which it produces in a living organism" (cf. Srivastava and Saxena, 1989). Bioassay mainly are of two types and they are '*in vitro*' assays (Prokaryotes like Salmonella strains/Animal tissue culture) and '*in vivo*' assays (Eukaryotic organisms like mice, rats, etc.).

Both the *in vitro* assays and the *in vivo*/rodent bioassays have all kinds of built-in limitations, assumptions and potential artifacts which are rarely acknowledged in the interpretations of 'positive' results. In other words, if a given agent can be shown to induce a 'positive' results in chromosomal aberration, micronuclei or sister chromatid exchanges *in vitro* and the same agent induces cancer in some rodent bioassay, it is automatically assumed by most that the agent is a genotoxic carcinogen.

The literature is now replete with numerous examples of agents which can induce 'positive' results in one or more of these short term 'genotoxicity' assays but which are obviously not genotoxic. With regard to chemical 'carcinogens', three

major concepts must be emphasized, namely, carcinogenesis is more than mutagenesis; carcinogenesis is a multi-step, multi-mechanism process and most chemical 'carcinogens' are not genotoxic (Trosko, 1997).

In vivo bone marrow chromosomal aberration test (Tjio and Whang, 1962), micronucleus (MN) test (Schmid, 1973; Schmid and Ledebur, 1973) and sperm morphology test (Wyrobek *et al.*, 1984) are the simple methods routinely used for the detection of mutagens. Short-term assays have been used effectively to screen for mutagens and potential carcinogens in the human environment. The same procedures are increasingly being used to identify antimutagens and potential anticarcinogens (Bruce and Heddle, 1977; Venitt and Parry, 1984).

1.9.1.Chromosomal aberration assay:

Chromosomal aberrations are due to lesions in DNA, which lead to discontinuities of the DNA double helix. Double strand breaks lead directly to chromosomal aberrations (Brewen and Stetka, 1982; Palitti, 1998). Depending on the time of induction within the cell cycle, the types of aberrations observed at the succeeding metaphase are of chromosome (from G_1) or chromatid (from G_2) nature i.e., involve both or only one chromatid respectively (Adler, 1984; Savage, 1998). The majority of aberrations are cell-lethal because genetic material is lost (Sudhakar *et al.*, 2000). However, non-lethal events may also occur in the form of balanced exchange of material between two chromosomes, so-called reciprocal translocations. The method is based on the following principles and observations:

Swiss albino mice (*Mus musculus*) contain 40 chromosomes, all of which are acrocentric (2n = 40). Mitotic arrest by a spindle poison such as colchicine is achieved by injecting the animals prior to killing. Hypotonic (KCl) treatment of the cellular material leads to the absorption of water by osmosis to equalize the salt concentration.

This serves to thin the viscous cytoplasm and nucleoplasm in order to obtain better spreading of the chromosomes. Fixation of the cell suspension using acetic methanol (Carnoy's fixative) helps in fixation of chromosomes and digestion of mucous components of the cytoplasm (Sharma and Sharma, 1990, 1994; Sharma, 1991).

Often the most critical step is preparing chromosome spreads on glass micro slides. All kinds of procedures can be used to obtain an even layer on the slide and good spreading of the chromosomes. Such methods include cooling the slides, warming them and immersing them in water/diluted acetic acid/or alcohol prior to the application of the cell suspension. One of the principles of good adherence of the cells to the glass surface is that the glass has to be absolutely clean and grease-free. Another principle of slide making is that the medium in which the cells are suspended (Carnoy's fixative) should dry as quickly as possible in order to obtain clear well preserved chromosomes. This can be achieved by flaming the alcohol on the slides or by drying the slides on an electric plate set to an appropriate temperature (40^0C) or by just gently blowing the glass surface.

For microscopic analysis counterstaining of the cytoplasm is required to facilitate scoring. Therefore, stains such as 5% Giemsa have been adopted. They preferentially stain nuclear material but do not leave the cytoplasm completely unstained. As a general rule it is advisable to analyse only chromosome spreads of excellent quality. Preparations from untreated samples can serve as quality controls. The treatment with the test compound can adversely affect the slide quality, for instance cell toxicity reduces the number of metaphases. The frequency of mitotic divisions (mitotic index) is estimated in preparations of somatic cells (Shyama and Rahiman, 1996; Vijayalaxmi and Rai, 1996; Yadav *et al.*, 2003). Aberrations are divided into chromatid-type and chromosome-type, the former involving only one

chromatid, the latter involving both chromatids at identical sites (Cohen and Hirschhorn, 1971).

1.9.1.1. Breaks and Gaps:

A gap is an unstained region of the chromatid which is smaller than the width of the chromatid. If the unstained region is larger, it would be considered as break (Savage, 1975). Breaks are true discontinuities with clearly dislocated fragments and also include fragments without obvious origin. They are to be distinguished from achromatic lesions (gaps) which do not represent true discontinuities in the DNA. It is generally assumed that gaps are sites of despiralization in the metaphase chromosome, which render the DNA non-visible under light microscopy. It has been proposed that a gap may actually be a single strand break in the DNA double helix as a result of incomplete excision repair and thus may represent a point of possible instability. Therefore, gaps are always noted but reported separately from true chromosomal aberrations (Adler, 1984).

1.9.1.2. Fragmentations:

The affected chromosomes, as seen at metaphase, appear to have broken up into many small pieces of varying length. Some of them can be seen to be minute acentric rings and other pieces may show partial exchange and isochromatid-type changes. Sometimes all the chromosomes in a cell are affected, but frequently a considerable number of them are affected.

1.9.1.3. Centric Fusion:

The association of two acrocentric chromosomes to form metacentric or submetacentric chromosome is known as centric fusion. Interchanges may also occur in the centromere regions of two chromosomes giving whole-arm exchanges.

1.9.1.4. Chromatid Exchanges (Translocation):

The exchange of chromosome parts after the occurrence and interaction of two lesions/damage is called chromatid exchanges (Savage, 1975).

1.9.1.5. Ring Chromosomes:

It is analogous to an asymmetrical interchange where the two arms involved belong to the same unduplicated chromosome (sister chromatid union), being separated by the centromere. Two lesions involved are being brought into proximity by a loop and in this case the loop contains the centromere. The consequence of the exchange is a ring-shaped centric chromosome.

1.9.1.6. Multiple Aberrations:

Sometimes the affected cells with five or more aberrations, as seen at metaphase, appear to have more than one type of aberration are grouped as multiple aberrations.

1.9.2. Bone marrow Micronucleus (MN) assay:

The micronucleus test is an *in vivo* assay for the detection of both clastogens and agents that induce aneuploidy (abnormal chromosomal segregation; i.e., non-disjunction) (Hayashi *et al.*, 2000). This test was initially developed in mouse bone marrow erythrocytes, but is also conducted in various mammals including rats, hamsters and monkeys. However, routinely it is conducted using the bone marrow erythrocytes of mice (Mathew *et al.*, 1990; Vijayalaxmi and Venu, 1999).

All common mice strains can be used for this assay. A typical micronucleus test consists of a dose range-finding assay and a micronucleus assay (Shyama and Rahiman, 1993; Hemavathi and Rahiman, 1996; Hayashi *et al.*, 2000). Male and female mice are dosed with the test agent, usually by intraperitoneal injection, but other routes of dosing are also acceptable.

Micronuclei are the smaller nuclei compared to the main nuclei of the cells. They are of two types and arise either from acentric chromosomal fragments resulting from chromosomal breaks or lagging of chromosomes (microtubule malfunctions in cell division). Small micronuclei are produced by the acentric chromosomal fragments, whereas the whole lagged chromosomes give rise to larger micronuclei. In the mouse micronucleus test, the target cells are the bone marrow erythroblasts. Chemically induced micronuclei in the erythroblasts are retained in the erythrocytes after the extrusion of the main nuclei from the cells during maturation and can be scored in polychromatic erythrocytes (PCE: young erythrocytes) and normochromatic erythrocytes (NCE: mature erythrocytes). An increase in micronuclei in these erythrocytes (PCE+NCE) indicates genotoxicity of the test agent.

Toxicity is also monitored by studies on bone marrow suppression. Bone marrow suppression is measured by the decrease of the ratio of PCE to NCE or to total erythrocytes/RBC (PCE+NCE) in the bone marrow, which is commonly referred to as the PCE/NCE or PCE/RBC ratio. The PCE/NCE ratio is normally 1:1 in untreated condition. If there is an increase in the NCE population, it is a signal of cytotoxicity of a drug or chemical (Adler, 1984).

Bone marrow smears are prepared on glass micro slides, stained with May-Grunwald- Giemsa or acridine orange and scored for micronucleated erythrocytes. As an additional advantage for the micronucleus test, young erythrocytes (PCE) stain differently from older forms (NCE). For the duration of their adolescence, lasting approximately 24 h, they do not stain reddish pink as NCE but bluish due to high RNA content. The micronuclei stain deep purple and are clearly distinguishable from the surrounding lightly stained cytoplasm.

The method is based on the following principles and observations: In anaphase, acentric chromosomal fragments or some of the whole chromosomes lag behind when the centric elements move towards the spindle poles. After telophase the undamaged chromosomes, as well as the centric fragments, give rise to regular daughter nuclei. Some of the lagged elements may be included in the daughter nuclei, but a considerable proportion of them are transformed into one or several secondary nuclei, which are much smaller than the principal nuclei and are therefore called micronuclei (Schmid, 1975, 1976; Muller and Streffer, 1994).

1.9.3. Sperm-shape abnormality assay (sperm morphology assay):

It is particularly relevant to study the genotoxic effects of various agents on germinal cells, because this is the only system in which transmissible genetic damage from one generation to another takes place (Au and Hsu, 1980; Wyrobek, 1982). The mouse sperm morphology test has been a commonly used sperm test for measuring spermatogenic damage induced by physical or chemical agents. So this test may be a valuable tool for identifying germ cell mutagens (Soares, 1979; Wyrobek *et al.*, 1984). Using this test many drugs, pesticides and other chemicals have been analysed in mammals for their mutagenic effects (Wyrobek and Bruce, 1975; Mathew *et al.*, 1992; Jayashree *et al.*, 1994; Giri *et al.*, 1998; Rai and Vijayalaxmi, 2001). In the mouse sperm morphology assay, assessment of effects on exposed mice is based on visually scoring for the percentage of sperms with abnormal head forms in smears of sperms from the epididymis or vas deferens. Varieties of abnormal sperms are observed such as amorphous, hookless, banana shaped, folded, double tailed, etc.

Testes weight is used as an additional measures of germ cell toxicity. This test complements sperm morphology assay and can be done in the same animal (Wyrobek, *et al.*, 1984).

1.10. CHOICE OF EXPERIMENTAL ANIMAL

As our ultimate concern is human welfare, the laboratory animals selected for such a study should be closely related to human species to enable meaningful extrapolation (Legator, 1972; Wolff, 1978; Truhaut, 1980). Swiss albino mice have several advantages for mutagenicity studies (Manna, 1991). Choice of mouse as experimental animal was done based on following reasons:

- Being a mammal, with most of its biochemical and physiological similarities to human beings, mutagenicity studies on them makes it relevant to human genetics and medicine and results of experiments could be easily extrapolated to the mammals of higher order including man.
- Mice are easy to identify and their classification and systematics are well established. Hence there is little risk of monitoring being confounded to uncertainities regarding the identification of, or relationship between the species being studied.
- They are particularly known experimental animals, with much research carried out, which provide background knowledge of toxicology thus reducing the risk of misinterpretation.
- They quickly acclimate to laboratory conditions and can tolerate the experimental stress.
- The ease of handling, housing, breeding and maintenance in healthy and normal

environment and cost of production is comparatively less than other mammals.

- Ease of experimentation within short duration.
- Its well established genetic background and karyotype are also advantageous characters for their use in mutagenicity studies (Miller and Miller, 1975).
- The time schedule of spermatogenesis and spermiogenesis from undifferentiated spermatogonia to the formation of mature spermatozoa lodged in the epididymis is worked out (Leonard, 1973).

1.11. GAVAGE

The route of exposure selected for toxicity studies should be the likely route of human exposure. Experimentation with treated and control groups should run concurrently. When the oral route is indicated, but feed and water are not appropriate vehicles, "gavage" (=administer by stomach tube) may be recommended method of administration (Lijnsky, 1990). An appropriate vehicle is one that is compatible to both test material and animal. When a vehicle is used to administer the test agent, a control group of animals should be administered an equal volume of the vehicle without the test substance (Prakash and Arora, 1998). Accordingly in the present study test materials (spices) were added to an appropriate vehicle (saline) and introduced into the esophagus with a tube attached to a graduated syringe.

1.12. CHEMICAL AGENT (POSITIVE CONTROL)

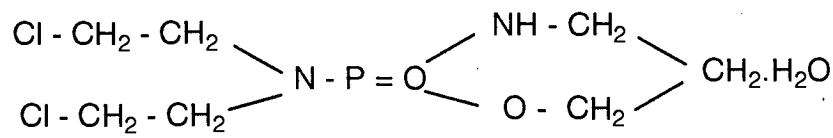
Chemical agents/positive controls are used to show the efficacy and reliability of the test system to confirm the sensitivity and specificity of the strain used. In the present investigation cyclophosphamide was used as a positive mutagenic agent and

cisplatin as a positive antitumour agent to confirm the sensitivity of the mice strain used.

1.12.1. Cyclophosphamide (CP):

Endoxan (ASTA) or cyclophosphamide being a well known mutagen is used as a positive control agent while performing genotoxicity studies. It is used in the treatment of various cancers such as Leukaemias, Waldenstrom's disease, malignant lymphomas, paraproteinaemias (e.g. plasmacytoma) as well as various malignant solid tumours including carcinoma of the ovaries or breast, neuroblastoma, seminoma, Ewing's sarcoma, etc. Further, it is also used in the treatment of "autoimmune diseases" (non-malignant diseases) like rheumatoid arthritis, autoimmune haemolytic anaemia, sclerodermia, nephrotic syndrome, etc.

Chemically CP is N, N-bis (dichloroethyl) N'O-Propylene phosphoric acid ester diamide monohydrate. Its molecular weight is 279.1 and empirical formula is $C_7H_{15}Cl_2N_2O_2P.H_2O$. Its structure is as follows:



It is a fine, white odourless crystalline powder with a slightly bitter taste and liquefies upon the loss of its water of crystallization. It discolours on exposure to light. It is an alkylating agent, which is soluble in water/alcohol and slightly soluble in ether. Alkylating agents have been found to exert a mutagenic effect on a great variety of organisms. Experiments proved the ability of alkylating agents to cause chromosome-type aberrations (Leonard, 1973). Side effects of CP includes

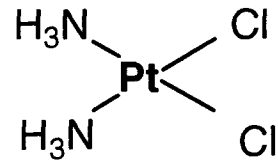
myelosuppression, pigmentation, carcinogenicity, alopecia, nausea and vomitting (Ferguson and Pearson, 1996).

Cyclophosphamide may be incompletely absorbed from the gastrointestinal tract. Peak concentration of CP appears about one hour after oral administration and it rapidly disappears from the plasma. It has to undergo metabolism in the liver before it becomes active. Irritant metabolites are excreted in the urine together with unchanged drug (Martindale, 1982). Being nucleophilic, CP reacts with the nucleic acid bases and inhibits DNA synthesis. It is one of the most studied drugs for its mutagenic effects.

1.12.2. Cisplatin (Csp):

The vast majority of clinically used antitumour drugs are either synthetic or natural product based organic compounds. The platinum antitumour agents are unique in that group and they are coordination complexes. The parent compound of this class, cisplatin contributes to the curative treatment of testicular teratoma and has significant activity against ovarian, head and neck, bladder, cervical and lung cancers. The compound is also highly toxic with the list of side effects including renal damage, severe nausea and vomitting, myelosuppression, ototoxicity and neurotoxicity (Ferguson and Pearson, 1996). Since the antitumour properties of Csp were reported by Rosenberg and coworkers in 1969, well over 1000 platinum analogues have been tested for antitumour activity (cf. Abrams, 1990).

Chemically, cisplatin is a cis-isomer of diamine dichloro platinum-II or cis-dichlorodiammineplatinum-II. It has a molecular weight of 300.1 and melting point of 207^0C. The empirical formula of the active compound is $Cl_2H_6N_2Pt$ and its structure is as follows:



Cisplatin is deep yellow in the crystalline solid condition and is very clear in the reconstituted solution form. It is an antineoplastic agent with biochemical properties similar to those of bifunctional alkylating agents. Its cytotoxic actions and antitumour activity is consistent with the hypothesis that major cytotoxic target of cisplatin is DNA. The drug covalently binds to DNA bases and disrupts DNA fraction. Csp appears to enter cells by diffusion. Hydrolysis of chloride in its structure is responsible for formation of activated species of the drug, which reacts with nucleic acids and proteins. Cisplatin exerts its action by forming both intrastrand crosslinks of complementary strands of DNA, thus inhibiting DNA synthesis. Protein and RNA synthesis are also inhibited to a lesser extent. Its antineoplastic actions are not cell-cycle specific (Sanderson *et al.*, 1996).

Following intravenous administration, Csp is rapidly distributed to all tissues, except the central nervous system (CNS), where it does not penetrate to an appreciable extent. The highest concentrations are reached in liver, kidneys, bladder, muscle, skin, testis and prostate. After intravenous administration, plasma elimination is biphasic with half-lives of 25-49 minutes (Phase-I) and 58-73 hours (Phase-II) respectively. Csp is eliminated through urine (27 to 43% of the administered dose is excreted in the urine within the first five days after administration).

1.13. CANCER AND ASCITES TUMOUR

Cancer is a major health problem of the world. It is a general term for all the varieties of carcinoma and sarcoma. The terms 'cancer' and 'carcinoma' appear to have the same origin (L. *cancer*, Gk. *karkinos* and Sanskrit *karakarata* = crab). Any abnormal, uncontrolled growth is cancer. According to Roe "Cancer is a disease of multicellular organisms which is characterized by the seemingly uncontrolled multiplication and spread within the organism of apparently abnormal forms of the organism's own cells" (cf. Kothari and Mehta, 1973). All cancers of epithelial (glandular, surface lining), neurectodermal and chorionic tissues should be referred to as "carcinoma", used as a separate term or suffixed to a cell-type or an organ e.g. hepatocarcinoma, choriocarcinoma or carcinoma of the chorion. Further, Dorland's dictionary draws a distinction between 'cancer' and 'carcinoma' based more on usage than on logic, by defining cancer as "a cellular tumour, the natural course of which is fatal and usually associated with formation of secondary tumours" and carcinoma as " a malignant new growth of epithelial cells tending to infiltrate the surrounding tissues and give rise to metastases" (Novak, 1995).

Structural features of cancer cells shows that majority of them are larger than the normal cells from which they are derived. However, some may be smaller and yet others may show no deviation from the normal.

1.13.1. Tumour:

A tumour is the result of a mutation/series of mutations in a gene/genes of a single cell or clones of that cell, which lead to loss of normal function, uncontrolled cell growth and often metastases (Roy and Sikdar, 2003). The term tumour (L. *tumere* = to swell) indicates the presence of a swelling due to any cause. It is basically a clinical term, meant to describe an alteration in morphology of body surface or the

surface of an organ whereby there arises a visible swelling, a protuberance or an excrescence (Couch, 1996; Wisniewski, 1996). According to Boyd, "a tumour or neoplasm is a growth of new cells which proliferate without relation to the needs of the body. The essence of the process is loss of control over two fundamental functions of the cell, namely reproduction and differentiation" (cf. Kothari and Mehta, 1973).

The term neoplasm (Gk. *neo* = new; *plasma* = formation) means newly formed tissue. Such a general term, though embracing both normal and abnormal new tissue formation, cannot be considered an improvement upon the term tumour. The behavioural qualities of any lesion are two types – 'Benign' means harmless and 'Malignant' indicates "the tendency to go from bad to worse" or to cause death. Benignancy or malignancy should be strictly determined by what the lesion does to the patient rather than what it looks under the microscope (Hajdu, 1998). These two qualities does not always correspond to each other. It cannot be denied that most cancers are malignant in their behaviour. However, not every cancer is malignant, nor is every non-cancerous lesion a benign one.

The suffixing of 'oma' to any organ or cell must imply a non-cancerous lesion consisting of increased number of normal cells (eg: hepatoma, adenoma, melanoma). This indicates that "benign" non-cancerous lesions made up of normal cells. All "benign" neoplasms genuinely constitute a lump or a tumour and hence the suffix-oma (Gk. *oma* = from onkoma, a swelling) is highly suitable.

Extensive perusal of cancer literature shows that neoplasm = tumour = cancer = malignancy = new growth.

It must be pointed out that the heightened search for preventive measures against cancer is owing to the dismal failure of modern medicine in the treatment of

most forms of cancer. The saying " Prevention is better than cure" is hardly applicable since a "cure" is almost not available.

'Cancer prevention' is not synonymous with 'cancer control'. Cancer control encompasses consideration of early diagnosis of malignant disease, whereas prevention means its avoidance in any form, early or late. Majority of human cancers are caused directly or indirectly by exogenous agents (cancerogens/carcinogens) and are thus theoretically preventable (Newberne and Zeiger, 1978; Weisburger *et al.*, 1986). Roe remarks, "more than 80% of all cancers are probably of environmental origin and therefore, potentially preventable" (cf. Kothari and Mehta, 1973). Phytochemicals of food and herbs are very potent antioxidants and free radical scavengers (Palmer and Bakshi, 1986). It is accepted that these chemicals minimize DNA damage by reacting with free radicals and in this way they could prevent cancer (Jankun *et al.*, 2003). The entire concept of cancer prevention would be revolutionized if we could identify proper anticarcinogens (Ahuja and Rajeshwari, 2003) and increase their use.

1.13.2. Dietary nutrient intervention/Chemoprevention:

Prevention of cancer and other mutation-related diseases can be pursued both by avoiding exposures to recognized mutagens/carcinogens and by favouring the intake of protective factors or fortifying physiological defense mechanisms. The latter approach, referred to as chemoprevention, is extremely delicate since it involves dietary or pharmacological intervention in the host organism (De Flora, 1998).

Cancer chemoprevention can be defined as the inhibition or reversal of carcinogenesis at a pre-malignant stage (Kelloff *et al.*, 1994). Therefore, chemoprevention falls within primary prevention (inhibition of occurrence of a disease) when it is addressed to healthy individuals and within secondary-prevention

(early diagnosis, possibly in a preclinical stage, followed by timely intervention) when it is addressed to individuals suffering from a preneoplastic situation in order to achieve its regression. Prevention of multiple primary tumours also falls within secondary prevention, since these tumours are expected to be in an advanced, preclinical stage at the time of intervention. Inhibition of invasion and metastasis is conversely outside the boundary of chemoprevention and falls within so-called tertiary prevention (De Flora, 1998)

The strategy of reducing the incidence of degenerative diseases by reducing the rate of DNA damage may lend itself to different approaches. Manipulating dietary components, (e.g., by increasing the dietary levels of foods with beneficial antioxidants) or by increasing the antioxidant levels in edible plants through genetic engineering, may be more biologically advantageous than that of drastically reducing energy intake (caloric restriction). Perhaps the relatively long life of humans has been achieved through the course of evolution not only by reduction of metabolic rate but also by reduction of oxidative damage by improved defense mechanisms. Although these studies offer intriguing inferences, the question of whether 'proper' diet can extend the maximum human life span remains unanswered; whether it can extend the average life span appears likely. Populations differ greatly not only in the pattern of cancer incidents but also in their dietary habits and environmental exposures. It is evident that some of the major human cancers are associated with complex, life style related causative, enhancing and inhibiting factors, several of which are related to the diet (Krishnaswamy and Polasa, 1995).

The relationship between specific dietary components and cancers are much less well established than that of diet and cardiovascular diseases. Though dietary influences in cancer risk have been estimated to be as important as other

environmental exposures, the role of diet in the causation of cancer has always been difficult to study and quantify partly because the diet encompasses a wide variety of foods, dietary traditions, habits and is a complex mixture of nutrients and non-nutrients. In theory there are several possible ways in which diet can influence human cancers, these are summarized in the Fig.1.2.

In order to understand the dietary factors implicated in cancer, it is necessary to know about the cancer process itself. Chemical carcinogenesis is characterized by three main stages viz., initiation, promotion and progression. The process of carcinogenesis consists of a distinct initiating process where an exogenous and endogenous carcinogen alters the genetic makeup of the cell and results in a lesion that confers upon the cell, the potential for neoplastic transformation.

The initiation process occurs following the metabolic activation of the pro-carcinogens, through the sequential enzymatic process which result in the formation of electrophiles or reactive substances which covalently bind to macro-molecules such as DNA protein. Unless these lesions are repaired effectively these are propagated.

The next step in carcinogenesis involves, alterations in gene expression and cell proliferation that transforms the initiated cell into a cancer cell. The promotional events can be inhibited by inhibitors which suppress neoplasia. Inhibitors/anti-promoters act by removing initiators or promoting agents, through detoxification or by regulating cellular receptors/differentiation or by inducing repair mechanisms (Fig.1.2). Progression is the expansion of the population of initiated cum promoted cancer cells. The process of chemical carcinogenesis, can be influenced by dietary factors at several stages. Diet-associated carcinogens, mutagens and inhibitors of the carcinogenic process play a vital role. The various dietary and nutritional factors implicated in cancer, by site, are summarized in Table 1.1.

Table 1.1. Dietary/nutritional factors in cancer.

Cancers	Dietary factors	Nutrients
Oral cavity	Alcohol Tobacco as shuff or through chewing	Beta-carotene Vitamin A, C, Riboflavin and Zn
Esophagus	Low intake of meat, fish, fruits and vegetables Corn as the staple alcohol Tobacco Bracken fern aflatoxins	Poor nutritional status Beta-carotene, vitamin A, riboflavin, Fe, Zn and Se
Stomach	Nitrates, nitrites and nitrosamines Pickles vegetables and salted substances Fried foods and smoked fish Undernutrition	Vitamin C and E Protein Complex carbohydrates Selenium
Colon and rectal	Low fibre Refined carbohydrates	High fat and protein Vitamin A and C
Larynx	Tobacco smoke Alcohol	Vitamin A
Lung	Cigarettes Low in green vegetables	Vitamin A and E
Bladder	Industrial chemicals Artificial sweeteners Coffee consumption	Vitamin A
Prostate	Low in green and yellow vegetables	Higher vitamin A Overnutrition High fat and protein
Breast	Obesity Low in vegetables	Beta-carotene Vitamin A High fat and protein
Cervical cancer	Low in vegetables	Vitamin A, C folic acid Poor nutrition
Pancreas	Tobacco smoke Coffee	Over nutrition
Liver	Aflatoxin	Poor nutrition

(**Source**: Krishnaswamy and Polasa, 1995)

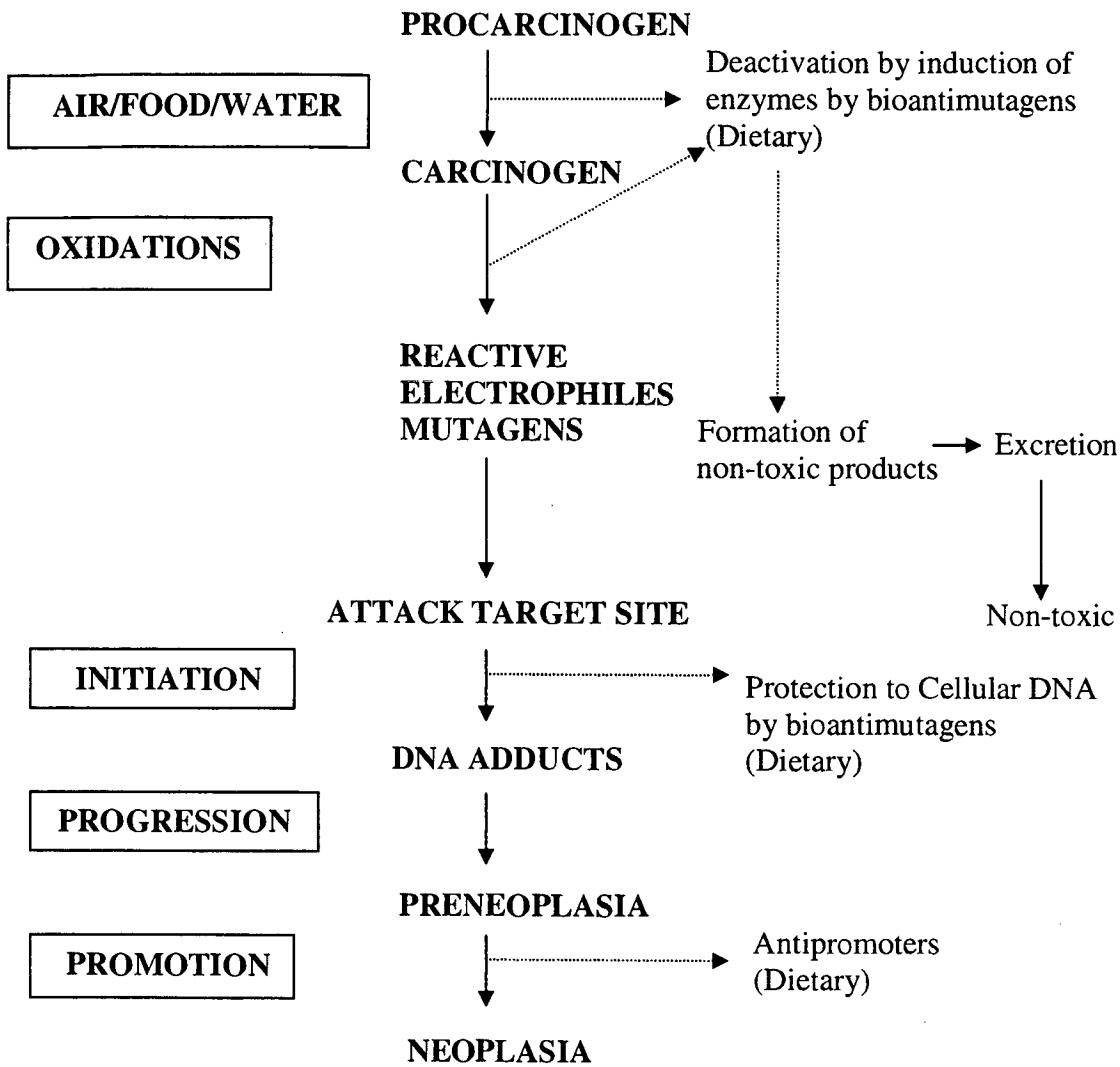


Fig. 1.2. Role of dietary factors in carcinogenic process.
(**Source**: Krishnaswamy and Polasa, 1995)

Though chemopreventive approach is recognized, it is better to have dietary or food based approaches for prevention of cancer. Public health action should be however, directed towards increasing consumption of dark green and yellow vegetables and fruits which possess the package of protective substances. It is important to realize that supplements of nutrients against the back drop of a poor diet in the long run cannot be expected to produce the desired outcome and hence it is important to lay stress on foods which are rich sources of microconstituents. In addition to supplying antioxidants in food, other non-nutrient components in such foods are known to play a significant role in inhibition of cancer.

Plant foods mainly the vegetables and fruits are rich in phenols, indoles, aromatic thiocyanates, coumarins, protease inhibitors and sterols which can inhibit neoplasia. Experimental evidences are now available to show that vegetables like cabbage, cauliflower, brussels sprouts, sweet potato and grapes have protective factos against carcinogenesis (Wattenberg, 1983). Further, spice like turmeric was evaluated as a potential chemopreventive agent (Krishnaswamy, 1993).

1.13.3. Ascites tumour:

An ascites tumour is defined as a neoplastic cell population able to grow in free suspension within the peritoneal fluid of the peritoneal body cavity. The ascites tumour can grow as either single cells or small aggregates in the peritoneal fluid in the absence of capillaries or stroma. Ascites tumours arise from solid tumours that have been finely minced and injected intraperitoneally. The conversion to ascites form is usually a long process and the result of serial passage of peritoneal exudate. It was found that the conversion from solid tumour to ascites form occurred either immediately or gradually (Medina, 1975).

The conversion to ascites form is stable and irreversible. Tumours, which were converted to ascites and then serially transplanted subcutaneously as solid tumours, retained the ability to form ascites tumours immediately on injection of the cells into the peritoneal cavity regardless of the length of time it took for the original ascites transformation. The cellular changes that were required for progressive growth in suspension were independent from those that facilitated survival in suspension. Thus the conversion from solid tumours to ascites tumour was the result of sequential progression of events.

It has been well established that the growth of experimental ascites tumours growing in the peritoneal cavity of mice gradually declines with increasing tumour mass and time after inoculation. With increasing age of tumours, "non proliferating" cells accumulate at an increasing rate and this is accompanied by increased cell loss, decreased growth fraction and prolongation of the mean cell cycle time (Potmesil *et al.*, 1977).

Crosses between tumourigenic and non-tumourigenic cell types resulted in highly malignant cells. One such parental line, which was derived from mouse fibroblasts was hybridized with a series of three highly tumourigenic ascites cell lines. The first hybrid originated as a mouse mammary carcinoma and is known as the "Ehrlich ascites tumour"; the second, designated SEWA, began as an osteogenic sarcoma induced by polyoma virus and the third, MSWBS, was a methylcholanthrene-induced sarcoma. The readily transplantable cancer - Walker carcinoma and Novikoff hepatoma in rats and the Ehrlich ascites carcinoma and sarcoma-180 in mice are varieties of tumours maintained in the laboratories only for experimental convenience (Kothari and Mehta, 1973; Kallman and Rockwell, 1977).

The simplest means of assessing tumour growth is to make direct serial measurements of tumour size and in solid tumours, size and weight are the directly measurable parameters. In dispersed or non-solid tumours, estimates of actual cell numbers can be obtained. For example, in the Ehrlich ascites tumour in mice, the number of tumour cells may be quantitated by aspiration and indicator dilution techniques (Lightdale and Lipkin, 1975).

1.13.4. Tumour reduction studies:

Based on the assumption that organs in animals and in man have similar metabolic patterns, a compound that is chemopreventive in an animal has a higher likelihood of being chemopreventive in man (Boone *et al.*, 1992).

Animal survival studies are routinely used to identify natural anticarcinogens (Unnikrishnan and Kuttan, 1990a; Rao and Umadevi, 1996a,b; Sharada *et al.*, 1996). Human populations are exposed over much of their lifetime to low doses of carcinogens from their environment and in food. Ideally, the animal models most analogous to human experience would be the so-called "complete carcinogenesis" models, in which the animals would be exposed to low doses of carcinogen over a long period of time (Boone *et al.*, 1992). Unfortunately, the expense and time required to use such models make them impractical. Hence animal survival is recorded upto 120 days and this is roughly equivalent to 5 year survival in man (Nias, 1990).

Tumour response is assessed on the basis of median survival time and tumour-free survival. Median survival time (MST) and percentage increase in life span (ILS) are calculated from the animals dying within 120 days and those surviving 120 days are excluded from these calculations (Sukumaran and Kuttan, 1991; Sharada *et al.*, 1996). During the antitumour study, body weights should be recorded on the day of tumour inoculation and at weekly intervals.

Some of the dietary constituents are known to inhibit carcinogenesis. Several naturally occurring compounds are known to suppress neoplastic growth, either by inhibiting the activation of carcinogens or by blocking/suppressing the carcinogenic agents (Wattenberg, 1985). Such compounds include several agents that are common constituents in vegetables and fruits such as carotenoids, polyphenolic materials, sulfur containing compounds, etc. Anticarcinogenic materials are also reported in spices such as turmeric, cinnamon, garlic, etc. which are commonly used in our country and the active ingredients in these preparations have been well established (Soudamini and Kuttan, 1989; Unnikrishnan and Kuttan, 1990a).

In the present investigation we have studied the antitumour activity of selected spices using Ehrlich ascites tumour.

1.14. PRESENT WORK

Many of the chemicals of daily use by man are reported to cause genetic damage and bring about mutation leading to various diseases including the dreadful cancer (Felton and Knize, 1990). The identification and if possible elimination of such chemicals from man's environment is of great concern of the day. On the other hand, some chemicals in human diet or environment nullify the genetic hazards of other toxic chemicals. Natural medicines and 'health foods' are therefore in greater demand today than ever before in the world. It is well known that 'Prevention is better than cure'. Hence, the quantification of optimum amount in the consumption of these classes of protective chemicals will be useful for the future planning of the treatment/prevention of heritable or genetic disorders. Therefore more emphasis should be given on the antimutagenic property of dietary factors so that a "safe diet"

(antimutagenic diet), which can inhibit the mutagenicity of genotoxic substances can be recommended.

In the present investigation an effort was made to study the mutagenic/antimutagenic effect of spices of common use (*P. nigrum, E. cardamomum, C. sativum* and *C. cyminum*) in Indian diet with following objectives:

- To study the mutagenic activity of spices.
- To study the mutagenically safe dose of different spices in the diet when they are fed alone.
- To analyse the antimutagenic activity of spices which are revealed as non-mutagenic by above studies against positive mutagens or standard mutagens like Cyclophosphamide or Endoxan.
- To analyse the antimutagenic activity of spices revealed as antimutagenic by above studies against mutagenic spices, if any.
- To study/assess the relation between potency (veerya) of spices and mutagenicity.
- To study thc tumour reducing ability of spices.

The thesis has been organized into **five** chapters. Besides an **Introduction** in this chapter, a **Review** of work so far done on mutagenicity, antimutagenicity and anticancer/tumour reducing activity of spices are presented in chapter-II. **Materials** used and **Methods** followed in the work have been elaborated in chapter-III. **Results** of the experiments are presented in chapter-IV of the thesis in three parts. Chapter-V consists of a detailed **Discussion** of the results. A brief **Summary** and a comprehensive list of **Bibliography** are at the end of the thesis.

PLATE - I

Photographs of the spices selected for study:

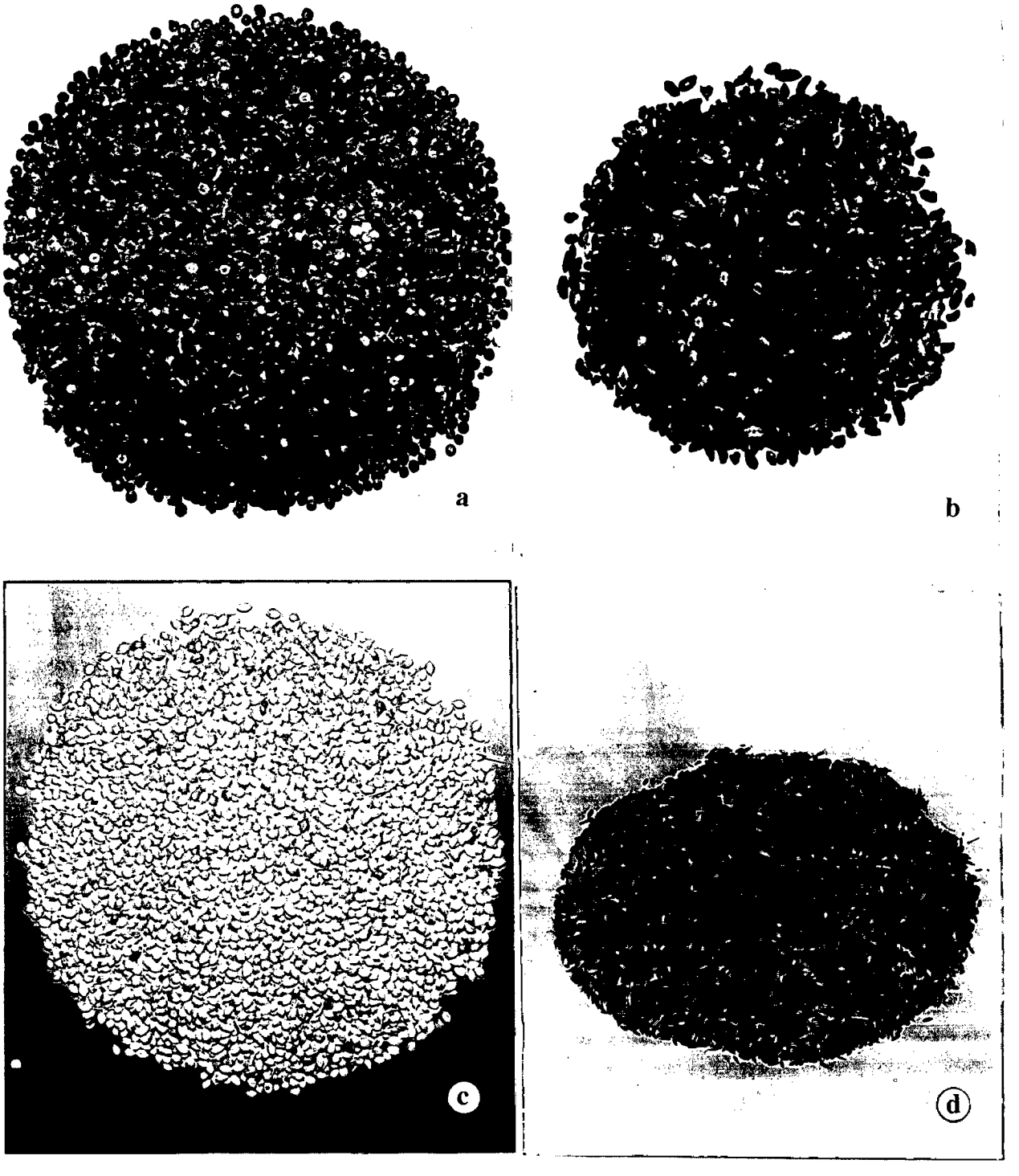
a) *Piper nigrum* Linn. (Black Pepper berries)

b) *Elettaria cardamomum* Maton. (Cardamom seeds)

c) *Coriandrum sativum* Linn. (Coriander seeds)

d) *Cuminum cyminum* Linn. (Cumin seeds)

PLATE : I



CHAPTER - II

REVIEW OF LITERATURE

Plants and plant products used extensively in Indian diet and traditional medicines are increasingly being screened for their role in modulating the activity of environmental mutagens. The property of preventing mutagenesis/carcinogenesis has been reported in many plant products. In screening for antimutagenic effects, extracts of different plant parts have been used, ranging from leafy vegetables, fruits, seeds and underground storage organs to whole plants. A number of medicinal plants and Ayurvedic formulations have been tested for the mutagenic/antimutagenic/antitumour effects on various test systems. The following review is confined to mutagenic, antimutagenic and anticarcinogenic/antitumour studies of plant products, particularly dietary constituents like spices.

A great majority of mutagens and carcinogens can only act through interaction with environmental factors. Exposure of the human population to mutagens and carcinogens is not limited to occupational settings since mutagens have been found in airborne particles, diesel engine emissions, beverages and food (Ames, 1983). Food is possibly the most chemically complex substance to which humans are exposed, undergoing various effects during storage, processing and cooking, often to form genotoxic compounds. 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 high doses (Sharma and Talukder, 1987). Total prevention from exposure to these harmful products is not always feasible. The simultaneous production of antimutagenic and desmutagenic factors has added to the possibility of identifying such factors and using the information to modify or alleviate toxic effects.

Awareness of human exposure to mutagenic and carcinogenic agents has led to a greater interest in natural antimutagenic and anticarcinogenic factors in dietary ingredients. A number of agents, known to suppress mutations and carcinogenesis in various test systems has been identified from different plant systems. Further a large amount of work has been carried out with the major individual components or isolated fractions of vegetable extracts. These include crude vegetable extracts and plant derived products such as plant pigments, flavonoids, vitamins and fibres.

Experiments with crude aqueous extracts of a large number of vegetables have been found to modify the effects of clastogenic chemicals in several tests systems both *in vivo* and *in vitro* and positive correlations between chromosomal aberrations, mutagenicity and carcinogenicity have been recognized. Effect of pretreatment with various vegetable juices, both fresh and boiled on 7, 12-dimethylbenzanthracene (DMBA) induced chromosomal aberrations in rat bone marrow cells *in vivo* was studied by Ito and his colleagues (Ito *et al.*, 1986). The vegetables included onion, burdrock, cabbage, eggplant, Welsh onion, lettuce, carrot, celery and pumpkin. Chromosomal aberrations (breaks, gaps and exchanges) were suppressed by both fresh and boiled juices from all the vegetables and significantly so from onion, burdrock, eggplant, cabbage and Welsh onion. The degree of suppression of mutagenic effects was same whether the juices were force-fed or given freely as drinking water. Short-term *in vivo* mouse bone marrow micronucleus test was carried out to evaluate the role of carrot and spinach in modulating the genetic damage induced by the commonly used chemotherapeutic drug, cyclophosphamide (CP) (Abraham *et al.*, 1986). The vegetable juices, whether administered before, after or simultaneously with various concentrations of CP, significantly suppressed the induction of micronuclei in the treated animals.

Extracts of *Phyllanthus emblica* L. fruits, a rich source of vitamin C and used in many Ayurvedic and Unani systems of medicine, when administered orally to Swiss albino mice together with known clastogens ($Zncl_2$-metallic salt, ethyl parathion-insecticide, metanil yellow-food additive) reduced the cytotoxic effects to a greater degree than a combination of the mutagens with vitamin C alone (Giri and Banerjee, 1986).

Populations are exposed mainly to environmental and dietary complex mixtures rather than to a single chemical. Thus, the counteraction of mutagenic effects of a large number of such mixtures as coal dust, tobacco (nitroso compounds), red wine and red grape juice (flavonoids), airborne and diesel emission particles (nitropyrenes), cigarette smoke, fried beef and fried shredded pork (aromatic amines and other polycyclic hydrocarbons) by chlorophyllin was extensively studied (Ong *et al.*,1986). Investigations carried out in bacterial mutation assays using *Salmonella typhimurium* TA 98, revealed that chlrorophyllin itself was neither toxic nor mutagenic, it inhibited the mutagenic activity of each of the complex mixtures in a dose-dependent manner, especially of the extracts of airborne particles, cigarette smoke, fried beef and fried shredded pork. The antimutagenic property of chlorophyllin was also found to be heat resistant.

Plant polyphenolic acids (ellagic, caffeic, chlorogenic and ferulic acids) have been observed to act as antimutagens towards Benzo(a)Pyrene and its mutagenic metabolites in bacterial mutation assays (Wood *et al.*, 1982). The phenolic compounds hydroxychavicol and eugenol separated from betel leaf chewed widely in India, exhibited a dose-dependent suppression of DMBA-induced mutagenesis (Amonkar *et al.*, 1986).

Nineteen vitamins including some derivatives (retinoids, riboflavin, folic acid, menadione, cyanocobalamin, ascorbic acid, pyridoxine, pyridoxamine, pyridoxal, thiamin, nicotinamide, pantothenic acid, FAD and FMN were tested for their ability to suppress the mutagenic activity of aflatoxin (AFB_1) (Battacharya *et al.*, 1987). The vitamins modified the microsome mediated mutagenic activation in Salmonella typhimurium strain TA100, with the first seven vitamins giving a significant difference. Ascorbic acid was found to inhibit mutagenicity of AFB_1 only at high dose. This finding however contradicts the reports of ascorbic acid being mutagenic at high doses. Nevertheless, vitamin C has been extensively studied as an antimutagen and anticarcinogen and has shown to decrease the chromosomal damage induced by cyclophosphamide in human leucocyte cultures (Gebhart *et al.*, 1985).

In bacterial mutation assays using *Salmonella typhimurium* TA98, fibres of a large number of vegetables (cabbage, burdrock, sweet pepper spinach, carrot, onion, bamboo shoot, Japanese radish) were found to inactivate pyrolysate mutagens derived from amino acids, by adsorbing them (Kada *et al.*, 1984).

2.1. MUTAGENICITY STUDIES

Cereals, pulses, vegetables, fruits and spices are natural foods which are rich in number of chemical compounds like flavonoids, alkaloids and furans. Some of the compounds are known mutagens. The chemical components of foods are altered by pre-ingestion processes like cooking, storage, or by the addition of certain food additives (Sugimura, 1982). The major components of foods which are known mutagens are shown in the Table 2.1.

Table 2.1. Classes of Mutagens in Foods*

Natural food contaminants:	Flavonoids, furans, alkaloids.
Food contaminants:	Pesticides, package materials, solvents, nitroso compounds, polycyclic aromatic hydrocarbons.
Food additives:	Food colours, food flavours, preservatives, sweeteners, anti-oxidants, miscellaneous food additives.
Mutagens generated by food processing:	Products of heating, smoking, boiling, curing, irradiation, solvent extraction.
Mutagens generated by food storage:	Malonaldehyde, fumigation products, mycotoxins.

(***Source**: Anonymous, ICMR Bulletin, 1987)

Dietary components and food dishes commonly consumed in South India were screened (Sivaswamy *et al.*, 1991) for their mutagenic activity. Kesari powder, calamus oil, palm drink, toddy and Kewra essence were found to be strongly mutagenic; garlic, palm oil, arrack, onion and paralyzed portions of bread toast, chicory powder were weakly mutagenic, while tamarind and turmeric were not mutagenic. Certain salted, sundried and oil fried food items were also mutagenic, *Cissus quadrangularis* was mutagenic, while 'decoctions' of cumin seeds, aniseeds and ginger were not showed mutagenicity. Several perfumes, essential oils and colouring agents, which are commonly used were also screened and many of them exhibited their mutagenic potential by inducing the reverse mutation in *Salmonella typhimurium* tester strains.

The cytotoxicity of the extracts from eight different spices used in Indian diet was determined (Unnikrishnan and Kuttan, 1988) using Dalton's lymphoma ascites tumour cells and human lymphocytes *in vitro* and Chinese Hamster Ovary cells

(CHO) and Vero cells in tissue culture. Alcoholic extracts of the spices were found to be more cytotoxic to these cells than their aqueous extracts. Ginger (*Zingiber officinale*), pippali (*Piper longum*) and black pepper (*Piper nigrum*) were the most cytotoxic but asafoetida (*Ferula asafoetida*), mustard (*Brassica compastris*), garlic (*Allium sativum*), sesame (*Sesamum indicum*) and horsegram (*Dolichos biflorus*) were much less cytotoxic.

Extracts of caraway, coriander and black pepper seeds were not mutagenic for *S. typhimurium* strains-TA98 and TA100 (Higashimoto *et al.*, 1993). However, the aqueous and methanolic extracts treated with nitrite were mutagenic for strain TA100. Black pepper showed highest mutagenicity and other two were moderate.

Chillies and their principal alkaloid Capsaicin along with turmeric powder were mutagenic in *S. typhimurium* (Nagabhushan and Bhide, 1986). The relationship between mutagenicity and the pungent properties of spices were studied (Azizan and Blevins, 1995 using *S. typhimurium* strains and assessed that there is no relationship between these two aspects. They observed that among six compounds associated with the pungent properties of specific spices (capsaicin, thymol, borneol, allyl isothiocyanate, eugenol and cinnamaldehyde), only capsaicin was mutagenic in *S. typhimurium* strain TA 100.

Pepper is widely incorporated in the diet of Asian and Western countries and it is also an important constituent of more than 150 Ayurvedic formulations (Karthikeyan and Rani, 2003). John and Abraham (1991) reported the ability of black pepper to induce chromosomal aberrations in Swiss albino mice. In another study spices like black pepper (*P. nigrum*), pippali (*P. longum*), ginger (*Z. officinale*) and mustard (*B. nigra*) increased the number of revertants in *S. typhimurium* strains indicating their mutagenic potential (Soudamini *et al.*, 1991, 1995).

Vijayalaxmi (1980) studied genetic effects of turmeric and curcumin in mice and rats using MN test, chromosomal aberration test and dominant-lethal assay. The results obtained with all three test systems showed that neither turmeric nor curcumin had any adverse cytogenetic and mutagenic effects when incorporated into the diets of mice and rats, in amounts which are normally consumed by man. Acute toxicity studies conducted (Shankar *et al.*,1980) on different species of animals including non-rodents (guinea pigs and monkeys) revealed that turmeric or its alcoholic extract consumption is not toxic even at very high level. Further, Kaushik *et al.* (1993) studied mutagenicity of turmeric on root tip cells of *Vicia faba*. They concluded that turmeric extract has strong mutagenic potential and is radiomimetic in higher concentration at chromosome levels.

Among the various kinds of spices tested, the aqueous extracts of dill weed (*Anethum graveolens* L.) and dill seeds (*A. sowa* D.C.) exhibited a mutagenicity to *S. typhimurium* strains TA98 and TA100 (Fukuoka *et al.*, 1980). A well known spice asafoetida (*Ferula narthex* Boiss) was found to be responsible for altering gestation period, litter size and sex ratio of the litter in albino rats (Borkar *et al.*, 1996). Study results of Ungsurungsie *et al.* (1982) showed mutagenic activity of crude extracts and water-heated/water-macerated residues of Ceylon cinnamon (bark of *Cinnamomum zeylanicum* Nees) in *Bacillus subtilis* strains H17 (rec+) and M45 (rec-).

2.2. ANTIMUTAGENICITY STUDIES

Food contains certain chemical components most of which have neither nutritional value nor any role in the normal metabolic processes. The components which are generally removed in the process of refining include fibres, polyphenols (which impart colour), saponins, lectins, tannins, coumarins, amines, flavonoids and

anthocyanins. Recent studies have revealed that most of these compounds have some beneficial effects like reducing blood cholesterol and triglycerides and other useful pharmacological properties like antidiabetic, antifertility, anticarcinogenic, antiallergic and antimutagenic effects. Wattenberg (1983) reported that foods contain large number of anticarcinogens and antimutagens i.e., compounds which counteract the effect of carcinogens. These compounds are polyphenols, aromatic isothiocyanates, methylated flavonoids, coumarins, plant sterols, selenium salts, protease inhibitors, ascorbic acid, tocopherols and retinols which are known to inhibit cancer formation. Epidemiological studies have shown that vegetarians have a lower risk of cancer than the non-vegetarians, as raw, green vegetables contain most of the above compounds. Wattenberg suggested that these compounds can reduce the risk of cancer.

Mustard is a spice used for flavouring and as a source of edible oil in India and all over the world. The leaves of this plant are consumed as vegetable. Mustard belongs to cruciferous family, other members of which are cabbage, broccoli, cauliflower, etc. All these vegetable extracts have the property of inactivating the mutagenicity of food mutagens like tryptophan pyrolysate (Kada *et al.*, 1978). The active principle of mustard, dithiolthiones can protect against liver toxicity induced by some chemicals. The antimutagenic property of mustard assessed in experimental animals, showed significant effects. Same report says that leafy vegetable cabbage and a known spice ginger contain antimutagenic factor(s) against tryptophan pyrolysate in bacterial strains. Further, they carried out a screening for bio-antimutagens in several plant specimens and found that the homogenate of Japanese green tea (*Camellia sinensis*) gave the highest bio-antimutagenic activity in the *Bacillus subtilis* NIG 1125 strain (Kada *et al.*, 1985).

Shinohara and Kuroki (1987) studied antimutagenic substances in vegetables and fruits on *S. typhimurium*. The dialyzates from broccoli, burdock, cucumber, eggplant, komatsuna, peaman, spinach, sarcocarps, pericarps and envelops of apple, amanatsu and natsudaidai showed antimutagenicity on Trp-P-2 (mutagen isolated from pyrolyzated tryptophan). This antimutagenic activity was retained even after heating them at 100^0C showing heat-stability.

Post treatment with vanillin, anisaldehyde, cinnamaldehyde and coumarin was effective in reducing the mutation frequencies induced by UV or X-rays in Chinese hamster V79 cells (Imanishi *et al.*, 1990). In another study chromosome aberrations were suppressed when vanillin, cinnamaldehyde or anisaldehyde was given orally to mice after X-ray irradiation. Further, dose-dependent decrease in MNPCEs was observed (Sasaki *et al.*, 1990).

Wu *et al.* (1990) reported that roasted ginger has inhibiting tendency on gastric ulcer in rats while the dry ginger has no such effects and suggests that water soluble constituents of the dry ginger have changed in the roasting process. Most of the heated vegetables showed greater inhibitory activity than unheated samples against the mutagenicity induced by chemicals in *S. typhimurium* system (Yamaguchi, 1992). Further the hot water extracts of caraway, coriander and black pepper reduced the mutagenicity induced by N-methyl-N-nitro-N-nitrosoguanidine significantly but did not show significant result against other mutagens tested in *S. typhimurium* (Higashimoto *et al.*, 1993).

Ruan *et al.* (1992) investigated antimutagenic effect of eight natural foods on moldy foods in a high liver cancer incidence area using Ames test. The results showed that these extracts (sesame, chest nut, dad-lily, laver, red Chinese date, bamboo shoots, kelp and green tea) had marked inhibitory effects on the mutagenic activity

induced by AFB or metabolic extracts from *Aspergillus versicolor* or *A. ochraceus*. Study results of Abbas *et al.* (1994) shows that aqueous extracts of green tea possess marked antimutagenic potential against a variety of important dietary and environmental mutagens in Wistar albino rats.

Garlic was found to inhibit the mutagenicity induced by direct acting mutagens such as N-methyl N'-nitro-N-nitrosoguanidine (MNNG) and sodium azide. Asafoetida, turmeric, curcumin (phenol present in turmeric) and eugenol (phenol present in clove) were found to inhibit microsomal activation dependent mutagenicity of 2-acetamidofluorene (Soudamini *et al.*, 1995). Further, Mitscher *et al.* (1996) reviewed natural antimutagenic agents and tumour chemopreventive agents. They concluded that spices like garlic (*A. sativum*), caraway (*Carum carvi* L.), coriander (*C. sativum*), black pepper (*P. nigrum*) and turmeric (*C. longa*) are showing antimutagenic activity in bacterial strains. But dietary curcumin and the glycoflavanoid hesperiden (both turmeric constituents) showed anticarcinogenic activity against various carcinogens.

Abraham *et al.* (1998) reported antigenotoxic effects and changes in glutathione-S-transferase (GST) activity of aqueous extracts of dietary vegetables, spices (cinnamon, *Cinnamomum zeylanicum* B.; pepper, *Piper nigrum*; cumin, *Cuminum cyminum*; clove, *Eugenia caryophyllus* and cardamom, *Elettaria cardamomum*), tea and coffee against Urethane (URE) in Swiss albino mice.

Meshram *et al.* (2000) studied mutagenicity and antimutagenicity of ginger (*Z. officinale*) extracts using Ames test. Both ethanolic and aqueous extracts found to be non-mutagenic and antimutagenic against various mutagens/carcinogens. Hussain *et al.* (2000) confirmed antimutagenic/anticarcinogenic activity of ajowan (*Carum*

copticum) using Ames test and *in vivo* bone marrow micronucleus test against various mutagens/carcinogens.

Shukla *et al.* (2002) evaluated the antimutagenic potential of certain dietary constituents like black tea extract, diallyl sulfide (component of garlic, *A. sativum*) and curcumin (component of turmeric, *C. longa*). They found that the antigenotoxicity of these dietary constituents towards cyclophosphamide induced chromosomal aberrations and micronuclei. In another study El Hamss *et al.* (2003) used the wing Somatic Mutation and Recombination Test (SMART) in *Drosophila melanogaster* to study the modulating action of bell pepper (*Capsicum annum*) and black pepper (*Piper nigrum*) in combination with the alkylating agent Methyl methanesulfonate (MMS) and the promutagen agent ethyl carbamate (EC). They observed that bell pepper was effective in reducing the mutational events induced by EC and MMS and black pepper was effective only against EC.

It has been reported that some kinds of plant essence, such as cinnamaldehyde, coumarin, umbelliferone, anisaldehyde, vanillin and tannic acid had antimutagenic effects on mutations induced by UV or chemicals in *Escherichia coli* (Ohta *et al.*, 1983a,b, 1986, 1988; Shimoi *et al.*, 1985). Further, Sukumaran and Kuttan (1995) observed inhibition of tobacco-induced mutagenesis by eugenol (constituent of clove) in Ames Salmonella/microsome assay and also noticed that eugenol inhibited the nitrosation of methyl urea in a dose-dependent manner. It was reported that capsaicin (a major pungent and irritating ingredient of hot pepper chilli) and diallyl sulfide (a thioether found in garlic) suppress vinyl carbamate (VC) and N-nitrosodimethylamine (NDMA) induced mutagenesis or tumourigenesis in *S. typhimurium* (surh *et al.*, 1995).

Simultaneous treatment of ICR170, a strong mutagen with safrole had no significant effect on mutations in Chinese hamster V79 cells. Pre-treatment and post-treatment with safrole slightly enhanced the frequency of mutations induced by ICR170 (Kuroda *et al.*, 1992). Study results of Farag and Abo-Zeid (1997) using Ames test proved that the mutagenicity of some spices due to the presence of safrole (ingredient of star anise, *Illticium verum* Hook.; cumin, *C. cyminum*; black pepper, *P. nigrum* and ginger, *Z. officinale*) can be destructed during drying of the washed seeds or during cooking either with or without any additional treatment as irradiation. Further they found that boiling whole seeds or powder of black pepper during cooking for few minutes (1-5 min) were more efficient in decreasing safrole content.

Allylisothiocyanate (AIT), a potent component of wasabi (*Wasabi japonica*) and horseradish (*Cholearia arnoracia*) showed antimutagenic activity toward 2-amino-3,8-dimethylimidazo[4,5-f]quinoxaline [MeIQx], a well-known mutagen/ carcinogen in broiled fish and meat. They also decreased His+ revertant colonies of 3-chloro-4-dichloromethyl-5-hydroxy-2(5H)-furanone (MX) in the Ames test (Kinae *et al.*, 2000). It was inferred that chlorophyll can successfully suppress the mutagenic activities of capsaicin and 2-aminoanthracene together with other antimutagenic factors that were present in the acetone extract of *Capsicum annum* in *S. typhimurium* TA 100 (Azizan and Blevins, 1995).

Vinitketkumnuen *et al.* (1994) found that ethanolic extract of lemon grass, *Cymbopogon citratus* Stapf (a Thai medicinal plant commonly used in the diet and in medicine) modifies mutation in *S. typhimurium* strains TA98 and TA100 which are induced by various known mutagens. *Ocimum sanctum* (Sanskrit: Tulasi), a sacred and well known medicinal plant in India affords *in vivo* protection against radiation induced cytogenetic damage in mice (Ganasoundari *et al.*, 1996). A survey of

scientific literature for the last 30 years has identified a number of medicinal plants and their components with antimutagenic and anticarcinogenic properties. This includes spices like garlic (*A. sativum*), mustard (*Brassica juncea*), turmeric (*C. longa*) and ginger (*Z. officinale*) (Devasagayam and Tilak, 2002).

It has already reported that curcumin, the major pigment in turmeric (important constituent of *curcuma longa*), possesses good anti-oxidant (Ruby *et al.*, 1994; Soni *et al.*, 1997a), anti-inflammatory (Ruby *et al.* 1998) and antitumour activity (Kuttan *et al.*, 1985; Menon *et al.*, 1995). Further it was tested for its antimutagenic and anticarcinogenic activity (Nagabhushan and Bhide, 1987) and found that potent inhibitors of mutagenesis in bacterial strains (Ruby *et al.*, 1996a). Both natural and synthetic curcumin showed antiinflammatory and cholesterol reducing properties in mice (Soudamini and Kuttan, 1992; Ruby *et al.*, 1998) and in man (Soni and Kuttan, 1992). Further they found curcumin to inhibit lipid peroxidation and superoxides (Nishigaki *et al.*, 1992; Inoue *et al.*, 1992; Thresiamma *et al.*, 1995).

Using Ames test, curcumin itself a non-mutagen, inhibited the mutagenic effects of chilli extract and capsaicin (Nagabhushan and Bhide, 1986). Similarly, curcumin was reported to inhibit the activity of known environmental mutagens which require metabolic activation, although it was reported to be ineffective against mutagens which do not require metabolic activation (Nagabhushan *et al.*, 1987; Nagabhushan and Bhide, 1987).

Turmeric and curcumin along with CP increased the life span of animals when compared to animals treated with CP alone (Soudamini *et al.*, 1992). Histopathological study revealed that both turmeric and curcumin are inhibitors of aflatoxin induced toxicity in experimental ducklings. Further extracts of turmeric,

garlic and asafoetida inhibited the aflatoxin production considerably (Soni *et al.*, 1992). Further, Turmeric and curcumin inhibited aflatoxin toxicity in ducklings (Soni *et al.*, 1993). In another study turmeric, curcumin, asafoetida and garlic were found to inhibit the mutagenesis induced by aflatoxin in Salmonella testers strains (Soni *et al.*, 1997b). There was a significant time-dependent reduction in the number of radiation-induced micronucleated polychromatic erythrocytes in mice with a single gavage doses of 5, 10 or 20 mg/kg b.w. curcumin in peanut oil (Abraham *et al.*, 1993).

Kuttan (1994) highlighted the antioxidant, antiinflammatory, liver protecting, antimutagenic, anticarcinogenic and antiteratogenic activities of turmeric and its constituent curcumin. Further, Shishu *et al.* (2002) studied the antimutagenic potential of various constituents of turmeric against the heterocyclic amine mutagens that are generated during cooking of muscle meats such as beef, fish and chicken using Ames test. Results indicated that natural curcuminoids are highly effective in a dose-dependent manner.

Turmeric has been attributed a number of medicinal properties in the traditional system of medicine. The major claims have been for use as antiseptic, cure for poisoning, eliminating body waste products, for dyspepsia, respiratory disorders and cure for a number of skin diseases including promotion of wound healing. Curcumin, curcuminoids and essential oils are the major active constituents. The main activities have been found to be anti-inflammatory, hepato-protective, antimicrobial, wound healing, anticancer, antitumour and antiviral (Srimal, 1997). Paper further added on the proper evaluation of antiviral properties of curcumin, particularly against HIV.

2.3. ANTICANCER/ANTITUMOUR STUDIES

The association between diet and cancer has received increasing attention and support in the last two decades as a result of data compiled by epidemiologists, clinicians and laboratory scientists. Dietary practices may either increase or decrease cancer risk depending on the intake of nutrients as well as non-nutrients and their interactions at several stages of carcinogenesis. Dietary substances can alter carcinogen metabolism, *in vivo* host response, damage to macro molecules, immune surveillance and modify promotion and progression of neoplasia (Krishnaswamy, 1991). The case control approach revealed that vitamin A, E, zinc and selenium have potential effects on cancer risk. However, several non-nutrient components in leafy vegetables and spices appear to offer protective role by inhibiting the process of carcinogenesis. Of these, turmeric, mustard, onion and garlic appear to be promising agents. Nutrient intervention (chemoprevention) of precancerous lesions appears to be an attractive alternative to prescription for prevention of cancer. Dietary modifications though difficult, could be more interesting as both the nutrients and non-nutrients can have an aggregate effect on cancer prevention.

In 1969, the International Agency for Research on Cancer (IARC) initiated the Monographs Programme to evaluate the carcinogenic risk of chemicals to humans. Results from short-term mutagenicity tests were first included in the IARC Monographs in the mid-1970s based on the observation that most carcinogens are also mutagens, although not all mutagens are carcinogens. Experimental evidence at that time showed a strong correlation between mutagenicity and carcinogenicity and indicated that short-term mutagenicity tests are useful for predicting carcinogenicity (Waters *et al.*, 1999). Although the strength of these correlations has diminished over

the past 30 years with the identification of putative nongenotoxic carcinogens and understanding mechanisms of carcinogenesis.

As effort continue toward better understanding of the various mechanisms involved in the induction of human cancer, results from short-term genetic tests will continue to provide valuable information for discriminating mutagenic and non-mutagenic mechanisms. Results from mammalian assays *in vivo*, followed by those from mammalian assays *in vitro* are considered more relevant than those from non-mammalian assays (Waters *et al.*, 1999).

Life style including dietary habits is one of the most important factors responsible for different types of cancer. The role of diet in human cancer has prompted many to analyse the food items for possible mutagens and carcinogens (Sivaswamy *et al.*, 1991). The limited data on herbs, spices and condiments come mainly from a few human case-control studies and some experimental animal studies. Human studies are limited by difficulties in quantifying intakes of individual items that are typically consumed in small quantities (Anonymous, 1997). A few studies have provided results relevant to spices in particular and herbs in general. These include *in vitro* studies and animal experiments.

Strong evidence suggests that consumption of fruits and vegetables results in decreased incidence of all types of cancer. They are known to contain variety of non-enzymatic antioxidants, namely carotenoids, tocopherols, ascorbic acid and plant polyphenols which exert their antimutagenic activity, even after subjected to the cooking process (Mathur, 1997). Diet consisting of food containing carbohydrates, proteins and fats but lacking fruits and vegetables, the level of DNA damage is higher than for diets including fruits and vegetables, which are rich in natural antioxidants (Simic and Bergtold, 1991).

The role of dietary factors on the development of tumours in animals (Boone *et al.*, 1990; Freedman *et al.*, 1990) and of antioxidants in reducing tumour incidence (Wattenberg, 1978) and the underlying free radical mechanisms (Simic, 1989) are supporting to the above observation and conclusion. Fruits and vegetables lower the level of oxidative DNA damage most likely reflects the interaction of plant antioxidants with O_2, thereby reducing the level of H_2O_2 and OH radicals. Other reactions may play a major role (e.g., inhibition of peroxyl radicals) but the correlations are not apparent (Simic and Bergtold, 1991).

Kuttan *et al.* (1985) reported that turmeric extract as a potential anticancer agent. This property is due to curcumin, a cytotoxic component present in turmeric (*C. longa*). Further the animal experiments showed that both turmeric extract and curcumin decreased the incidence of tumour formation in experimental mice. An ethanolic extract of turmeric as well as an ointment of curcumin (its active ingredient) were found to produce remarkable symptomatic relief in patients with external cancerous lesions (Kuttan *et al.*, 1987). In another study Soudamini and Kuttan (1988) reported that both aqueous and alcoholic extracts of turmeric were cytotoxic to various cell lines *in vitro* and tumour reducing in Swiss albino mice bearing Dalton's lymphoma ascites tumour.

Comparable anti-tumour effects were observed in animal studies with turmeric. A 1% dietary turmeric inhibited the formation of BP-induced forestomach tumours in female Swiss mice by 58% and lowered the incidence of spontaneous mammary tumours in C3H Jax mice by 60% (Nagabhushan and Bhide, 1987). Anti-tumour effect of curcumin is supported in another study of skin carcinogenesis in mice. Repeated applications of turmeric extract and curcumin in the promotion phase

produced a reduction in the expression of papillomas in mouse skin induced by 7,12 dimethylbenzanthracene (Soudamini and Kuttan, 1989).

Strong antioxidant effects of several components of turmeric result in an inhibition of carcinogenesis. Extracts of the spice may play a role as chemoprotectant, which limit the development of cancers (Liberti, 1993). Curcumin showed a reasonable increase in the lifespan of Ehrlich tumour bearing mice (Ruby *et al.*, 1994). Further it also showed a significant reduction on solid tumours in mice when injected intraperitoneally.

Later studies of Ruby *et al.* (1995, 1996b) showed that natural and synthetic curcuminoids (especially those having the phenolic structure) possess the anticancer and antioxidant activities evinced by curcumin and also act as potent antipromoters. They increased the life span of animals bearing Ehrlich ascites carcinoma. Further, dietary administration of food additives such as turmeric, garlic and curcumin to rats treated with aflatoxin B_1 inhibited the development of hepatocellular neoplasm (Soni *et al.*, 1997b).

Dhar *et al.* (1968) screened ethanolic extracts of 285 botanically identified plant materials including spices with 61 tests including anticancer tests. Results obtained includes anticancer potential of several plants. They noticed that a number of plants showed activity with the crude extract which could not be confirmed on fractionation.

Out of 20 spices/leafy vegetables screened for their influence on the carcinogen-detoxifying enzyme, glutathione-S-transferase (GST) in Swiss mice, spices like cumin seeds, poppy seeds, asafoetida and turmeric showed protective activity against carcinogenesis (Aruna and Sivaramakrishnan, 1990). They significantly suppressed (*in vivo*) the chromosome aberrations caused by B(a)P in

mouse marrow cells. But spices like fenugreek seeds, coriander seeds and ginger did not show significant result.

Chemical components isolated from vegetables and spices such as cauliflower, citrus fruits, tomatoes, green chillies, pineapples, strawberries, garlic, onion, soya and red chillies were studied for their anticancer activities (Madhavankutty, 1994). Components like sulphoraphane, indole-3-crbinol, flavonoids, coumaric acid, chlorogenic acid, allylic sulphides, genistein, and capsaicin showed anticancer ability.

Tumour reducing activity of extracts of eight commonly used spices in India were studied in mice transplanted intraperitoneally with Ehrlich ascites tumour (Unnikrishnan and Kuttan, 1990). Oral administration of extracts of black pepper (*P. nigrum*), asafoetida (*Ferula asafoetida*), pippali (*Piper longum*) and garlic (*Allium sativum*) could increase the percentage of life span but intraperitoneal administration of spice extracts did not produce any significant reduction in tumour growth except of sesame (*Sesamum indicum*). Asafoetida and garlic were not only found to increase the life span of tumour bearing animals but also was shown to inhibit chemical carcinogenesis. Other spices like ginger (*Z. officianale*), mustard (*Brassica compastris*) and horsegram (*Dolichos biflorus*) did not show any positive response against ascites tumour.

Painting and feeding of mice with black pepper extract results in a significant increase of the number of tumour bearing mice. Further, feeding of mice with powder of black pepper in diet has no impact on carcinogenesis (Shwaireb *et al.*, 1990). Force feeding of d-limonene (a pepper terpenoid) for a long time to the mice showed anticarcinogenic activity against above constituents and methylcholanthrene (MCA). But piperine (one of black pepper alkaloid) was ineffective. In another study El-Mofty *et al.* (1988, 1991) speculated that one or more constituents of black pepper may be

responsible for tumour induction in the organs of the Egyptian toad, *Bufo regularis* fed with suspension of black pepper. Further studies showed metastatic deposits of hepatocellular carcinomas in the spleen, kidney, fat body and ovary. Wrba *et al.* (1992) observed that in mice, injection of safrole and tannic acid (constituents of black pepper) during the pre-weaning period induced tumours in different organs. Piperine could inhibit the pulmonary metastasis induced by B16F10 melanoma cells in mice and also observed significant increase in life span of tumour bearing animals treated with piperine (Pradeep and Kuttan, 2002).

Among the vegetables/spices, those belonging to the allium family have received increased attention in recent times. Onion and garlic are commonly consumed through the diet. They contain sulphur compounds like diallylsulphide and diallyl disulphide. Onion (*Allium cepa* L.) extract was found to be cytotoxic to ascites cells (MFS-180) under *in vitro* condition and also it could check the ascites tumour growth in mice when administered along with transplantation of tumour cells (Nerkar *et al.*, 1981). Study results of Unnikrishnan *et al.* (1990) showed chemoprotection of garlic (*Allium sativum*) extract towards cyclophosphamide toxicity in mice with an increase in life span. Garlic extract alone does not have any tumour reducing activity, but it reduced the toxicity of cyclophosphamide and increased its therapeutic efficacy significantly. Further, it was found that garlic extract reverse the toxicity induced weight loss in animals and free radical scavenging significantly.

Samman *et al.* (1998) observed that mint (*Mentha arvensis* L.) has a chemopreventive effect against shamma (Shamma, a complex mixture of powdered tobacco, slaked lime, ash, oils, spices and other additives, has been linked to oral cancer in Saudi Arabia) induced carcinogenesis in hamsters. Menon *et al.*(1998) studied two dietary soybean isoflavones, genistein and daidzein for the inhibition of

lung metastasis induced by B16F-10 melanoma cells in mice. Genistein inhibited lung tumour nodule formation and also increased the life span of the tumour-bearing animals. But daidzein had no significant effect on the reduction of lung metastasis. Sukumaran and Kuttan (1991) studied antitumour potential of ferns in mice bearing Ehrlich ascites tumour using animal survival studies.

In recent years, several observations have strongly implicated nitrate, nitrite and nitrosamines in the development of tumours in man. Nitrates are ubiquitous in water and food. Though nitrates are not harmful it can be reduced to nitrite under certain circumstances both food and in the body which combine with amines in food to form nitroso compounds. The nitrate content of foods show that it is high in spices followed by vegetables particularly the non-tuberous variety followed by roots and tubers (Gundimeda *et al.*, 1993). *In vitro* studies shows several spices such as pepper, red chillies, and cumin yield significant amount of nitroso compounds. Substances such as turmeric and tomato can inhibit *in vitro*, nitrosations (Krishnaswamy and Polasa, 1995). Vitamin C is a potent inhibitor of *in vivo* nitrosations. High nitrite containing foods with high salt intake may increase the risk of gastric cancer.

In an attempt to find natural products with antitumour/radiosensitizing properties, extracts of medicinal plants were screened in experimental tumour systems. It was found that *Withania somnifera*, popularly known as Ashwagandha (Sanskrit) in India, has properties that may prove useful in clinical cancer therapy. Sharada *et al.* (1996) studied the antitumour and radiosensitizing effects of Withaferin A (WA), a steroidal lactone from *W. somnifera* on mouse Ehrlich ascites carcinoma *in vivo*. Increase in life span and tumour free survival were studied up to 120 days. Important findings of the study were the higher *in vivo* tumour killing when WA treatment was combined with irradiation. The drug inhibited tumour growth and

increased survival, which was dependent on the WA dose per fraction rather than the total dose.

The alcoholic extract of the dried Withania roots as well as the active component Withaferin A isolated from the extract showed significant antitumour and radiosensitizing effects in experimental tumours *in vivo*, without any noticeable systemic toxicity (Umadevi, 1996). Further, Withaferin A showed significant growth inhibitory and cytocidal effects on exponentially growing mouse Ehrlich ascites carcinoma *in vivo* (Umadevi, 1996). Shohat *et al.* (1967, 1970) found that Withaferin A obtained from Withania leaves inhibited the *in vivo* growth of Ehrlich ascites carcinoma in the mouse. In another study Umadevi *et al.* (1992, 1993) observed that ethanolic extract of Withania roots was very effective against transplantable mouse sarcoma-180 solid tumour *in vivo* and that the tumour killing effect significantly increased by combining Ashwagandha treatment with irradiation and hyperthermia. Further, crude extract was also effective in prolonging the life span of mice bearing Ehrlich ascites tumour (Umadevi *et al.*, 1994).

Rao and Umadevi (1996a) studied *in vivo* response of mouse sarcoma-180 to different doses of Cisplatin in combination with radiation (RT) and hyperthermia (HT). On the basis of tumour cure (CR), volume doubling time (VDT), regrowth delay (RD) and animal survival up to 120 days; it was concluded that combination of a moderate dose of HT with low dose of cisplatin could enhance the tumour cure and prolong survival of mice. Further, *in vivo* response of mouse sarcoma-180 to multimodality treatment using AK-2123 (AK), hydralazine (HDZ), irradiation (RT) and hyperthermia (HT) was analysed (Rao and Uma devi, 1996b). Multimodality approach using AK, RT and HT with the inclusion of HDZ was more effective than

the bimodality and the trimodality treatments without HDZ based on CR, VDT, RD and animal survival up to 120 days.

Umadevi and Ganasoundari (1995) studied radioprotective effect of leaf extract of *Ocimum sanctum* using Swiss albino mice. Animal survival was studied up to 30 days and the aqueous extract was more effective in increasing survival than the alcohol extract. Further, intraperitoneal (ip) administration gave the best protection (70% survival) and other routes (im, iv, and po) were less effective and produced (37-47%) survival. Menon *et al.* (1997) reported increase in life span of mice bearing metastatic tumour treated with herbal drugs like brahma rasayana and aswagandha rasayana.

Table 2.2. Summary of studies on the mutagenic potential of different dietary substances.

Exposure/ Test material	Test object	Test system	Mutagenicity (+ / -)	References
Edible oil	*S. typhimurium* TA98 & TA100	Frame shift and base pair mutations	-	Gastel, *et al.*, 1984.
Capsaicin	Mice-Germ cells and testes	SSA, DLT	-	Muralidhara and Narasimhamurthy, 1988.
Theophylline (alkaloid in tea)	Mice-Germ cells	CAT	+	Kameshwari *et al.*, 1991.
Capsaicin	Mice-Bonemarrow	MN	+ -	Bhide *et al.*,1993. Villasener *et al.*, 1993.
Eugenol	*S. typhimurium* TA100, TA102, TA1535	Revertants	-	Sukumaran and Kuttan, 1995.
Mustard and horse radish	*S. typhimurium* TA100	Revertants	-	Kassie *et al.*, 1996.
Piperine	Mice-Bone marrow & *S. typhimurium*	MN, SSA & DLT, Revertants	-	Karekar *et al.*, 1996.
Mint	*S. typhimurium*	Revertants	-	Samman *et al.*, 1998.
Chilli oleoresin & Capsaicin	*S. typhimurium*	Revertants	-	Gupta *et al.*, 2000

Note:- CAT = Chromosomal aberration test ; **DLT** = Dominant lethal test;
MN = Micronucleus test; **SSA** = Sperm-shape abnormality test.

Table 2.3. Summary of studies on antimutagenicity of different dietary substances.

Exposure/ Test material	Analysed biomarker (mammals)	Analysed biomarker (other than mammals)	Mutagens used	Protective effect/ Antimut agenicity (+ / -)	References
Indian gooseberry (Emblica officinalis G.)	-	Ames test – *S. typhimurium*	SA, 4-NPDA	+	Grover and Kaur, 1989.
Chlorophylls (Aquatic plants)	-	Ames test – *S. typhimurium*	B(a)P	-	Sato *et al.*, 1990.
Turmeric (*C. longa*) and Curcumin	Wistar rats-liver (DNA) Golden hamsters oral pouches	-	B(a)P DMBA	+ +	Mukundan *et al.*, 1993. Krishnaswamy *et al.*, 1998.
Mustard seed (*Brassica nigra*)	Wistar rats-urine	Ames test – *S. typhimurium*	B(a)P	+	Polasa *et al.*, 1994.
Chlorophylls	-	*D. melanogaster*	4-NQO	+	Negishi *et al.*, 1997.
Vitamins	Review Article	-	-	+ (Vit.A, C,E)	Odin, 1997.
Ascorbic acid (Vit.C)	Mice-CAT, MN, SSA	-	CIS	+	Giri et al, 1998.
Carotenoids	Mice-MN	Ames test – *S. typhimurium*	B(a)P, AF, CP, IQ	+	Raucher *et al.*, 1998.
Mint (*Mentha arvensis* L.)	-	Ames test – *S. typhimurium*	MMS, B(a)P, AFB_1	+	Samman *et al.*, 1998.
Tea polyphenols	Review Article	-	-	+	Kuroda and Hara, 1999.
Chilli (*C. annum*) & Capsaicin	-	Ames test – *S. typhimurium*	NQO, SA, MMC, MG, CP, DMBA	+ (Except SA)	Gupta *et al.*, 2000.
Saffron (*Crocus sativus* L.)	Mice-MN, GST activity	-	CP, CIS, MMC, URE	+	Premkumar *et al.*, 2001.

Contd....

Exposure/ Test material	Analysed biomarker (mammals)	Analysed biomarker (other than mammals)	Mutagens used	Protective effect/ Antimut agenicity (+ / -)	References
Tea (*Camellia sinensis* & *C. assamica*)	Review Article	-	-	+	Gupta *et al.*, 2002.
Curcumin	Wistar rats-CAT	-	CP	+	Taneja *et al.*, 2002.
Vitamins (A, C, E)	Wistar rats-Blood serum and liver	-	2,4-DAB	+	Velanganni and Balasundara, 2003.

Note:- **AF** = Aflatoxin; **2-AAF** = 2-acetamidofluorene; **B(a)P** = Benzo (a) pyrene; **CIS** = Cisplatin; **CP** = Cyclophosphamide; **DAB** = Dimethylaminoazobenzene; **DMBA** = 7,12-dimethylbenzanthracene; **IQ** = 2-amino-3-methylimidazo (4,5f)quinoline; **GST** = Glutathione S-transferase; **MMC** = Mitomycin; **MG** = Methylglyoxal; **MMS** = Methylmethane sulfonate; **MNNG** = N-methyl-N-nitro-N-nitroso guanidine; **4-NPDA** = 4-nitro-O-phenylenediamine; **NQO** = 4-nitroquinoline, 1-oxide; **SA** = Sodium azide; **URE** = Urethane; **CAT** = Chromosomal aberration test; **MN** = Micronucleus test; **SSA** = Sperm-shape abnormality test.

Table 2.4. Summary of studies on anticarcinogenicity/antitumour activity of dietary substances.

Exposure/ Test material	Carcino gens/ Tumour	Test object/ Animals	Organs	Anticarcin ogenicity (+ / -)	References
Cinnamoyl methanes (analogs of curcumin)	DLA cells	Albino mice and CHA cell culture	Cells	-	Rao *et al.*, 1987.
Curcumin	BMBA, TPA	Albino mice	Skin	+	Nishio *et al.*, 1992.
Capsaicin	BHC	Dawley rats and golden hamsters	Cheek pouch	-	Bhide *et al.*, 1993.
Chilli, cumin & black pepper	DMH	Wistar rats	Colon	Chilli (-), cumin & black pepper (+)	Nalini *et al.*, 1998.
Tea polyphenols	Review	Wistar rats & Albino mice	-	+	Kuroda and Hara, 1999.
Saffron	Review	-	-	+	Abdullaev, 2002.

Note:- BHC = Benzene hexachloride; **CHO** = Chinese hamster ovary;
DLA = Dalton's lymphoma ascites; **DMBA** = 7,12-dimethylbenzanthracene;
DMH = 1,2-dimethylhydrazine; **20-MC** = 20-methylcholanthrene;
TPA = 12-O-tetradeonylphorbol-13-acetate.

CHAPTER - III

MATERIALS & METHODS

Mutagenic and antimutagenic effect of selected spices were studied in mice by employing the following parameters.

(a) Chromosomal aberrations in bone marrow cells.

(b) Micronucleus assay of bone marrow erythrocytes.

(c) Sperm-shape abnormality assay.

Further, the 'mitotic index' values were analysed along with chromosomal aberration test and the 'PCE/NCE ratios' were noted along with MN test. Body weight and testes weights were also studied along with sperm-shape abnormality assay.

Antitumour activity of spices was carried out using Ehrlich ascites tumour cells.

Following materials and methods were used for the above studies.

3.1. ASSAY ANIMALS

In the present investigation, Swiss albino mice *Mus musculus* (2n=40) were used as experimental animals. The animals were bred and maintained as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) Chennai, India (Balaram, 1998; Ramalingaswami, 1998). Mice were initially procured from the Sri Venkateshwara Enterprises, Bangalore, India and were acclimatized for two weeks, reared and inbred in the animal house of Zoology Department (Plate - IIa). From their progeny, 8-10 weeks old healthy individuals (25-30 g body weight) were selected for the present study. Animals of both the sexes were used except for sperm abnormality test. The animals were randomized into groups of 5 animals each and housed in opaque, grill topped polypropylene cages (290 x 220 x 140 mm) bedded with paddy husk. The mice were

maintained at ambient laboratory conditions viz. temperature 28^{0}C (±2^{0}C), relative humidity 65% (±5%) and photoperiod 12L:12D (Raghuramulu *et al.*, 1983). They were fed with standard mice pellets ('Amrut' Laboratory animal feeds Sangli-Maharashtra, India, supplied by Kamadhenu Agencies, Bangalore) and water *ad libitum.*

3.2. PREPARATION OF SPICE EXTRACT

3.2.1. Sources of Spices:

a) *Piper nigrum* — Mr. Kongot Narayana Bhat, Nileshwar, Kerala, India.

b) *Elettaria cardamomum* — 'Kalbavi Exporters', Mangalore, Karnataka, India.

c) *Coriandrum sativum*
d) *Cuminum cyminum* — 'Chandu Traders', Panaji, Goa, India.

3.2.2. Extraction of Spices:

Spices were extracted following slightly modified method of Unnikrishnan and Kuttan (1988). The method of extraction is common for all four spices. After the spices were brought to the laboratory, undesirable substances were separated, if any. They were directly powdered using mortar and pestle just before extraction. Fine powders of spices were collected by using 210 µ size sieve ("Kumar" B.S.S. 72, India). Care was taken to clean and dry the sieve, mortar and pestle thoroughly after powdering a particular spice and before starting to powder a new spice to avoid mixing up of spices. The powdered spices were extracted with ethanol as described below.

One gram of dry powder was weighed ("Sartorius" BP 110S, Germany) and stirred with 100 ml of 90% ethanol on a magnetic stirrer ("Remi"2MLH-210, India) at

40^0C. The extract was evaporated until a syrupy liquid was left out. This concentrated extract was diluted to 100 ml in isotonic saline (0.9%).

3.3. DOSE AND ROUTE OF ADMINISTRATION

3.3.1. Spices:

Three different doses viz. low (L), normal (N) and high (H) were selected for the present study. Normal (N) doses were selected based on human consumption of various spices. This was calculated by referring publications of National Institute of Nutrition (NIN), Hyderabad, India (Pasricha and Rebello, 1977; Srikantia, 1977; Pasricha, 1989; Rao *et al.*, 1989; Varadarajan, 1990; Pasricha and Thimmayamma, 1992; Swaminathan, 1992; Mohanram, 1996; Krishnaswamy, 1998) and by interviewing various families belonging to different regions of the country. Ten times higher and lower to normal dose were selected as the higher (H) and lower (L) doses respectively (Table - 3.1). Extracts of spices prepared as above were directly administered to stomach though stomach tube ('gavage') (Plate - IIc).

Two doses i.e., normal (N) and high (H) doses of spices were selected for antimutagenicity and tumour reduction studies.

The antimutagenic effect of cumin against the mutagenic effect of black pepper was studied employing 2 doses (N&H) of black pepper and a single dose (H) of cumin which were found to be significantly mutagenic and antimutagenic respectively.

3.3.2. Cyclophosphamide (CP):

Cyclophosphamide, because of its pronounced mutagenic side effects, was selected as a positive control. In the present investigation 50 mg/kg body weight dose of CP was administered intraperitoneally. Since this dose yields high frequency of

MN and chromosomal aberrations in mouse and shows less cytotoxicity (Krishna, *et al.*, 1995; Shyama and Rahiman, 1993, 1996), this particular dose was chosen for the present study. Cyclophosphamide solution was prepared by dissolving 0.125 g of CP powder in 10 ml of distilled water just before use.

3.4. TREATMENT PROTOCOL

3.4.1. Mutagenicity Studies:

All three doses (Low, Normal and High) of the spice extracts were orally administered to the experimental animals in equal quantity (1.5 ml) for 7 days at 24 h time intervals.

A solvent control group of animals administered with 1.5 ml of saline (0.9%) (Nagabhushanam *et al.*, 1981) for seven days was also maintained. This group formed the negative controls.

Another group of animals treated intraperitoneally (i.p) with 50 mg/kg body weight of CP (0.1 ml) were used as positive controls.

3.4.2. Antimutagenicity Studies:

Two different doses (Normal and High) of the spice extracts were used for antimutagenicity studies. Above mentioned doses of the extracts were orally administered (pre-treatment) for 7 days. On the 7^{th} day, animals were injected intraperitoneally with selected dose of the positive mutagen (CP-50 mg/kg body wt.), one hour after the last dose of the extract treatment.

Sperm-shape abnormality assay groups of animals were treated with CP on the day one. From the 2^{nd} day onwards for 7 consecutive days they were administered (post-treatment) with spice extracts. Even though Wyrobek *et al.* (1984) recommended a 5-day dose schedule for the sperm abnormality assay, in the present

study CP was administered only once, to correlate the results with other parameters used i.e., bone marrow MN test and chromosomal aberration assay.

Antimutagenic activity of cumin (antimutagenic spice) against mutagenic spice i.e., black pepper was also studied. Both the spice extracts were administered alternately for 10 days at 24h time interval (simultaneous treatment).

Table 3.1. Selected doses of spices and treatment protocol

Treatment	Mutagenicity Experiments			Antimutagenicity Experiments	
I. Spices	(mg/kg body wt. in 1.5ml saline for 7 days)			(mg/kg body wt. in 1.5 ml saline for 7 days + 50 mg CP in 0.1 ml distilled water on the 7th day)	
	L	N	H	N	H
P. nigrum (PN)	3.8	38	380	-------	
E.cardamomum (EC)	2.7	27	270	27	270
C. sativum (CS)	3.4	34	340	34	340
C. cyminum (CC)	6.1	61	610	61	610
II. Controls					
Negative control (saline)	1.5 ml			1.5 ml	
Positive control (CP)	50 mg/kg in 0.1 ml distilled water, once			-------	
III. Combined				(mg/kg body wt. in 1.5 ml saline for 10 alternate days)	
P. nigrum + *C. cyminum* (PN+CC)	-------			38 + 610 380 + 610	

3.5. EXPERIMENTAL PROTOCOL

Four sampling times i.e., 12, 24, 48 and 96 h after the final treatment were used in the present investigation to study time-response effect. For antimutagenicity

experiments (i.e., combined treatment of spice and CP) only 24 h sampling time was used. For sperm-shape abnormality assay 5 weeks (35 days) post-treatment sampling time was used. Five animals were used for each treatment and control group. In combined treatment of black pepper and cumin 24 h post treatment sampling time was used and the result was correlated with 96 h black pepper alone treatment (7 days) in mutagenicity studies.

3.5.1. Chromosomal aberration test/assay:

Bone marrow chromosomal preparations were made following the method of Tjio and Whang (1962).

3.5.1.1. Preparation of slides/metaphase plates:

About 0.25 ml of 0.025% colchicine was injected intraperitoneally to control and treated animals at the above mentioned time intervals to arrest the dividing cells at metaphase stage. After 90 minutes they were sacrificed by cervical dislocation, the femur and tibia bones were collected and cleaned well. Both ends of the bones were cut open and the bone marrow was flushed into a centrifuge tube with about 2-3 ml of prewarmed (37^0C) hypotonic potassium chloride solution (0.56%) by using a syringe and a needle within the minimum possible time. The flushed cells collected in the centrifuge tube were mixed well with KCl solution and were incubated ("Remi"6A/6S, India) at 37^0C for 20 minutes for the cells to swell. The time of the hypotonic treatment is critical. The incubated suspension was centrifuged at 1000 rpm ("Remi" R-23, India) for 8 minutes. The supernatant was discarded and the pellet was fixed in 2-3 ml of prechilled Carnoy's fixative (1:3 aceticacid:methanol) and kept for an hour to allow the suspended cells to react with fixative. The contents of the tube were centrifuged again, supernatant was discarded and residue was resuspended in 2-3 ml of fresh fixative, incubated for 15 minutes and centrifuged. The process was

repeated thrice using fresh fixative and at the end, the pellet was dispersed in a small amount of fresh fixative to form a thick cell suspension.

'Blue Star' superdelux microslides were washed well, immersed in distilled water and chilled in the refrigerator ("Samsung" SR/A19NFO, India) at 20^0C. These prechilled absolutely clean slides were taken from the storage and drained. Test slides were prepared employing the flame dry technique with the cell suspension. About 3-4 drops of the suspension were dropped with a pasteur pipette on a cold wet slide held at an angle of 45^0 and at a height of about 2 feet from the pasteur pipette. Rapid evaporation of the fixative and the rupture of the swollen cells were then accomplished by quickly passing the slide though the flame. This flame dry technique results in good spreading-apart of the chromatid arms and enhances the appearance of the chromosome spread. Four slides were prepared for each animal. The prepared slides were then coded using a diamond point glass marker and allowed to dry well before staining with Giemsa.

3.5.1.2. Staining:

The bone marrow slides were stained with buffered Giemsa of pH 6.8. The Giemsa stain was diluted with buffer as and when required for staining. The bone marrow slides were kept in this diluted stain for 20 minutes. Excess stain was washed in the buffer solution. Slides were dried and observed under microscope.

3.5.1.3. Preparation of reagents and stain:

♦ Colchicine (0.025%):

Stock solution (0.1%) was prepared by dissolving 0.1 g of colchicine powder in 100 ml of distilled water and stored in a refrigerator.

The working solution (0.025%) was prepared whenever required by adding 1 ml of the stock solution to 3 ml of distilled water (4 times dilution).

♦ Potassium chloride (0.56%):

0.56 g of KCl was dissolved in 100 ml of distilled water and stored in an incubator at 37^0C.

♦ Carnoy's fixative (1:3 acetic methanol):

Carnoy's fixative was prepared by mixing 1 part of glacial acetic acid with 3 parts of methanol and chilled before use. This was prepared fresh just before use.

♦ Phosphate Buffer (pH 6.8):

2.366 g of disodium hydrogen phosphate (Na_2HPO_4) and 2.27 g of potassium dihydrogen phosphate (KH_2PO_4) were dissolved in 1 litre of distilled water.

♦ Giemsa stain:

Stock Giemsa stain was prepared by dissolving 1 g of Giemsa powder in 54 ml of glycerol and stirring it on a magnetic stirrer at 56^0C for two hours. After cooling, 84 ml of methanol was added to it and placed on a magnetic stirrer overnight at room temperature. The solution was finally filtered using Whatman's filter paper and stored as stock solution in a dark bottle.

The working solution was then prepared as and when required for staining by mixing 2 ml of the stock solution and 2 ml of buffer (pH 6.8) with 46 ml of distilled water.

3.5.2. Micronucleus assay:

Micronucleus (MN) test was performed by modified method of Schmid (1973). Here, instead of the foetal calf serum of Schmid's method, bovine serum albumin (BSA) was used as the suspending medium (Seetharam *et al.*, 1983).

3.5.2.1. Preparation of smears/slides:

The control and treated animals were sacrificed at the above mentioned time intervals by cervical dislocation, the femur and tibia bones were dissected out and cleaned off from the tissues. For the collection of bone marrow, both the ends of these bones were cut to leave small openings. The bone marrow was flushed into a clean cavity block using 0.5 ml of the suspending medium (5% BSA) taken in a syringe. About 2-3 ml of the media was used to collect the marrow of a single mouse. A fine suspension was made by gently mixing the contents in the cavity block using the syringe and then centrifuged at 1000 rpm for 8 minutes. The supernatant was discarded and a thick suspension was made in 2-3 drops of BSA solution. A drop of suspension was placed on a clean dry slide with the aid of a pasteur pipette and smeared according to the standard hematological preparations. Four slides were prepared for each animal. The smears were air dried and fixed in methanol for 10 minutes. The air-dried smears were stained between 3-24 h after the preparation.

3.5.2.2. Staining:

The smeared slides were placed in undiluted May-Grunwald's stain for 3 minutes and were later on transferred to May-Grunwald's stain diluted with distilled water (1:1) and were allowed to react for 3 minutes. The smears were later washed thoroughly in distilled water by giving two changes. These slides were transferred to diluted Giemsa stain with buffer (pH 6.8), 1:6 v/v and kept for 10 minutes. Excess of the stain was removed by rinsing the smear in buffer solution 3-4 times. Later on the smears were placed in buffer solution for 5 minutes for the proper differentiation of erythrocytes. Finally backsides of these slides were cleaned with methanol and the smears were allowed to dry. The dried smears were observed under microscope.

3.5.2.3. Preparation of reagents and stains:

♦ Bovine serum albumin (5%):

5% bovine serum albumin (BSA) solution was prepared by dissolving the required quantity of BSA powder in phosphate-buffered saline (PBS) of pH 7.2. While dissolving BSA powder care was taken by adding the powder little by little to the solvent with thorough mixing, to avoid any protein clumps and to form a clear solution. 2-3 drops of 1% sodium azide was added as preservative. BSA solution was prepared fresh in required quantity just before use.

♦ Phosphate-buffered saline (PBS, pH 7.2):

Disodium hydrogen phosphate (Na_2HPO_4) solution was prepared by dissolving 1.065 g in 50 ml of distilled water and sodium dihydrogen phosphate (NaH_2PO_4) solution was prepared by dissolving 1.71 g in 50 ml of distilled water. To prepare working buffer solution (pH 7.4), 41 ml of Na_2HPO_4 solution was mixed with 9 ml of NaH_2PO_4 solution. 50 ml of this buffer solution was mixed with 50 ml of normal saline (0.9%), to form phosphate-buffered saline (PBS) of pH 7.2.

♦ Normal saline (0.9%):

Isotonic saline/normal saline (0.9%) was prepared by dissolving 0.9 g of sodium chloride (NaCl) in 100 ml of distilled water.

♦ Sodium azide solution (1%):

1 g of sodium azide (NaN_3) was dissolved in 100 ml of distilled water.

♦ May-Grunwald's stain:

May-Grunwald's stain was prepared by dissolving 0.2 g of the stain powder in 100 ml of acetone free methanol with slight heating and stirring. This was filtered later and was ready for use.

♦ Giemsa stain:

Giemsa stain was prepared as per the procedure described earlier in this chapter.

3.5.3. Sperm-shape abnormality assay:

For sperm-shape abnormality assay/sperm morphology assay, technique of Wyrobek *et al.* (1984) was adopted.

3.5.3.1. Preparation of smears/slides:

The control and treated animals were weighed ("Ohaus"CS 2000, USA) (Plate - IIb) and sacrificed at the above mentioned time interval (i.e., 5 weeks) by cervical dislocation and dissected out. The reproductive tract was exposed and both the caudae epididymides were removed and placed in a petri-dish containing 2 ml of phosphate-buffered saline (PBS, pH 7.2). The caudae epididymides were minced with a blade in the phosphate-buffered saline and the resulting suspension was filtered though two layers of muslin cloth to remove tissue debris. The suspension was stained with 1% aqueous eosin Y for 30 minutes and smears were made on clean dry slides. These slides were dried and observed under the microscope. Testes weight were also determined.

3.5.3.2. Preparation of reagent and stain:

♦ Phosphate-buffered saline (PBS, pH 7.2):

PBS was prepared as per the procedure described earlier under micronucleus assay in this chapter.

♦ Aqueous eosin Yellow (1%):

Eosin Y stain was prepared by dissolving 1 g of eosin yellow powder in 100 ml of distilled water. This was filtered and used for staining.

3.6. SCREENING OF SLIDES

The slides of chromosomal aberration test, Micronucleus assay and sperm morphology assay were screened in horizontally overlapping rows employing a compound trinocular research microscope ("Olympus" CH-30, Japan). They were first focussed under low powered (10x) objective and further under appropriate objectives of higher magnification as per the requirement.

The well spread metaphase plates found in 10x objective were finally analysed under high power (100x) with oil immersion objective. Hundred (100) metaphase plates of each animal were screened for chromosomal aberrations. Different types of aberrations such as breaks, gaps, fragments, centric fusions, chromatid exchanges, ring chromosomes and multiple aberrations were scored and recorded. However stickiness and pulverization were not included in the present study. Screening 2000 cells per animal determined the 'mitotic index' values of each group.

The smears of MN assay were scanned under high power objective (100x) with oil immersion for the presence of MN in PCEs and NCEs. About 2000 PCEs and corresponding number of NCEs from each animal were screened. The P/N ratio was also determined/computed from the total number of erythrocytes scored per animal.

The smears of sperm morphology assay were screened under 40x objective for abnormal sperms. 2000 sperms per animal were examined from each treatment and control group for the presence of morphological abnormalities.

Photomicrography was done using trinocular microscope ("Olympus" BX-40, Japan) with inverted camera and 35 mm films ("Kodak" Max 400, India).

3.7. TUMOUR REDUCTION STUDIES

The antitumour studies were carried out using Ehrlich ascites tumour in an *in vivo* mouse system following the method of Unnikrishnan and Kuttan (1990b); Ruby *et al.* (1995) and Sharada *et al.* (1996).

3.7.1. Ehrlich ascites carcinoma cells:

Ehrlich ascites carcinoma cells were originally brought as ascites tumours in Swiss albino mice from the Department of Applied Zoology, Mangalore University, Mangalagangothri, Karnataka, India. Tumour cells were propagated and maintained by injecting 0.1 ml of tumour cells to healthy animals (2-3) at regular time intervals (15 days) (Plate - IX).

3.7.2. Cisplatin (Csp) - The positive control:

Cisplatin is a potent chemotherapeutic agent, which has gained a widespread use against various malignant tumours in different experimental animals and in a variety of human malignancies (Prasad and Giri, 1994). A dose of 4 mg of Csp/kg body weight yields effective chemotherapeutic result (Giri *et al.*, 1998) and hence this dose was selected for the present study.

3.7.3. Induction of Ehrlich ascites tumour:

0.1 ml tumour cells were drawn from the tumoured mouse using disposable syringe, made upto 1 ml (10 times dilution) (Plate - IIIa) using normal saline and was further diluted to 200 times (Plate - IIIb). From this, 0.1 ml solution was injected to healthy mice for regular maintenance of tumour cells.

In order to inject optimum number of tumour cells ($1x10^6$) into the experimental animals, the concentration of the cells in the intraperitoneal fluid was analysed employing 'trypan blue exclusion method' (trypan blue stains dead cells and stained cells were excluded from cell counting) as follows:

Two-three drops of trypan blue stain was added to 0.1 ml of diluted (200 times) (Plate - III) tumour cell suspension. After 5 minutes the number of live cells were counted using WBC counting chambers of hemocytometer ("Neubauer"HBG, Germany). The suspension was diluted further so as to get $1x10^6$ live cells in 0.1 ml of saline. Ascites tumours were induced in experimental animals using the following method.

3.7.4. Experimental protocol:

Experimental animals (*Mus musculus*) were weighed, assigned to 11 groups and each group caged individually. 12 animals were used for each treatment and control group. They were injected intraperitoneally 0.1 ml of Ehrlich ascites tumour suspension with viable cells (10^6). After 24 h of tumour implantation each of the group 1-8 were orally administered with different spices of study in normal and high doses for 7 days. Group 9 was orally treated with normal saline for 7 days. 10^{th} group was used as positive control and administered intraperitoneally 4 mg/kg body weight of cisplatin in 0.2 ml single dose on 3^{rd} day (after 72 h). Last group served as negative control i.e., tumour alone treated group.

After a week, the animals were weighed and the changes in body weights were noted. Mortality of animals dying of tumour was noted up to 120 days (4 months) and average increase in life span (% ILS) was calculated using the formula; % ILS = [(T - C)/C] x 100, where T is the number of days treated animals survived and C is the number of days control animals survived (Menon *et al.*, 1998).

3.7.5. Preparation of reagent and stain:

◆ Normal saline (0.9%):

Normal saline was prepared as per the procedure described earlier in this chapter.

♦ Trypan blue stain:

Trypan blue stain was prepared by dissolving 0.1 g of trypan blue powder in 100 ml of normal saline.

• Precaution:

Sufficient care was taken while performing experiment. Disposable syringes, hand glouses and dead animals were incinerated every time. Glasswares were kept in boiling water bath for 20-30 minutes and then washed.

3.8. STATISTICAL ANALYSIS

Statistical significance of the results were determined by comparing the MN frequency and chromosomal aberration in spice treated groups with that of control groups by using Student's 't' test. Values obtained for sperm-shape abnormality test were compared with control values by using Mann Whitney 'U' test. Tumour reduction data were analysed using Kruskal and Wallis test.

3.8.1. Student's 't' test:

Unpaired/pooled 't' test (Mahajan, 1997) was used to analyse the significance of the results obtained for mutagenicity and antimutagenicity studies. This test is applied to unpaired data of independent observation made on individuals of two different samples, to test if the difference between the two means is real or it can be attributed to sampling variability such as between means of the control and experimental groups.

As per null hypothesis (H_0), it is assumed that there is no real difference between the means of two samples, if the samples are taken at random and drawn independently from the same population. Following steps are taken to test the significance of difference.

- Find the observed difference between means of two samples $(\overline{X}_1-\overline{X}_2)$ and calculate the SE of difference between these means. This measure of variation in a variable will determine the limits of chance or biological variation.

  Standard Error (SE) = SD x $\sqrt{1/n_1 + 1/n_2}$

  Standard Deviation (SD) = $\sqrt{SD^2}$

  $$\text{Combined Variance } (SD^2) = \frac{\Sigma(X_1 - \overline{X}_1)^2 + \Sigma(X_2 - \overline{X}_2)^2}{n_1+n_2 - 2}$$

- Calculate the 't' value, i.e., the ratio between the observed difference and its SE by substituting the above values in the formula,

  $$t = \overline{X}_1 - \overline{X}_2 / SE.$$

- Determine the pooled degrees of freedom from the formula

  $$df = (n_1-1) + (n_2-1) = n_1 + n_2 - 2.$$

- Compare calculated value with the table value at particular degrees of freedom to find the level of significance in two-tailed test.

If the calculated 't' value is higher than table value and the difference between means of two samples are significant or real, then it is sensible to reject the null hypothesis. Further, it can be concluded that test materials (spices) are responsible for the difference in increase of MN and chromosomal aberrations in experimental groups.

3.8.2. Analysis of variance (ANOVA) test:

The mutagenicity study data obtained by chromosomal aberration test and micronucleus test of treated groups of animals at different time intervals were compared with that of control groups using two way ANOVA (**Sigmastat Version-2**). This test gives collective results of all the means of three different doses studied at

four different time intervals. In the present investigation it compared time and dose interaction of spice treatments in mice with that of control.

3.8.3. Mann-Whitney 'U' test:

The data obtained by sperm abnormality test of treated group of animals were compared with that of the control groups by using Mann-Whitney 'U' test (Siegel, 1956 and Udupa, 2001). This is one of the most powerful of the non-parametric tests and is a most useful alternative to the parametric 't' test.

Let n_1 represent the number of cases in the smaller of two independent groups and n_2 represent the number of cases in the larger group. To apply the 'U' test the scores from the both the groups (control and treated) are ranked according to their algebric size, the lowest ranks are assigned to the largest negative numbers, if any. R_1 and R_2 represent total ranks of n_1 and n_2 respectively. The statistic used for making the test is,

$$U_1 = n_1.n_2 + n_1 (n_1+1) / 2 - R_1$$

$$U_2 = n_1.n_2 + n_2 (n_2+1) / 2 - R_2 = n_1.n_2 - U_1$$

$U = \text{min.}\ (U_1, U_2) = n_1.n_2 - U_x$ (Where, U_x is larger value among U_1 and U_2).

For values of n_1 and n_2, Mann-Whitney had given a table of exact probabilities. If the computed value of 'U' is less than or equal to the tabulated value, then we reject the null hypothesis at the stated level of significance.

3.8.4. Kruskal and Wallis test:

Kruskal and Wallis test (Hassard, 1991 and Udupa, 2001) was used to analyse tumour reduction studies. This test is a substitute for one-way analysis of variance technique when data is classified according to one main factor incompletely randomized designs.

Under null hypothesis (H_0), there is no difference among treatments/groups (k), ranks are given for each observation (irrespective of treatments) keeping the identity of each treatment to get rank totals. If there is no significant difference among populations, all rank totals need to be same which are not so due to chance factors. To test this, 'H' statistic is used and is given by,

$$H = 12 / n (n+1) \times (\Sigma R_i^2/n_i) - 3 (n+1),$$

Where, $n = n_1+n_2+n_3+.....+n_k$

R_i = rank total of i^{th} treatment/group

The calculated 'H' value is compared with the Chi-square (X^2) distribution table with k-1 degrees of freedom. If the value of 'H' is more than table value, null hypothesis (H_0) is rejected, otherwise H_0 is accepted.

3.8.5. Computer Softwares:

Computer software programmes were also used for the analysis of data and for graph plotting. '**Prism Demo Software**' (www.graphpad.com) was used for unpaired 't' test. Jandel Corporation's '**Sigmastat Version-2**' was used for two way ANOVA test. Using Microcal '**Origin-6**' Kaplan-Maeier survival curve of antitumour study was plotted. Microsoft '**Excel**' was used for mutagenicity and antimutagenicity graphs.

PLATE - II

Assay animals:

a) Swiss albino mice (*Mus musculus*) colony in animal house of Zoology Department.

b) Weighing the experimental animal using 'Ohaus' CS 2000 balance

c) Gavage dosing with tube attached to a graduated syringe

PLATE : II

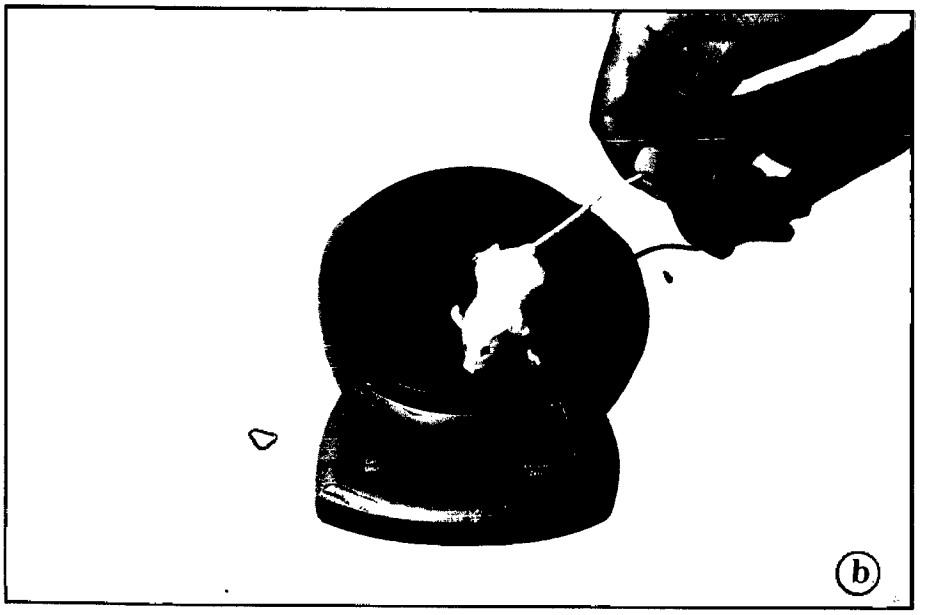


PLATE - III

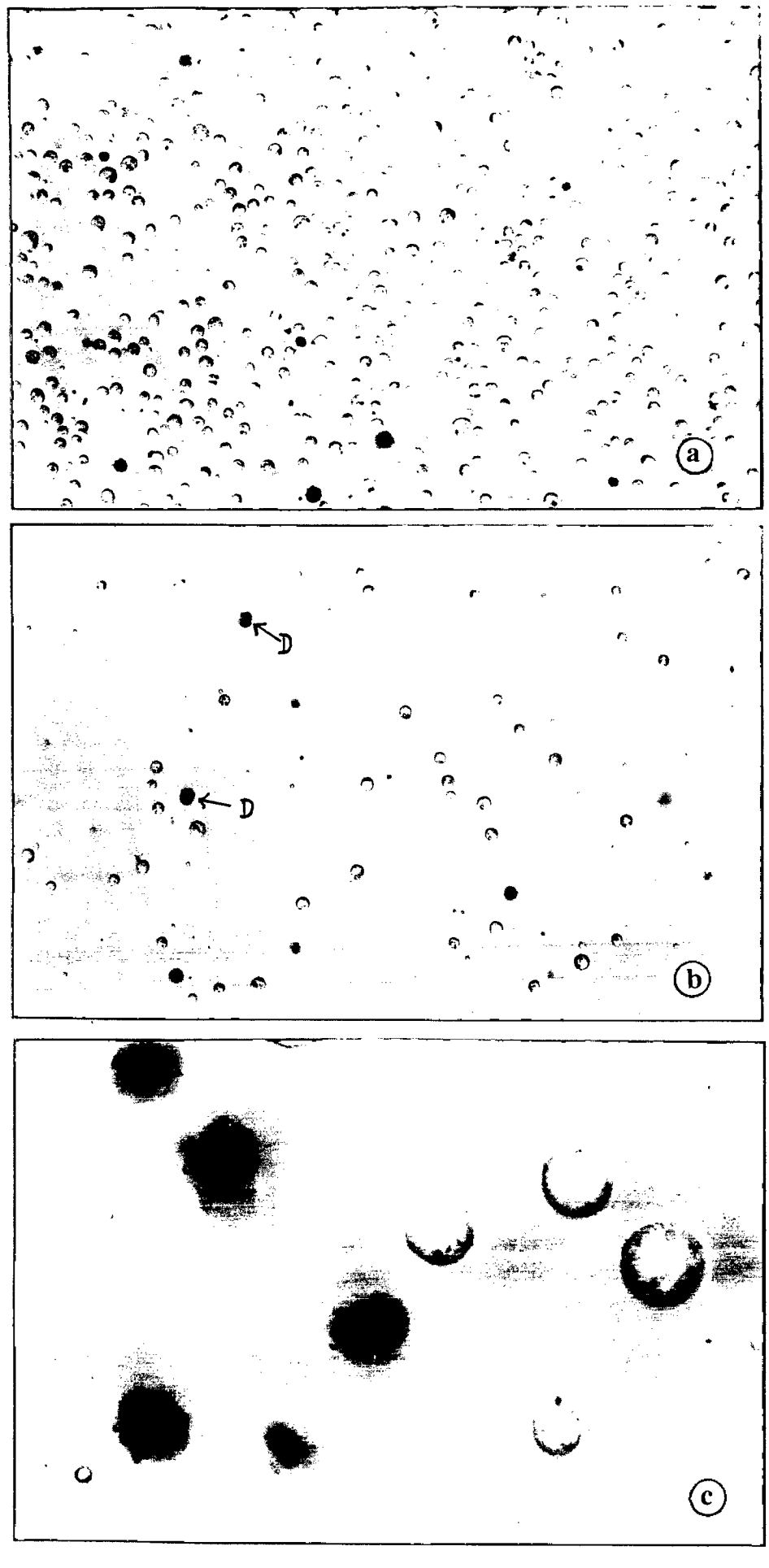
Ehrlich ascites tumour cells:

a) Live and dead cells - 10 times dilution (Magnification: 10 x 10X)

b) Live and dead (D) cells in "Neubauer" chamber of haemocytometer - 200 times dilution (Magnification: 10 x 10X)

c) Enlarged view of live and dead cells (Magnification: 10 x 40X)

PLATE : III



CHAPTER - IV

RESULTS

The results of the present investigation are represented in three sections viz., Mutagenicity, Antimutagenicity and Antitumour studies, supported with tables, figures and plates.

4.1. MUTAGENICITY OF SPICES

The data obtained for mutagenicity study are depicted in Tables 4.1 to 4.10 and represented in the form of Figures 4.1 to 4.16.

4.1.1 Chromosomal aberration assay: [Plate - IV & V]

The results of chromosomal aberration (CA) assay in mitotic chromosomes of bone marrow cells of *M. musculus* treated with different spices are presented in Tables 4.1 to 4.4. 'Mitotic index' (MI) values are also presented in the same tables. The time-yield effect and dose-yield effect for chromosomal aberrations without gap and with gap are graphically represented in Fig. 4.5 and Fig. 4.6 respectively.

The different types of aberrations observed were gaps, breaks, fragmentations, centric fusions, chromatid exchanges, chromatid rings and multiple aberrations. Chromatid breaks and gaps were observed very frequently than other types of aberrations.

At all four time intervals (12, 24, 48 and 96 h) studied the positive control group (CP, 50 mg/kg) of mice showed highly significant difference ($P \leq 0.001$) in their mean values of aberrations (without gap and with gap) compared to that of the respective control group of mice (Table 4.1).

A dose-dependent increase in total aberrations (without gap and with gap) was noted in mice treated with black pepper (*Piper nigrum*) extract at all time intervals (Table 4.1). Among the three doses studied both normal (38 mg/kg) and high (380 mg/kg) doses of pepper showed statistically significant aberrations ($P \leq 0.05$ &

$P \leq 0.001$) compared to controls except the lowest dose (3.8 mg/kg). The maximum yield of aberration was observed at 24 h (Fig. 4.5). Although there was a slight declining trend in the aberration frequency at later time intervals, the yield of aberration was significant even at 96 h.

Highest dose (270 mg/kg) of cardamom (*Elettaria cardamomum*) showed statistically significant ($P \leq 0.05$) aberrations (without gap and with gap) at 12, 24 and 48 h time intervals but at 96 h it was not significant (Table 4.2). Maximum aberration was observed at 24 h compared to other time intervals.

Animals treated with three different doses (3.4, 34 and 340 mg/kg) of coriander seed (*Coriandrum sativum*) extract did not show significant increase in aberrations compared to controls at all time intervals (Table 4.3). Results obtained by the treatment of cumin seed (*Cuminum cyminum*) extract at all time intervals showed non-mutagenic in chromosomal aberration assay (Table 4.4).

Maximum aberrations were observed at 24 h compared to all other time intervals in all the four spices selected for study. There was a declining trend in the aberration frequency at later time intervals. Two way ANOVA test results of chromosomal aberration (without gap and with gap) assay showed statistically significant difference in mean values among the various time intervals ($P = 0.001$ & 0.01) as well as various doses ($P = 0.001$). But there was no statistically significant interaction between time and dose.

Lower values of 'mitotic index' (MI) were observed in CP treated (positive control) animals compared to controls at all time intervals. A dose dependent decrease in MI values were observed in black pepper treated animals. Among the three doses normal and high doses showed noticeable decrease in MI compared to controls. Animals treated with high dose of cardamom showed considerable decrease in MI

value at 24 h time interval. In all other time interval groups there was not much change in MI value compared to controls. Animals treated with different doses of coriander and cumin extracts did not show decrease in MI values compared to control at all time intervals.

4.1.2. Micronucleus (MN) assay: [Plate – VI]

Micronuclei (MN) were observed both in polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE). The frequencies of micronucleus in bone marrow erythrocytes of mice induced by different spices and controls are given in Tables 4.5 to 4.8 and Figures 4.7 to 4.10. P/N ratios are also given in the same tables and graphically represented in Fig. 4.13. The time-yield effect and dose-yield effect on the frequencies of MN in PCE and total percentage MN in erythrocytes are graphically represented in Figures 4.11 and 4.12.

In comparison to controls, the frequency of MN was significantly increased ($P \leq 0.001$) in cyclophosphamide treated mice with the maximum MN being induced in PCEs at all time intervals (Table 4.5). P/N ratio of erythrocytes treated with CP was declined significantly at all time intervals.

Statistically significant MN in PCE were observed (Table 4.5) at all time intervals for normal ($P \leq 0.05$) and high doses ($P \leq 0.001$) of black pepper except a slight increase (insignificant) at low dose. Pepper induced maximum number of MN in PCE at 24 h time interval which gradually declined later on. It failed to induce significant % MN in erythrocytes with normal dose at 12 h time interval. A statistically significant reduction in P/N ratio ($P \leq 0.05$ & $P \leq 0.001$) was observed at all the time intervals treated with normal and high doses of black pepper (Table 4.5).

The animals treated with high dose of cardamom extract showed statistically significant increase ($P \leq 0.05$) in % MN in PCE at 12 and 24 h time intervals (Table

4.6). Mice treated with high dose of cardamom showed significant increase ($P \leq 0.05$) in total % MN in erythrocytes only at 24 h time interval. P/N ratio of erythrocytes of mice treated with high dose of cardamom were significant ($P \leq 0.05$) at all time intervals except 96 h compared to controls.

Animals treated with all three doses of coriander extract did not show significant increase in % MN in PCE or total % MN in erythrocytes compared to their respective controls (Table 4.7). Cumin showed its non-genotoxic effect at all time intervals with regard to frequency of MN in PCE, total % MN in erythrocytes and P/N ratio of erythrocytes (Table 4.8) compared to control groups.

ANOVA results of percentage MN in PCE, total % MN in erythrocytes and P/N ratio showed statistically significant ($P = 0.001$) difference in mean values among the various time intervals as well as various doses tested. But except P/N ratio ($P = 0.001$) none of the results showed statistically significant interaction between time and dose.

4.1.3. Sperm-shape abnormality assay: [Plate - VII & VIII]

The results obtained for the sperm-shape abnormality assay are presented in Table 4.4 and in Fig. 4.14.

Various forms of abnormal sperms i.e., amorphous shaped, banana shaped, hookless, folded, double headed and double tailed sperms were observed. Analysis of these abnormal sperm shapes showed that overall amorphous types and hookless sperms were more prevalent in different treatment and control groups than other types of abnormal sperms.

From the results, it was observed that there was a significant ($P = 0.004$) increase in the induction of abnormal sperms in animals treated with CP (positive agent) against the control. The yield of abnormal sperms was significant in normal

(P = 0.048) and high (P = 0.008) doses of black pepper treated groups. A dose-dependent increase in abnormal sperms was observed in animals treated with all spices studied, eventhough they were not significant compared to control for the spices other than black pepper.

The effect of spices on the body weight and testes weight after 5 weeks are shown in Table 4.10, Figures 4.15 and 4.16.

Drastic decrease in body weight was observed in CP treated and high doses of pepper treated groups compared to control groups. Slight decrease in body weight was noticed in animals treated with low dose of pepper, high dose of cardamom, normal and high doses of coriander. In cumin treated groups drastic increase in percentage body weight was observed.

A decreasing trend in testes weight was observed in CP and pepper treated groups of animals compared to controls. Cardamom failed to induce appreciable decrease in testes weight. But an increasing trend of testes weight was noticed in animals treated with all the doses of coriander and cumin extracts except the group treated with low dose of cumin.

4.2. ANTIMUTAGENICITY OF SPICES

The results obtained in antimutagenicity study are summarized in Tables 4.11 to 4.18 and in Figures 4.17 to 4.32.

The spice that was found mutagenic (black pepper) did not use for antimutagenicity study. The doses of other spices, which showed maximum response (normal and high doses) were selected for antimutagenicity screening. Only one time interval (24 h), which showed maximum response in mutagenicity study was selected for antimutagenicity study.

4.2.1. Chromosomal aberration assay:

The data of chromosomal aberration assay in bone marrow cells of Swiss albino mice are presented in Table 4.11. The antimutagenic effect for chromosomal aberrations are graphically represented in Fig. 4.17 (without gap) and 4.18 (with gap). The saline treated control group showed similar result as CP alone treated group.

Chromatid and isochromatid breaks, gaps, fragmentations, centric fusions, chromatid exchanges, chromatid rings and multiple aberrations were observed. Chromatid breaks and gaps were more very frequent than other types of aberrations. Endoxan (CP) induced fragmentations were observed more in number compared to mutagenicity studies.

Among the three spices studied (cardamom, *E. cardamomum*; coriander, *C. sativum* and cumin, *C. cyminum*) the pre-treatment of animals with high dose (610 mg/kg) of cumin extract followed by CP (50 mg/kg) showed a significant ($P \leq 0.05$) decrease in the percentage of aberrations (without gap and with gap) compared to control (CP alone treated group) showing the protective ability. Animals treated with cardamom showed an increasing trend in aberrations (without gap) in a dose-dependent manner compared to CP alone treated control groups. But this trend was not noticed in aberrations included with gap. A dose-dependent decrease in total aberrations (without gap and with gap) was noticed in mice treated with coriander extract even though not significant.

'Mitotic index' (MI) values were decreased in cardamom treated groups in a dose-dependent manner compared to CP alone treated control. But coriander and cumin treated groups showed increase in MI values in a dose-dependent manner. High dose of cumin showed significant increase in MI value compared to control group.

4.2.2. Micronucleus (MN) assay:

The results obtained are presented in Table 4.12. Micronuclei frequencies in PCE and total % MN in erythrocytes are graphically represented in Figures 4.22 and 4.23 respectively. P/N ratios are graphically represented in Fig. 4.24. Saline treated group showed almost similar result as that of control.

Of the different spices tested, high dose of cumin was found to be significant in reducing the mutagenicity induced by CP. There was a significant reduction in the percentage MN in PCE ($P \leq 0.05$) and total % MN ($P \leq 0.01$). Cardamom treated group showed slight increase in MN/PCE values. But in total % MN values only high dose of cardamom showed increasing trend compared to control. Mice treated with normal dose of coriander extract along with CP showed slight increase in MN/PCE value but high dose showed slight decreasing trend. A dose-dependent decrease in total % MN values were observed in coriander treated mice. P/N ratio was decreased in high dose of cardamom treatment and in all other cases increasing trend was noticed. High dose of cumin showed significant P/N ratio.

4.2.3. Sperm-shape abnormality assay:

The results of sperm morphology assay are presented in Table 4.13 and in Fig. 4.22. Decrease in abnormal sperms were noticed in the mice groups treated with coriander and cumin extracts. A statistically significant ($P = 0.048$) result obtained in high dose cumin treatment. In cardamom treatment groups an increasing trend of abnormal sperms were noticed.

The effect of spices on the body weight and testes weight of *M. musculus* treated with cyclophosphamide are shown in Table 4.14. Graphically it is given in Figures 4.23 and 4.24. Slight increase in percentage body weight was noticed in coriander treated group and low dose cardamom treated group. But in cumin treated

animals it was more than double the percentage increase that of control group. Except high dose cardamom treated group, all other groups showed increase in testes weight compared to control group and high dose cumin treated group was significant among them.

4.2.4. Antimutagenicity of cumin (*C. cyminum*) against pepper (*P. nigrum*) induced mutagenicity:

Pepper (normal and high doses), which showed mutagenic potential and cumin (high dose), which showed significant antimutagenic ability were used for this study. The results of combined effect studies are given in Tables 4.15 to 4.18 and in Figures 4.25 to 4.32.

The high dose of cumin showed statistically significant ($P \leq 0.05$ & $P \leq 0.01$) antimutagenicity against pepper (both normal and high doses) induced chromosomal aberrations (without gap and with gap) in *M. musculus* (Table 4.15). MI values were also increased compared to pepper alone treated groups.

In micronucleus assay animals treated with high doses of pepper and cumin showed significant ($P \leq 0.05$ & $P \leq 0.001$) decrease in MN/PCE values. Eventhough the % MN in PCE reduced in mice treated with normal dose of pepper and high dose of cumin compared to control group, the value was not significant (Table 4.16). In both the experimental groups significant decrease in total % MN were observed. The value was not significant eventhough P/N ratio was increased in mice treated with normal dose of pepper and high dose of cumin. But a significant increase was observed in other group that was treated with high doses of pepper and cumin.

In sperm-shape abnormality assay a significant decrease ($P = 0.048$) in abnormal sperms were observed in mice treated with normal dose of pepper and high dose of cumin. But in other group eventhough percentage of abnormal sperms

reduced, the value was not significant (Table 4.17). Increase in body weights and testes weights were observed in both the cases compared to respective pepper alone treated groups (Table 4.18).

4.3. TUMOUR REDUCTION STUDIES [Plate – IX]

The data obtained for antitumour study are depicted in Tables 4.19 and 4.20. The results are also represented in the Figures 4.33 and 4.34.

There was a sharp increase in body weight in tumour alone and tumour plus saline treated groups of animals. But a slight increase in body weight was noticed in cisplatin (Csp, 4 mg/kg) treated control group. In spice treated groups increase in body weight was marginal (Table 4.19).

No death was observed within16 days after the inoculation of Ehrlich ascites tumour cells (Table 4.20). The untreated control animals survived 17-23 days and the saline treated group survived 18-26 days. Out of 12 animals only 3 died in cisplatin (Csp) treated group during study period and 75% survived beyond 120 days (Table 4.19, 4.20) and was highly significant statistically ($P \leq 0.001$).

Among spice treated groups, animals treated with high dose of cumin survived maximum period i.e., 21-38 days (29 days MST) which was highly significant compared to all other spice treated groups (Table 4.20). A moderate survival period was noticed in cardamom (24 days MST) and coriander (25 days MST) treated animals eventhough they were not significant. Survival time of pepper treated groups were very close to control group (19 days MST). About 50% increase in life span was noticed in the case of cumin (high dose) treated groups and about 30% ILS was observed in coriander (both doses) treated animals (Table 4.19).

Table 4.1. Percentage[a] of chromosomal aberrations (% CA) in *M. musculus* bone marrow erythrocytes induced by various doses of *P. nigrum* and controls at different time intervals [5 animals/group].

Treatment	Dose[b]	Time (h)	MI[c] ± SEM	BS	F	CF	EX	R	MA	TA ± SEM	% CA/ Cell	GS	TAG ± SEM	% CA/ Cell
Control	-	12	4.85 ± 0.16	3.6	-	0.2	0.2	0.4	0.2	4.6 ± 0.24	0.05	1.4	6.0 ± 0.32	0.06
CP	50	12	2.73 ± 0.13	26.2	8.0	4.4	7.2	3.6	9.4	58.8 ± 0.73***	0.59	11.6	70.4 ± 1.60***	0.70
	3.8	12	4.75 ± 0.10	3.4	-	0.6	0.4	0.4	0.4	5.2 ± 0.20	0.05	1.6	6.8 ± 0.37	0.07
P. nigrum	38	12	4.29 ± 0.10	4.2	0.2	0.6	0.4	0.6	0.6	6.6 ± 0.68*	0.07	1.2	7.8 ± 0.37*	0.08
	380	12	3.69 ± 0.11	7.4	0.8	1.2	1.0	1.8	1.4	13.6 ± 0.51***	0.14	4.4	18.0 ± 1.00***	0.18
Control	-	24	5.16 ± 0.14	3.4	-	0.2	0.4	0.4	0.4	4.8 ± 0.37	0.05	1.6	6.4 ± 0.24	0.06
CP	50	24	3.22 ± 0.13	27.6	7.2	4.0	6.2	4.8	10.0	59.8 ± 0.97***	0.60	13.4	73.2 ± 1.07***	0.73
	3.8	24	4.62 ± 0.15	3.6	-	0.6	0.4	1.0	0.8	6.4 ± 0.60	0.06	1.0	7.4 ± 0.51	0.07
P. nigrum	38	24	4.34 ± 0.09	4.2	0.4	0.6	0.4	0.8	0.8	7.2 ± 0.66*	0.07	1.2	8.4 ± 0.51**	0.08
	380	24	3.72 ± 0.08	7.8	1.0	1.6	1.4	2.4	2.0	16.2 ± 0.58***	0.16	4.8	21.0 ± 0.71***	0.21
Control	-	48	4.98 ± 0.15	3.2	-	0.4	0.4	0.4	0.2	4.6 ± 0.40	0.05	1.8	6.4 ± 0.24	0.06
CP	50	48	3.30 ± 0.16	29.0	7.8	4.6	5.8	4.4	9.6	61.2 ± 1.24***	0.61	11.0	72.2 ± 1.43***	0.72
	3.8	48	4.88 ± 0.12	3.8	-	0.6	0.4	0.8	0.6	6.2 ± 0.58	0.06	1.0	7.2 ± 0.37	0.07
P. nigrum	38	48	4.38 ± 0.08	4.4	0.4	0.6	0.4	0.8	0.6	7.2 ± 0.73*	0.07	1.0	8.2 ± 0.37**	0.08
	380	48	3.79 ± 0.07	8.0	0.8	1.6	1.6	2.0	1.8	15.8 ± 0.73***	0.16	3.6	19.4 ± 0.51***	0.19
Control	-	96	5.12 ± 0.10	3.4	-	0.2	-	0.4	0.4	4.4 ± 0.24	0.04	1.8	6.2 ± 0.49	0.06
CP	50	96	3.45 ± 0.08	28.2	8.2	4.6	6.2	4.4	8.4	60.0 ± 0.71***	0.60	11.0	71.0 ± 1.87***	0.71
	3.8	96	5.11 ± 0.14	3.2	-	0.4	0.6	0.6	0.4	5.2 ± 0.37	0.05	2.2	7.4 ± 0.24	0.07
P. nigrum	38	96	4.46 ± 0.11	3.8	0.2	0.4	0.4	0.6	0.6	6.0 ± 0.45*	0.06	2.0	8.0 ± 0.45*	0.08
	380	96	3.81 ± 0.19	7.6	0.6	1.6	1.8	2.0	1.6	15.2 ± 0.58***	0.15	3.0	18.2 ± 0.58***	0.18

Note:- a = From 100 metaphases/animal; **b** = mg/kg body wt.; **c** = From 2000 cells/animal; MI = Mitotic index; BS = Breaks; F = Fragmentations; CF = Centric fusions; EX = Exchanges; R = Chromatid rings; MA = Multiple aberrations; TA = Total aberration without gap; GS = Gaps; TAG = Total aberration with gap; CP = Cyclophosphamide; * = P values ≤ 0.05; ** = P values ≤ 0.01; *** = P values ≤ 0.001.

Table 4.2. Percentage[a] of chromosomal aberrations (% CA) in *M. musculus* bone marrow erythrocytes induced by various doses of *E. cardamomum* and controls at different time intervals [5 animals/group].

Treatment	Dose[b]	Time (h)	MI[c] ± SEM	BS	F	CF	EX	R	MA	TA ± SEM	% CA/ Cell	GS	TAG ± SEM	% CA/ Cell
Control	-	12	4.85 ± 0.16	3.6	-	0.2	0.2	0.4	0.2	4.6 ± 0.24	0.05	1.4	6.0 ± 0.32	0.06
CP	50	12	2.73 ± 0.13	26.2	8.0	4.4	7.2	3.6	9.4	58.8 ± 0.73***	0.59	11.6	70.4 ± 1.60***	0.70
E. cardamomum	2.7	12	4.81 ± 0.09	3.6	-	0.4	0.2	0.6	0.4	5.2 ± 0.37	0.05	1.0	6.2 ± 0.37	0.06
	27	12	4.78 ± 0.13	3.4	-	0.4	0.4	0.6	0.6	5.4 ± 0.40	0.05	1.6	7.0 ± 0.32	0.07
	270	12	4.54 ± 0.08	4.0	-	0.6	0.2	0.6	0.4	5.8 ± 0.37*	0.06	1.4	7.2 ± 0.37*	0.07
Control	-	24	5.16 ± 0.14	3.4	-	0.2	0.4	0.4	0.4	4.8 ± 0.37	0.05	1.6	6.4 ± 0.24	0.06
CP	50	24	3.22 ± 0.13	27.6	7.2	4.0	6.2	4.8	10.0	59.8 ± 0.97***	0.60	13.4	73.2 ± 1.07***	0.73
E. cardamomum	2.7	24	4.99 ± 0.09	4.0	0.2	0.4	0.4	0.6	0.6	6.0 ± 0.45	0.06	0.4	6.4 ± 0.24	0.06
	27	24	4.90 ± 0.15	3.4	0.2	0.6	0.4	0.8	0.6	6.0 ± 0.45	0.06	1.4	7.4 ± 0.51	0.07
	270	24	4.66 ± 0.11	3.6	0.2	0.6	0.6	1.0	0.6	6.6 ± 0.40*	0.07	1.4	8.0 ± 0.45*	0.08
Control	-	48	4.98 ± 0.15	3.2	-	0.4	0.4	0.4	0.2	4.6 ± 0.40	0.05	1.8	6.4 ± 0.24	0.06
CP	50	48	3.30 ± 0.16	29.0	7.8	4.6	5.8	4.4	9.6	61.2 ± 1.24***	0.61	11.0	72.2 ± 1.43***	0.72
E. cardamomum	2.7	48	5.08 ± 0.12	4.4	-	0.4	0.2	0.6	0.4	6.0 ± 0.63	0.06	1.0	7.0 ± 0.32	0.07
	27	48	4.98 ± 0.10	3.2	-	0.4	0.4	0.8	0.8	5.6 ± 0.24	0.06	1.6	7.2 ± 037	0.07
	270	48	4.62 ± 0.10	3.6	0.2	0.6	0.6	0.8	0.6	6.4 ± 0.51*	0.06	2.0	8.4 ± 0.60*	0.08
Control	-	96	5.12 ± 0.10	3.4	-	0.2	-	0.4	0.4	4.4 ± 0.24	0.04	1.8	6.2 ± 0.49	0.06
CP	50	96	3.45 ± 0.08	28.2	8.2	4.6	6.2	4.4	8.4	60.0 ± 0.71***	0.60	11.0	71.0 ± 1.87***	0.71
E. cardamomum	2.7	96	5.10 ± 0.07	3.2	-	0.6	0.4	0.8	0.6	5.6 ± 0.51	0.06	1.0	6.6 ± 0.24	0.07
	27	96	5.03 ± 0.12	3.2	-	0.4	0.4	0.8	0.6	5.4 ± 0.40	0.05	1.6	7.0 ± 0.32	0.07
	270	96	4.98 ± 0.12	4.0	-	0.4	0.2	0.6	0.4	5.6 ± 0.51	0.06	2.0	7.6 ± 0.40	0.08

Note:- a = From 100 metaphases/animal; **b** = mg/kg body wt.; **c** = From 2000 cells/animal; MI = Mitotic index; BS = Breaks; F = Fragmentations; CF = Centric fusions; EX = Exchanges; R = Chromatid rings; MA = Multiple aberrations; TA = Total aberration without gap; GS = Gaps; TAG = Total aberration with gap; CP = Cyclophosphamide; * = P values ≤ 0.05; *** = P values ≤ 0.001.

Table 4.3. Percentage[a] of chromosomal aberrations (% CA) in *M. musculus* bone marrow erythrocytes induced by various doses of *C. sativum* and controls at different time intervals [5 animals/group].

Treatment	Dose[b]	Time (h)	MI[c] ± SEM	BS	F	CF	EX	R	MA	TA ± SEM	% CA/ Cell	GS	TAG ± SEM	% CA/ Cell
Control	-	12	4.85 ± 0.16	3.6	-	0.2	0.2	0.4	0.2	4.6 ± 0.24	0.05	1.4	6.0 ± 0.32	0.06
CP	50	12	2.73 ± 0.13	26.2	8.0	4.4	7.2	3.6	9.4	58.8 ± 0.73***	0.59	11.6	70.4 ± 1.60***	0.70
C. sativum	3.4	12	4.87 ± 0.07	3.6	-	0.4	0.2	0.6	0.4	5.2 ± 0.37	0.05	1.0	6.2 ± 0.20	0.06
	34	12	4.65 ± 0.08	3.4	-	0.4	0.4	0.6	0.4	5.2 ± 0.20	0.05	1.6	6.8 ± 0.58	0.07
	340	12	4.67 ± 0.10	3.6	-	0.4	0.4	0.6	0.6	5.6 ± 0.51	0.06	1.4	7.0 ± 0.32	0.07
Control	-	24	5.16 ± 0.14	3.4	-	0.2	0.4	0.4	0.4	4.8 ± 0.37	0.05	1.6	6.4 ± 0.24	0.06
CP	50	24	3.22 ± 0.13	27.6	7.2	4.0	6.2	4.8	10.0	59.8 ± 0.97***	0.60	13.4	73.2 ± 1.07***	0.73
C. sativum	3.4	24	5.04 ± 0.08	3.6	-	0.6	0.4	0.6	0.4	5.6 ± 0.40	0.06	1.0	6.6 ± 0.40	0.07
	34	24	4.72 ± 0.08	3.6	-	0.6	0.4	0.8	0.6	6.0 ± 0.45	0.06	1.2	7.2 ± 0.37	0.07
	340	24	4.78 ± 0.11	3.4	0.2	0.6	0.6	1.0	0.6	6.4 ± 0.60	0.06	1.0	7.4 ± 0.40	0.07
Control	-	48	4.98 ± 0.15	3.2	-	0.4	0.4	0.4	0.2	4.6 ± 0.40	0.05	1.8	6.4 ± 0.24	0.06
CP	50	48	3.30 ± 0.16	29.0	7.8	4.6	5.8	4.4	9.6	61.2 ± 1.24***	0.61	11.0	72.2 ± 1.43***	0.72
C. sativum	3.4	48	5.00 ± 0.08	3.6	-	0.4	0.2	0.6	0.6	5.4 ± 0.51	0.05	1.2	6.6 ± 0.40	0.07
	34	48	4.79 ± 0.11	4.0	-	0.4	0.4	0.6	0.4	5.8 ± 0.37	0.06	1.2	7.0 ± 0.45	0.07
	340	48	4.85 ± 0.14	3.4	-	0.8	0.4	0.8	0.6	6.0 ± 0.55	0.06	1.4	7.4 ± 0.51	0.07
Control	-	96	5.12 ± 0.10	3.4	-	0.2	-	0.4	0.4	4.4 ± 0.24	0.04	1.8	6.2 ± 0.49	0.06
CP	50	96	3.45 ± 0.08	28.2	8.2	4.6	6.2	4.4	8.4	60.0 ± 0.71***	0.60	11.0	71.0 ± 1.87***	0.71
C. sativum	3.4	96	5.10 ± 0.10	3.0	-	0.4	0.4	0.6	0.4	4.8 ± 0.37	0.05	1.8	6.6 ± 0.24	0.07
	34	96	4.85 ± 0.09	3.6	-	0.6	0.2	0.6	0.4	5.4 ± 0.51	0.05	1.8	7.2 ± 0.20	0.07
	340	96	4.96 ± 0.13	3.4	-	0.4	0.2	0.6	0.4	5.0 ± 0.45	0.05	2.4	7.4 ± 0.24	0.07

Note:- a = From 100 metaphases/animal; **b** = mg/kg body wt.; **c** = From 2000 cells/animal; MI = Mitotic index; BS = Breaks; F = Fragmentations; CF = Centric fusions; EX = Exchanges; R = Chromatid rings; MA = Multiple aberrations; TA = Total aberration without gap; GS = Gaps; TAG = Total aberration with gap; CP = Cyclophosphamide; *** = P values ≤ 0.001.

Table 4.4. Percentage[a] of chromosomal aberrations (% CA) in *M. musculus* bone marrow erythrocytes induced by various doses of *C. cyminum* and controls at different time intervals [5 animals/group].

Treatment	Dose[b]	Time (h)	MI[c] ± SEM	BS	F	CF	EX	R	MA	TA ± SEM	% CA/ Cell	GS	TAG ± SEM	% CA/ Cell
Control	-	12	4.85 ± 0.16	3.6	-	0.2	0.2	0.4	0.2	4.6 ± 0.24	0.05	1.4	6.0 ± 0.32	0.06
CP	50	12	2.73 ± 0.13	26.2	8.0	4.4	7.2	3.6	9.4	58.8 ± 0.73***	0.59	11.6	70.4 ± 1.60***	0.70
C. cyminum	6.1	12	4.81 ± 0.11	3.6	-	0.4	0.2	0.4	0.2	4.8 ± 0.20	0.05	1.6	6.4 ± 0.24	0.06
	61	12	4.78 ± 0.12	3.4	-	0.2	0.4	0.6	0.4	5.0 ± 0.32	0.05	2.2	7.2 ± 0.58	0.07
	610	12	4.66 ± 0.08	3.4	-	0.4	0.2	0.6	0.6	5.2 ± 0.37	0.05	1.6	6.8 ± 0.20	0.07
Control	-	24	5.16 ± 0.14	3.4	-	0.2	0.4	0.4	0.4	4.8 ± 0.37	0.05	1.6	6.4 ± 0.24	0.06
CP	50	24	3.22 ± 0.13	27.6	7.2	4.0	6.2	4.8	10.0	59.8 ± 0.97***	0.60	13.4	73.2 ± 1.07***	0.73
C. cyminum	6.1	24	5.04 ± 0.15	3.8	-	0.2	0.2	0.4	0.4	5.0 ± 0.32	0.05	1.0	6.0 ± 0.32	0.06
	61	24	4.92 ± 0.12	3.6	-	0.4	0.2	0.4	0.6	5.2 ± 0.37	0.05	1.4	6.6 ± 0.40	0.07
	610	24	4.74 ± 0.12	3.8	-	0.4	0.4	0.6	0.6	5.8 ± 0.37	0.06	1.2	7.0 ± 0.32	0.07
Control	-	48	4.98 ± 0.15	3.2	-	0.4	0.4	0.4	0.2	4.6 ± 0.40	0.05	1.8	6.4 ± 0.24	0.06
CP	50	48	3.30 ± 0.16	29.0	7.8	4.6	5.8	4.4	9.6	61.2 ± 1.24***	0.61	11.0	72.2 ± 1.43***	0.72
C. cyminum	6.1	48	5.00 ± 0.09	3.2	-	0.4	0.2	0.4	0.4	4.6 ± 0.24	0.05	2.0	6.6 ± 0.24	0.07
	61	48	4.86 ± 0.13	3.6	-	0.2	0.2	0.6	0.4	5.0 ± 0.45	0.05	2.0	7.0 ± 0.32	0.07
	610	48	4.83 ± 0.11	3.8	-	0.6	0.4	0.6	0.4	5.8 ± 0.49	0.06	1.6	7.4 ± 0.51	0.07
Control	-	96	5.12 ± 0.10	3.4	-	0.2	-	0.4	0.4	4.4 ± 0.24	0.04	1.8	6.2 ± 0.49	0.06
CP	50	96	3.45 ± 0.08	28.2	8.2	4.6	6.2	4.4	8.4	60.0 ± 0.71***	0.60	11.0	71.0 ± 1.87***	0.71
C. cyminum	6.1	96	5.06 ± 0.11	2.6	-	0.6	0.4	0.6	0.4	4.6 ± 0.40	0.05	2.2	6.8 ± 0.37	0.07
	61	96	5.03 ± 0.14	3.0	-	0.4	0.4	0.6	0.2	4.6 ± 0.40	0.05	2.4	7.0 ± 0.32	0.07
	610	96	4.93 ± 0.15	3.8	-	0.4	0.2	0.4	0.2	5.0 ± 0.32	0.05	2.0	7.0 ± 0.32	0.07

Note:- a = From 100 metaphases/animal; **b** = mg/kg body wt.; **c** = From 2000 cells/animal; MI = Mitotic index; BS = Breaks; F = Fragmentations; CF = Centric fusions; EX = Exchanges; R = Chromatid rings; MA = Multiple aberrations; TA = Total aberration without gap; GS = Gaps; TAG = Total aberration with gap; CP = Cyclophosphamide; *** = P values ≤ 0.001.

Table 4.5. Percentage[a] frequencies of *M. musculus* bone marrow MN induced by various doses of *P. nigrum* and controls at different intervals [5 animals/group].

Treatment	Dose[b]	Time (h)	Mean % PCE	Mean % NCE	% MN in PCE ± SEM	Total MN (%) ± SEM	P/N ratio ± SEM
Control	-	12	51.17	48.83	0.28 ± 0.03	0.22 ± 0.02	1.048 ± 0.004
CP	50	12	46.16	53.84	2.04 ± 0.13***	1.11 ± 0.06***	0.857 ± 0.002***
P. nigrum	3.8	12	51.00	49.00	0.32 ± 0.03	0.24 ± 0.02	1.041 ± 0.003
	38	12	50.72	49.28	0.38 ± 0.03*	0.28 ± 0.02	1.029 ± 0.003**
	380	12	49.98	50.02	0.52 ± 0.03***	0.38 ± 0.02***	0.999 ± 0.003***
Control	-	24	50.69	49.31	0.32 ± 0.03	0.25 ± 0.01	1.028 ± 0.004
CP	50	24	43.40	56.60	2.42 ± 0.08***	1.26 ± 0.03***	0.767 ± 0.004***
P. nigrum	3.8	24	50.89	49.11	0.34 ± 0.02	0.25 ± 0.02	1.036 ± 0.003
	38	24	50.23	49.77	0.46 ± 0.03*	0.35 ± 0.03**	1.009 ± 0.004**
	380	24	49.49	50.51	0.64 ± 0.04***	0.45 ± 0.03***	0.980 ± 0.005***
Control	-	48	51.26	48.74	0.31 ± 0.03	0.23 ± 0.02	1.052 ± 0.006
CP	50	48	44.65	55.35	2.18 ± 0.11***	1.16 ± 0.04***	0.807 ± 0.003***
P. nigrum	3.8	48	50.93	49.07	0.33 ± 0.03	0.25 ± 0.02	1.038 ± 0.003
	38	48	50.50	49.50	0.42 ± 0.02*	0.31 ± 0.02*	1.022 ± 0.005**
	380	48	49.60	50.40	0.58 ± 0.04**	0.42 ± 0.03**	0.988 ± 0.006***
Control	-	96	50.95	49.05	0.29 ± 0.02	0.21 ± 0.01	1.039 ± 0.007
CP	50	96	47.54	52.46	2.09 ± 0.08***	1.16 ± 0.05***	0.906 ± 0.007***
P. nigrum	3.8	96	50.89	49.11	0.32 ± 0.01	0.23 ± 0.01	1.036 ± 0.003
	38	96	50.52	49.48	0.39 ± 0.03*	0.30 ± 0.02**	1.021 ± 0.002*
	380	96	49.75	50.25	0.50 ± 0.03***	0.36 ± 0.02***	0.990 ± 0.005***

Note:- a = From 2000 PCE/animal; **b** = mg/kg body wt.; PCE = Polychromatic erythrocytes; NCE = Normochromatic erythrocytes; MN = Micronucleus; P/N ratio = PCE/NCE ratio; CP = Cyclophosphamide; * = P values ≤ 0.05; ** = P values ≤ 0.01; *** = P values ≤ 0.001.

Table 4.6. Percentage[a] frequencies of *M. musculus* bone marrow MN induced by various doses of *E. cardamomum* and controls at different time intervals [5 animals/group].

Treatment	Dose[b]	Time (h)	Mean % PCE	Mean % NCE	% MN in PCE ± SEM	Total MN (%) ± SEM	P/N ratio ± SEM
Control	-	12	51.17	48.83	0.28 ± 0.03	0.22 ± 0.02	1.048 ± 0.004
CP	50	12	46.16	53.84	2.04 ± 0.13***	1.11 ± 0.06***	0.857 ± 0.002***
E. cardamomum	2.7	12	51.06	48.94	0.31 ± 0.03	0.22 ± 0.03	1.043 ± 0.004
	27	12	50.97	49.03	0.32 ± 0.03	0.24 ± 0.02	1.040 ± 0.003
	270	12	50.81	49.19	0.37 ± 0.03*	0.27 ± 0.02	1.033 ± 0.004*
Control	-	24	50.69	49.31	0.32 ± 0.03	0.25 ± 0.01	1.028 ± 0.004
CP	50	24	43.40	56.60	2.42 ± 0.08***	1.26 ± 0.03***	0.767 ± 0.004***
E. cardamomum	2.7	24	50.78	49.22	0.32 ± 0.03	0.25 ± 0.02	1.032 ± 0.004
	27	24	50.65	49.35	0.36 ± 0.03	0.27 ± 0.03	1.027 ± 0.005
	270	24	50.29	49.71	0.45 ± 0.04*	0.34 ± 0.04*	1.012 ± 0.004*
Control	-	48	51.26	48.74	0.31 ± 0.03	0.23 ± 0.02	1.052 ± 0.006
CP	50	48	44.65	55.35	2.18 ± 0.11***	1.16 ± 0.04***	0.807 ± 0.003***
E. cardamomum	2.7	48	50.92	49.08	0.32 ± 0.02	0.24 ± 0.01	1.038 ± 0.004
	27	48	50.90	49.10	0.34 ± 0.03	0.24 ± 0.03	1.037 ± 0.005
	270	48	50.80	49.20	0.41 ± 0.03	0.28 ± 0.02	1.032 ± 0.002*
Control	-	96	50.95	49.05	0.29 ± 0.02	0.21 ± 0.01	1.039 ± 0.007
CP	50	96	47.54	52.46	2.09 ± 0.08***	1.16 ± 0.05***	0.906 ± 0.007***
E. cardamomum	2.7	96	50.92	49.08	0.31 ± 0.04	0.22 ± 0.03	1.037 ± 0.004
	27	96	50.95	49.05	0.33 ± 0.03	0.23 ± 0.03	1.039 ± 0.005
	270	96	50.84	49.16	0.35 ± 0.02	0.25 ± 0.02	1.034 ± 0.003

Note:- a = From 2000 PCE/animal; **b** = mg/kg body wt.; PCE = Polychromatic erythrocytes; NCE = Normochromatic erythrocytes; MN = Micronucleus; P/N ratio = PCE/NCE ratio; CP = Cyclophosphamide; * = P values ≤ 0.05; *** = P values ≤ 0.001.

Table 4.7. Percentage[a] frequencies of *M. musculus* bone marrow MN induced by various doses of *C. sativum* and controls at different time intervals [5 animals/group].

Treatment	Dose[b]	Time (h)	Mean % PCE	Mean % NCE	% MN in PCE ± SEM	Total MN (%) ± SEM	P/N ratio ± SEM
Control	-	12	51.17	48.83	0.28 ± 0.03	0.22 ± 0.02	1.048 ± 0.004
CP	50	12	46.16	53.84	2.04 ± 0.13***	1.11 ± 0.06***	0.857 ± 0.002***
C. sativum	3.4	12	51.06	48.94	0.29 ± 0.02	0.22 ± 0.01	1.043 ± 0.004
	34	12	51.09	48.91	0.31 ± 0.03	0.23 ± 0.02	1.045 ± 0.003
	340	12	51.03	48.97	0.31 ± 0.03	0.23 ± 0.02	1.042 ± 0.002
Control	-	24	50.69	49.31	0.32 ± 0.03	0.25 ± 0.01	1.028 ± 0.004
CP	50	24	43.40	56.60	2.42 ± 0.08***	1.26 ± 0.03***	0.767 ± 0.004***
C. sativum	3.4	24	50.89	49.11	0.32 ± 0.03	0.24 ± 0.01	1.036 ± 0.005
	34	24	50.66	49.34	0.33 ± 0.03	0.25 ± 0.02	1.027 ± 0.004
	340	24	50.54	49.46	0.33 ± 0.03	0.26 ± 0.02	1.022 ± 0.006
Control	-	48	51.26	48.74	0.31 ± 0.03	0.23 ± 0.02	1.052 ± 0.006
CP	50	48	44.65	55.35	2.18 ± 0.11***	1.16 ± 0.04***	0.807 ± 0.003***
C. sativum	3.4	48	51.00	49.00	0.31 ± 0.03	0.23 ± 0.02	1.041 ± 0.005
	34	48	51.04	48.96	0.32 ± 0.03	0.23 ± 0.01	1.043 ± 0.005
	340	48	50.90	49.10	0.35 ± 0.02	0.25 ± 0.01	1.037 ± 0.004
Control	-	96	50.95	49.05	0.29 ± 0.02	0.21 ± 0.01	1.039 ± 0.007
CP	50	96	47.54	52.46	2.09 ± 0.08***	1.16 ± 0.05***	0.906 ± 0.007***
C. sativum	3.4	96	51.03	48.97	0.30 ± 0.02	0.21 ± 0.02	1.042 ± 0.005
	34	96	50.95	49.05	0.31 ± 0.04	0.22 ± 0.02	1.039 ± 0.004
	340	96	50.93	49.07	0.33 ± 0.03	0.23 ± 0.01	1.038 ± 0.005

Note:- a = From 2000 PCE/animal; **b** = mg/kg body wt.; PCE = Polychromatic erythrocytes; NCE = Normochromatic erythrocytes; MN = Micronucleus; P/N ratio = PCE/NCE ratio; CP = Cyclophosphamide; *** = P values ≤ 0.001.

Table 4.8. Percentage[a] frequencies of *M. musculus* bone marrow MN induced by various doses of *C. cyminum* and controls at different time intervals [5 animals/group].

Treatment	Dose[b]	Time (h)	Mean % PCE	Mean % NCE	% MN in PCE ± SEM	Total MN (%) ± SEM	P/N ratio ± SEM
Control	-	12	51.17	48.83	0.28 ± 0.03	0.22 ± 0.02	1.048 ± 0.004
CP	50	12	46.16	53.84	2.04 ± 0.13***	1.11 ± 0.06***	0.857 ± 0.002***
C. cyminum	6.1	12	51.09	48.91	0.30 ± 0.03	0.22 ± 0.02	1.045 ± 0.006
	61	12	51.14	48.86	0.29 ± 0.02	0.23 ± 0.01	1.047 ± 0.005
	610	12	51.09	48.91	0.31 ± 0.02	0.24 ± 0.01	1.044 ± 0.005
Control	-	24	50.69	49.31	0.32 ± 0.03	0.25 ± 0.01	1.028 ± 0.004
CP	50	24	43.40	56.60	2.42 ± 0.08***	1.26 ± 0.03***	0.767 ± 0.004***
C. cyminum	6.1	24	50.64	49.36	0.31 ± 0.02	0.23 ± 0.02	1.026 ± 0.009
	61	24	50.68	49.32	0.32 ± 0.03	0.25 ± 0.01	1.028 ± 0.004
	610	24	50.66	49.34	0.33 ± 0.02	0.26 ± 0.02	1.027 ± 0.009
Control	-	48	51.26	48.74	0.31 ± 0.03	0.23 ± 0.02	1.052 ± 0.006
CP	50	48	44.65	55.35	2.18 ± 0.11***	1.16 ± 0.04***	0.807 ± 0.003***
C. cyminum	6.1	48	51.18	48.82	0.31 ± 0.01	0.24 ± 0.01	1.049 ± 0.007
	61	48	51.32	48.68	0.33 ± 0.02	0.24 ± 0.01	1.054 ± 0.005
	610	48	51.19	48.81	0.32 ± 0.02	0.24 ± 0.02	1.049 ± 0.005
Control	-	96	50.95	49.05	0.29 ± 0.02	0.21 ± 0.01	1.039 ± 0.007
CP	50	96	47.54	52.46	2.09 ± 0.08***	1.16 ± 0.05***	0.906 ± 0.007***
C. cyminum	6.1	96	51.02	48.98	0.30 ± 0.03	0.21 ± 0.01	1.042 ± 0.007
	61	96	50.90	49.10	0.34 ± 0.02	0.24 ± 0.01	1.037 ± 0.008
	610	96	50.94	49.06	0.30 ± 0.02	0.21 ± 0.01	1.039 ± 0.005

Note:- a = From 2000 PCE/animal; **b** = mg/kg body wt.; PCE = Polychromatic erythrocytes; NCE = Normochromatic erythrocytes; MN = Micronucleus; P/N ratio = PCE/NCE ratio; CP = Cyclophosphamide; *** = P values ≤ 0.001.

Table 4.9. Frequencies of different type of abnormal sperms[a] in *M. musculus*, 5 weeks after the treatment with various doses of selected spices and controls [5 animals/group].

Treatment	Dose[b]	Normal	AM	BA	HL	FO	DH/DT	Total AS	% AS ± SEM
Control	-	9764	109	22	65	28	12	236	2.36 ± 0.11
CP	50	9345	358	54	148	66	29	655	6.55 ± 0.10***
P. nigrum	3.8	9745	128	21	68	26	12	255	2.55 ± 0.08
	38	9736	120	21	75	34	14	264	2.64 ± 0.05*
	380	9711	135	25	77	34	18	289	2.89 ± 0.10**
E. cardamomum	2.7	9756	114	23	68	26	13	244	2.44 ± 0.09
	27	9747	124	20	71	25	13	253	2.53 ± 0.09
	270	9733	123	23	74	32	15	267	2.67 ± 0.12
C. sativum	3.4	9762	111	21	69	28	09	238	2.38 ± 0.13
	34	9754	116	23	68	26	13	246	2.46 ± 0.11
	340	9752	123	21	67	26	11	248	2.48 ± 0.07
C. cyminum	6.1	9763	115	21	65	27	09	237	2.37 ± 0.08
	61	9760	113	23	66	25	13	240	2.40 ± 0.05
	610	9755	112	22	69	27	15	245	2.45 ± 0.07

Note:- a = From 2000 sperms/animal; **b** = mg/kg body wt.; AM = Amorphous; BA = Banana; HL = Hookless; FO = Folded; DH/DT = Double headed/Double tailed; AS = Abnormal sperms; CP = Cyclophosphamide; P values: * = 0.048; ** = 0.008; *** = 0.004.

Table 4.10. Effect of various doses of selected spices and controls on body weight & testes weight of *M. musculus* after 5 weeks [5 animals/group].

Treatment	Dose[a]	IBW (g) ± SEM	SBW (g) ± SEM	% INBW	Testes wt. (g) ± SEM	% ITW
Control	-	26 ± 0	31.6 ± 0.40	21.54	0.211 ± 0.011	-
CP	50	28 ± 0	31.2 ± 0.58	11.43	0.197 ± 0.006	-6.91
P. nigrum	3.8	27 ± 0	33.6 ± 0.60	24.44	0.207 ± 0.012	-2.08
	38	28 ± 0	32.2 ± 0.66	15.00	0.210 ± 0.010	-0.85
	380	28 ± 0	31.8 ± 0.66	13.57	0.200 ± 0.007	-5.58
E. cardamomum	2.7	28 ± 0	32.8 ± 1.39	17.14	0.214 ± 0.008	1.28
	27	26 ± 0	31.2 ± 0.49	20.00	0.214 ± 0.014	1.09
	270	28 ± 0	32.4 ± 0.75	15.71	0.217 ± 0.020	2.41
C. sativum	3.4	29 ± 0	34.8 ± 0.86	20.00	0.232 ± 0.010	9.56
	34	29 ± 0	33.6 ± 0.40	15.86	0.224 ± 0.007	6.10
	340	30 ± 0	34.6 ± 1.08	15.33	0.243 ± 0.010	14.90
C. cyminum	6.1	27 ± 0	33.6 ± 0.75	24.44	0.217 ± 0.011	2.41
	61	28 ± 0	35.2 ± 0.58	25.71	0.234 ± 0.006	10.69
	610	29 ± 0	35.0 ± 0.32	20.69	0.243 ± 0.005	15.09

Note:- a = mg/kg body wt.; IBW = Initial body weight; SBW = Sacrifice body weight;
% INBW = Percentage increase in body weight; % ITW = Percentage increase in testes weight;
CP = Cyclophosphamide.

Table 4.11. Antimutagenic effect of two different doses of selected spices on chromosomal aberrations[a] induced by cyclophosphamide (CP) in *M. musculus* bone marrow cells [5 animals/group].

Treatment	Dose[b]	MI[c] ± SEM	BS	F	CF	EX	R	MA	TA ± SEM	% CA/ Cell	% INH	GS	TAG ± SEM	% CA/ Cell	% INH
CP	50	3.22 ± 0.13	27.60	7.2	4.0	6.2	4.8	10.0	59.8 ± 0.97	0.60	-	13.4	73.2 ± 1.07	0.73	-
CP + Saline	50	3.21 ± 0.12	30.60	7.0	4.8	4.0	5.2	9.0	60.6 ± 1.50	0.61	-1.34	13.6	74.2 ± 2.06	0.74	1.37
E. cardamomum	27	3.18 ± 0.13	31.00	6.8	4.8	4.4	5.6	8.6	61.2 ± 1.36	0.61	-2.34	11.2	72.4 ± 1.50	0.72	1.09
	270	3.04 ± 0.10	30.60	7.6	5.6	4.8	5.2	8.8	62.6 ± 1.36	0.63	-4.68	12.0	74.6 ± 1.81	0.75	1.91
C. sativum	34	3.36 ± 0.08	31.20	5.8	3.8	4.6	4.8	7.4	57.6 ± 0.51	0.58	3.68	13.6	71.2 ± 1.28	0.71	2.73
	340	3.59 ± 0.06	28.60	6.4	4.8	4.0	4.2	8.0	56.0 ± 1.52	0.56	6.35	13.6	69.6 ± 1.29	0.70	4.92
C. cyminum	61	3.41 ± 0.07	28.80	6.6	4.8	4.0	4.8	8.4	57.4 ± 1.21	0.57	4.01	12.6	70.0 ± 1.92	0.70	4.37
	610	3.74 ± 0.07	28.00	6.0	3.6	4.0	5.2	8.0	54.8 ± 1.59*	0.55	8.36	12.8	67.6 ± 1.36*	0.68	7.65

Note:- a = From 100 metaphases/animal; **b** = mg/kg body wt.; **c** = From 2000 cells/animal; MI = Mitotic index; BS = Breaks; F = Fragmentations; CF = Centric fusions; EX = Exchanges; R = Chromatid rings; MA = Multiple aberrations; TA = Total aberration without gap; GS = Gaps; TAG = Total aberration with gap; % CA = Percentage chromosomal aberration; % INH = Percentage inhibition; * = P values ≤ 0.05.

Table 4.12. Inhibitory effect of two different doses of selected spices on induction of MN in polychromatic erythrocytes[a] and total micronuclei by cyclophosphamide (CP) in *M. musculus* bone marrow cells [5 animals/group].

Treatment	Dose[b]	Mean % PCE	Mean % NCE	% MN in PCE ± SEM	% INH	Total MN (%) ± SEM	% INH	P/N ratio ± SEM
CP	50	43.40	56.60	2.42 ± 0.08	-	1.26 ± 0.03	-	0.767 ± 0.004
CP + Saline	50	43.42	56.58	2.47 ± 0.13	-2.07	1.28 ± 0.06	-1.35	0.768 ± 0.006
E. cardamomum	27	43.52	56.48	2.44 ± 0.08	-0.83	1.25 ± 0.04	0.79	0.771 ± 0.004
	270	43.34	56.66	2.53 ± 0.08	-4.55	1.30 ± 0.04	-3.26	0.765 ± 0.004
C. sativum	34	43.48	56.52	2.43 ± 0.07	-0.41	1.24 ± 0.04	1.27	0.769 ± 0.004
	340	43.56	56.44	2.41 ± 0.06	0.41	1.23 ± 0.03	2.14	0.772 ± 0.004
C. cyminum	61	43.73	56.27	2.38 ± 0.05	1.65	1.22 ± 0.03	3.49	0.777 ± 0.005
	610	44.35	55.65	2.13 ± 0.06*	11.98	1.10 ± 0.02**	12.31	0.797 ± 0.009*

Note:- a = From 2000 PCE/animal; **b** = mg/kg body wt.; PCE = Polychromatic erythrocytes; NCE = Normochromatic erythrocytes; MN = Micronucleus; TMN = Total micronuclei; P/N ratio = PCE/NCE ratio; % INH = Percentage inhibition; * = P values ≤ 0.05; ** = P values ≤ 0.01.

Table 4.13. Inhibitory effect of two different doses of selected spices on sperm-shape abnormality[a] in *M. musculus* induced by cyclophosphamide (CP) [5 animals/group].

Treatment	Dose[b]	Normal	AM	BA	HL	FO	DH/DT	Total AS	% AS ± SEM	% INH
CP	50	9345	358	54	148	66	29	655	6.55 ± 0.10	-
CP + Saline	50	9344	359	49	147	73	28	656	6.56 ± 0.08	-0.15
E. cardamomum	27	9338	357	52	151	72	30	662	6.62 ± 0.09	-1.07
	270	9336	366	51	147	73	27	664	6.64 ± 0.06	-1.37
C. sativum	34	9352	372	44	142	68	22	648	6.48 ± 0.12	1.07
	340	9372	363	42	139	63	21	628	6.28 ± 0.14	4.12
C. cyminum	61	9360	369	45	142	61	23	640	6.40 ± 0.09	2.29
	610	9379	362	38	142	62	17	621	6.21 ± 0.08*	5.19

Note:- a = From 2000 sperms/animal; **b** = mg/kg body wt.; AM = Amorphous; BA = Banana; HL = Hookless; FO = Folded; DH/DT = Double headed/Double tailed; AS = Abnormal sperms; % INH = Percentage inhibition; P value: * = 0.048.

Table 4.14. Effect of two different doses of selected spices on body weight & testes weight of *M. musculus* treated with cyclophosphamide (CP) after 5 weeks [5 animals/group].

Treatment	Dose[a]	IBW (g) ± SEM	SBW (g) ± SEM	% INBW	Testes wt. (g) ± SEM	% ITW
CP	50	28 ± 0	32.2 ± 0.49	11.43	0.197 ± 0.006	-
CP + Saline	50	24 ± 0	26.8 ± 0.86	11.67	0.184 ± 0.006	-6.40
E. cardamomum	27	27 ± 0	30.6 ± 0.93	13.33	0.204 ± 0.004	3.40
	270	28 ± 0	31.4 ± 1.03	12.14	0.189 ± 0.008	-3.81
C. sativum	34	28 ± 0	31.6 ± 0.51	12.86	0.207 ± 0.006	5.08
	340	25 ± 0	29.2 ± 0.86	16.80	0.206 ± 0.005	4.83
C. cyminum	61	26 ± 0	32.2 ± 1.07	23.85	0.214 ± 0.005	8.69
	610	28 ± 0	34.8 ± 0.73	24.29	0.229 ± 0.002	16.31

Note:- a = mg/kg body weight.; IBW = Initial body weight; SBW = Sacrifice body weight; % INBW = Percentage increase in body weight; TW = Testes weight; % ITW = Percentage increase in testes weight.

Table 4.15. Antimutagenic effect of *C. cyminum* on chromosomal aberrations[a] induced by *P. nigrum* in *M. musculus* bone marrow cells [5 animals/group].

Treatment	Dose[b]	MI[c] ± SEM	BS	F	CF	EX	R	MA	TA ± SEM	% CA/ Cell	% INH	GS	TAG ± SEM	% CA/ Cell	% INH
PN-N	38	4.46 ± 0.11	3.8	0.2	0.4	0.4	0.6	0.6	6.0 ± 0.45	0.06	-	2.0	8.0 ± 0.45	0.08	-
PN-N + CC-H	38 + 610	4.76 ± 0.12	3.2	-	0.4	0.4	0.6	-	4.6 ± 0.40*	0.05	23.3	1.4	6.0 ± 0.55*	0.06	25.0
PN-H	380	3.81 ± 0.19	7.6	0.6	1.6	1.8	2.0	1.6	15.2 ± 0.58	0.15	-	3.0	18.2 ± 0.58.	0.18	-
PN-H + CC-H	380 + 610	4.26 ± 0.13	4.8	-	1.4	1.2	1.6	0.8	9.8 ± 0.58***	0.10	35.5	4.6	14.4 ± 0.81**	0.14	20.9

Note:- a = From 100 metaphases/animal; **b** = mg/kg body wt.; **c** = From 2000 cells/animal; MI = Mitotic index; BS = Breaks; F = Fragmentations; CF = Centric fusions; EX = Exchanges; R = Chromatid rings; MA = Multiple aberrations; TA = Total aberration without gap; GS = Gaps; TAG = Total aberration with gap; % CA = Percentage chromosomal aberration; % INH = Percentage inhibition; PN = *P. nigrum*; CC = *C. cyminum*; N = Normal; H = High; * = P values ≤ 0.05; ** = P values ≤ 0.01; *** = P values ≤ 0.001.

Table 4.16. Inhibitory effect of *C. cyminum* on induction of MN in polychromatic erythrocytes[a] and total micronuclei by *P. nigrum* in *M. musculus* bone marrow cells [5 animals/group].

Treatment	Dose[b]	Mean % PCE	Mean % NCE	% MN in PCE ± SEM	% INH	Total MN (%) ± SEM	% INH	P/N ratio ± SEM
PN-N	38	50.52	49.48	0.39 ± 0.03	-	0.30 ± 0.02	-	1.021 ± 0.002
PN-N +CC-H	38 + 610	50.97	49.03	0.30 ± 0.03	23.08	0.21 ± 0.02*	30.00	1.040 ± 0.003
PN-H	380	49.75	50.25	0.50 ± 0.03	-	0.36 ± 0.02	-	0.990 ± 0.005
PN-H +CC-H	380 + 610	50.36	49.64	0.40 ± 0.02*	20.00	0.29 ± 0.01*	19.44	1.014 ± 0.005**

Note:- a = From 2000 PCE/animal; **b** = mg/kg body wt.; PCE = Polychromatic erythrocytes; NCE = Normochromatic erythrocytes; MN = Micronucleus; TMN = Total micronuclei; P/N ratio = PCE/NCE ratio; % INH = percentage inhibition; PN = *P. nigrum*; CC = *C. cyminum*; N = Normal; H = High; * = P values < 0.05; ** = P values ≤ 0.01.

Table 4.17. Inhibitory effect of *C. cyminum* on sperm-shape abnormality[a] in *M. musculus* induced by *P. nigrum* [5 animals/group].

Treatment	Dose[b]	Normal	AM	BA	HL	FO	DH/DT	Total AS	% AS ± SEM	% INH
PN-N	38	9736	120	21	75	34	14	264	2.64 ± 0.05	-
PN-N + CC-H	38 + 610	9759	125	20	61	26	9	241	2.41 ± 0.10*	8.71
PN-H	380	9711	135	25	77	34	18	289	2.89 ± 0.10	-
PN-H + CC-H	380 + 610	9731	125	22	75	33	14	269	2.69 ± 0.13	6.92

Note:- a = From 2000 sperms/animal; **b** mg/kg body wt.; AM = Amorphous; BA = Banana; HL = Hookless; FO = Folded; DH/DT = Double headed/Double tailed; AS = Abnormal sperms; % INH = Percentage inhibition; PN = *P. nigrum*; CC = *C. cyminum*; N = Normal; H = High; P value: * = 0.048.

Table 4.18. Effect of combined treatment of *P. nigrum* and *C. cyminum* on body weight & testes weight of *M. musculus* after 5 weeks [5 animals/group].

Treatment	Dose[a]	IBW (g) ± SEM	SBW (g) ± SEM	% INBW	TW (g) ± SEM	% ITW
PN-N	38	28 ± 0	32.2 ± 0.66	15.00	0.21 ± 0.010	-
PN-N + CC-H	38 + 610	26 ± 0	30.4 ± 0.51	16.92	0.22 ± 0.001	3.01
PN-H	380	28 ± 0	31.8 ± 0.66	13.57	0.20 ± 0.007	-
PN-H + CC-H	380 + 610	28 ± 0	32.4 ± 0.51	15.71	0.22 ± 0.005	9.87

Note:- a = mg/kg body weight.; IBW = Initial body weight; SBW = Sacrifice body weight; % INBW = Percentage increase in body weight; TW = Testes weight; % ITW = Percentage increase in testes weight; PN = *P. nigrum*; CC = *C. cyminum*; N = Normal; H = High.

Table 4.19. Effect of selected spices and controls on Ehrlich ascites tumour reduction in *M. musculus* [12 animals/group].

Treatment	Dose (mg/kg body wt.)	IBW (%)	MST (days)	ILS (%)	Survival on 120th day (%)
Tumour Alone (Control)	-	18.33	19.42	-	-
Tumour + Saline	-	10.83	22.67	16.74	-
Tumour + Cisplatin	4	2.88	23.00	18.43	75
Tumour + *P. nigrum*	38	5.26	19.83	2.11	-
	380	8.90	21.25	9.42	-
Tumour + *E. cardamomum*	27	6.32	24.33	25.28	-
	270	5.12	23.58	21.42	-
Tumour + *C. sativum*	34	5.17	24.67	27.03	-
	340	6.86	25.33	30.43	-
Tumour + *C. cyminum*	61	7.11	23.25	19.72	-
	610	6.68	28.83	48.46	-

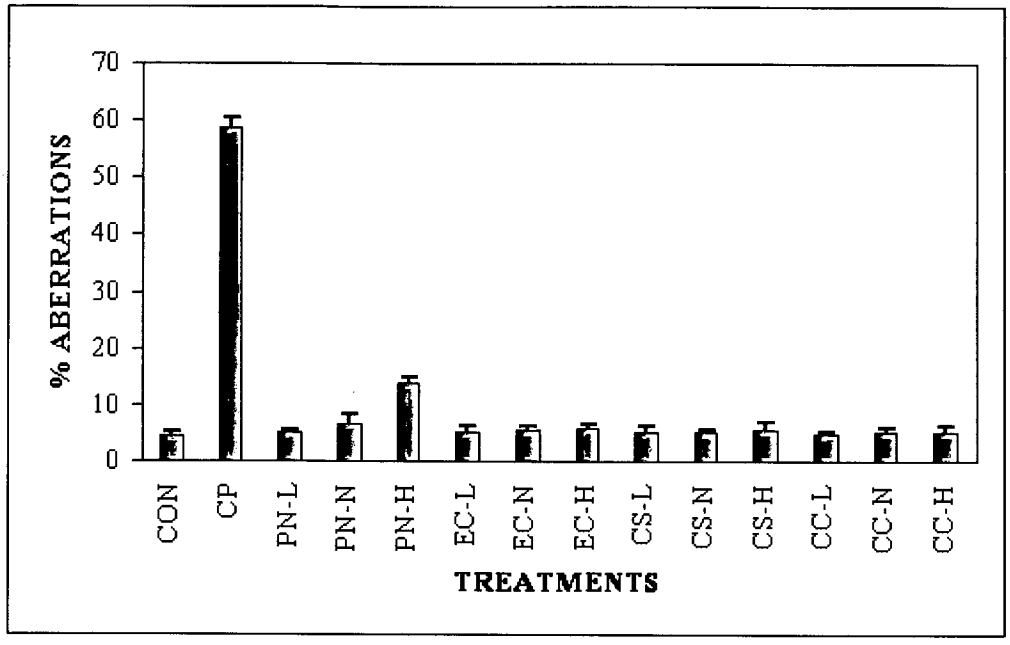
Note:- IBW = Increase in body wt.; MST = Mean survival time; ILS = Increase in life span.

Table 4.20. Effect of selected spices and controls on survival time of *M. musculus* bearing Ehrlich ascites carcinoma [12 animals/group; Figures in the parentheses are ranks].

	T (Con)	T + Sa	T + Csp	T + BP-N	T + BP-H	T + CM-N	T + CM-H	T + CR-N	T + CR-H	T + CU-N	T + CU-H
Dose[a]	-	-	4	38	380	27	270	34	340	61	610
1	17 (8)	18 (18)	20 (35.5)	17 (8)	16 (2)	17 (8)	16 (2)	17 (8)	16 (2)	21 (46.5)	21 (46.5)
2	17 (8)	19 (27)	24 (77.5)	17 (8)	17 (8)	19 (27)	18 (18)	18 (18)	21 (46.5)	21 (46.5)	23 (69)
3	17 (8)	21 (46.5)	25 (85)	18 (18)	17 (8)	19 (27)	18 (18)	20 (35.5)	21 (46.5)	22 (58)	23 (69)
4	18 (18)	22 (58)	120 (128)	18 (18)	18 (18)	20 (35.5)	19 (27)	22 (58)	21 (46.5)	22 (58)	24 (77.5)
5	18 (18)	22 (58)	120 (128)	19 (27)	18 (18)	20 (35.5)	19 (27)	22 (58)	23 (69)	22 (58)	28 (99.5)
6	19 (27)	22 (58)	120 (128)	20 (35.5)	18 (18)	22 (58)	20 (35.5)	23 (69)	25 (85)	23 (69)	28 (99.5)
7	20 (35.5)	23 (69)	120 (128)	21 (46.5)	20 (35.5)	26 (93)	24 (77.5)	23 (69)	26 (93)	23 (69)	30 (108)
8	20 (35.5)	23 (69)	120 (128)	21 (46.5)	20 (35.5)	26 (93)	28 (99.5)	25 (85)	28 (99.5)	24 (77.5)	31 (112)
9	21 (46.5)	25 (85)	120 (128)	21 (46.5)	22 (58)	29 (104.5)	28 (99.5)	25 (85)	28 (99.5)	24 (77.5)	31 (112)
10	21 (46.5)	25 (85)	120 (128)	25 (85)	23 (69)	31 (112)	29 (104.5)	33 (116.5)	30 (108)	25 (85)	33 (116.5)
11	22 (58)	26 (93)	120 (128)	29 (104.5)	24 (77.5)	31 (112)	30 (108)	34 (119.5)	31 (112)	26 (93)	36 (122)
12	23 (69)	26 (93)	120 (128)	29 (104.5)	25 (85)	32 (115)	34 (119.5)	34 (119.5)	34 (119.5)	26 (93)	38 (123)
ΣRi	378	759.50	1350	548.00	432.50	820.50	736.00	841.00	927.00	831.00	1154.50
ΣRi^2	142884	576840.25	1822500	300304	187056.25	673220.25	541696	707281	859329	690561	1332870.25
$\Sigma Ri^2/ni$	11907	48070.02	151875***	25025.33	15588.02	56101.69	45141.33	58940.08	71610.75	57546.75	111072.52***

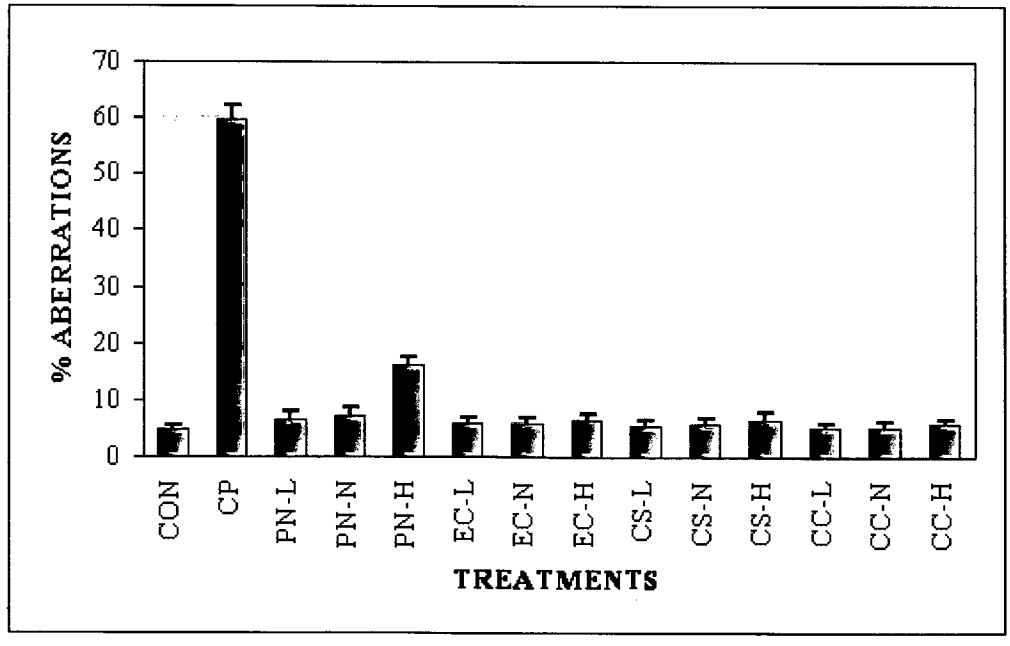
Note:- a = mg/kg body wt.; T = Tumour; Con = Control; Sa = Saline; Csp = Cisplatin; BP = Black pepper (*P. nigrum*); CM = Cardamom (*E. cardamomum*); CR = Coriander (*C. sativum*); CU = Cumin (*C. cyminum*); N = Normal; H = High; Ri = Rank; ni = Number of observations; *** = P value ≤ 0.001.

Fig. 4.1. Percentage of chromosomal aberrations (without gap) in bone marrow erythrocytes of *M. musculus* induced by different doses of selected spices at 12 h.



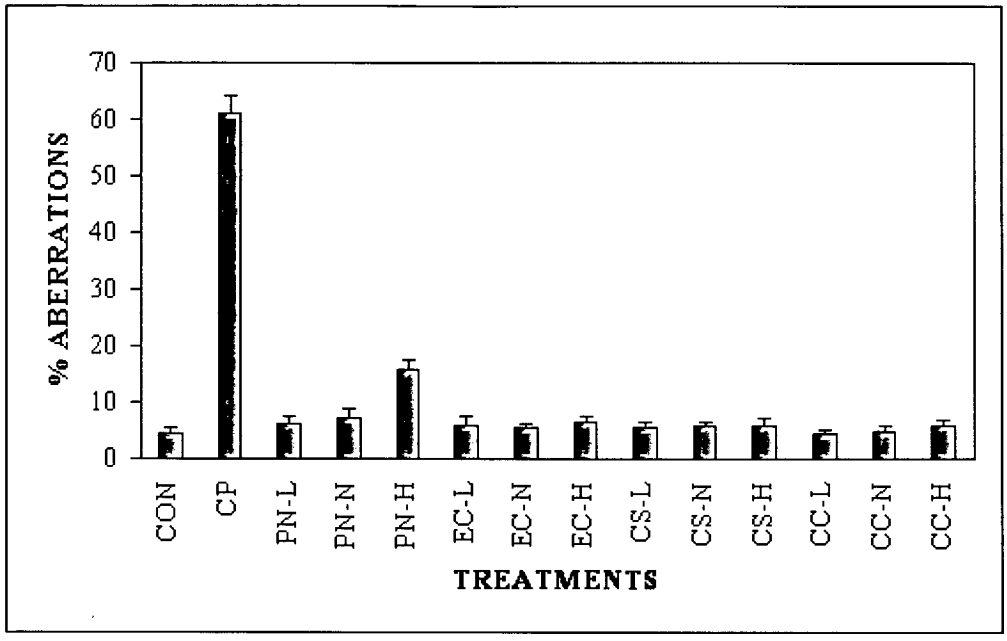
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.2. Percentage of chromosomal aberrations (without gap) in bone marrow erythrocytes of *M. musculus* induced by different doses of selected spices at 24 h.



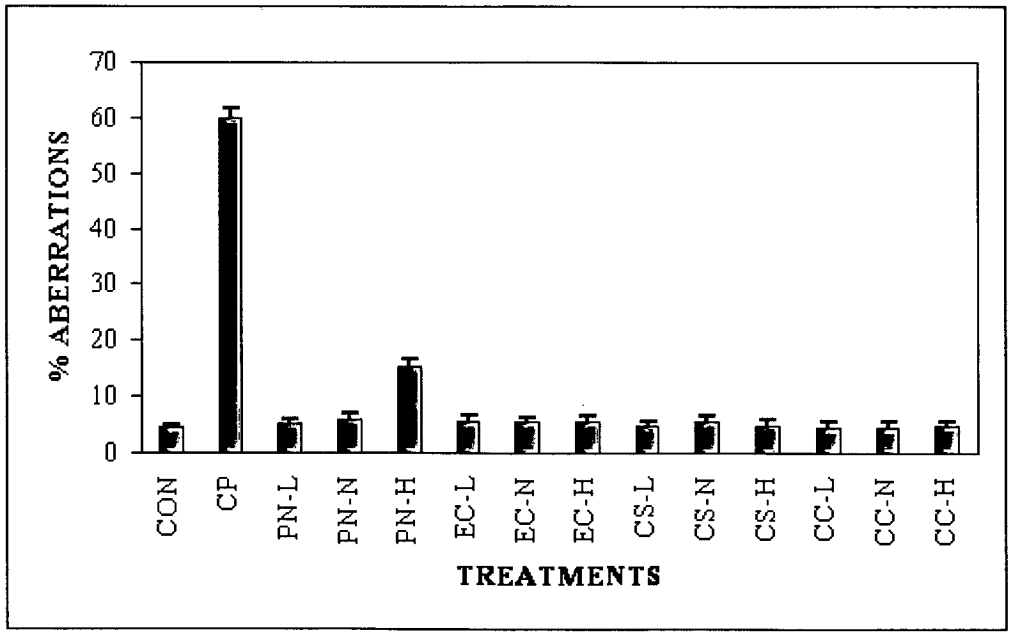
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.3. Percentage of chromosomal aberrations (without gap) in bone marrow erythrocytes of *M. musculus* induced by different doses of selected spices at 48 h.



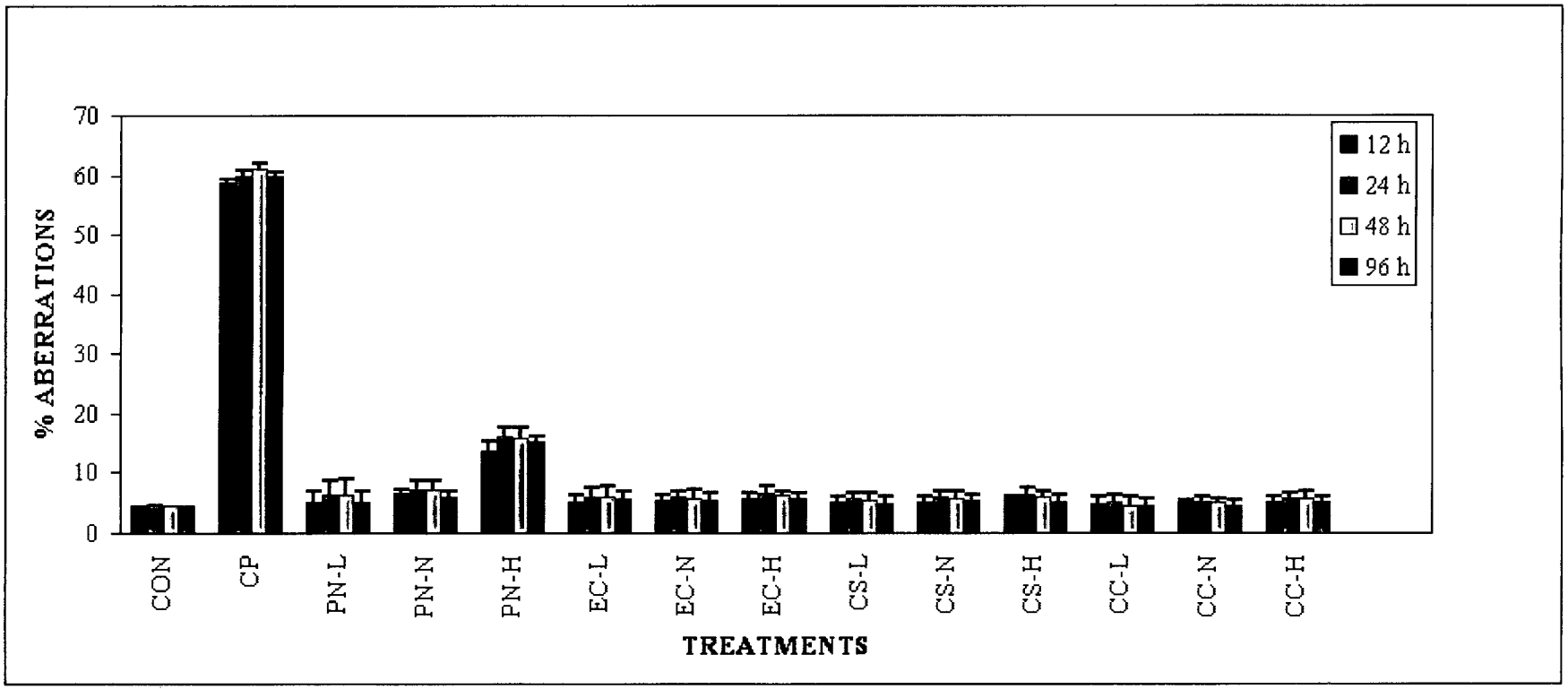
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.4. Percentage of chromosomal aberrations (without gap) in bone marrow erythrocytes of *M. musculus* induced by different doses of selected spices at 96 h.



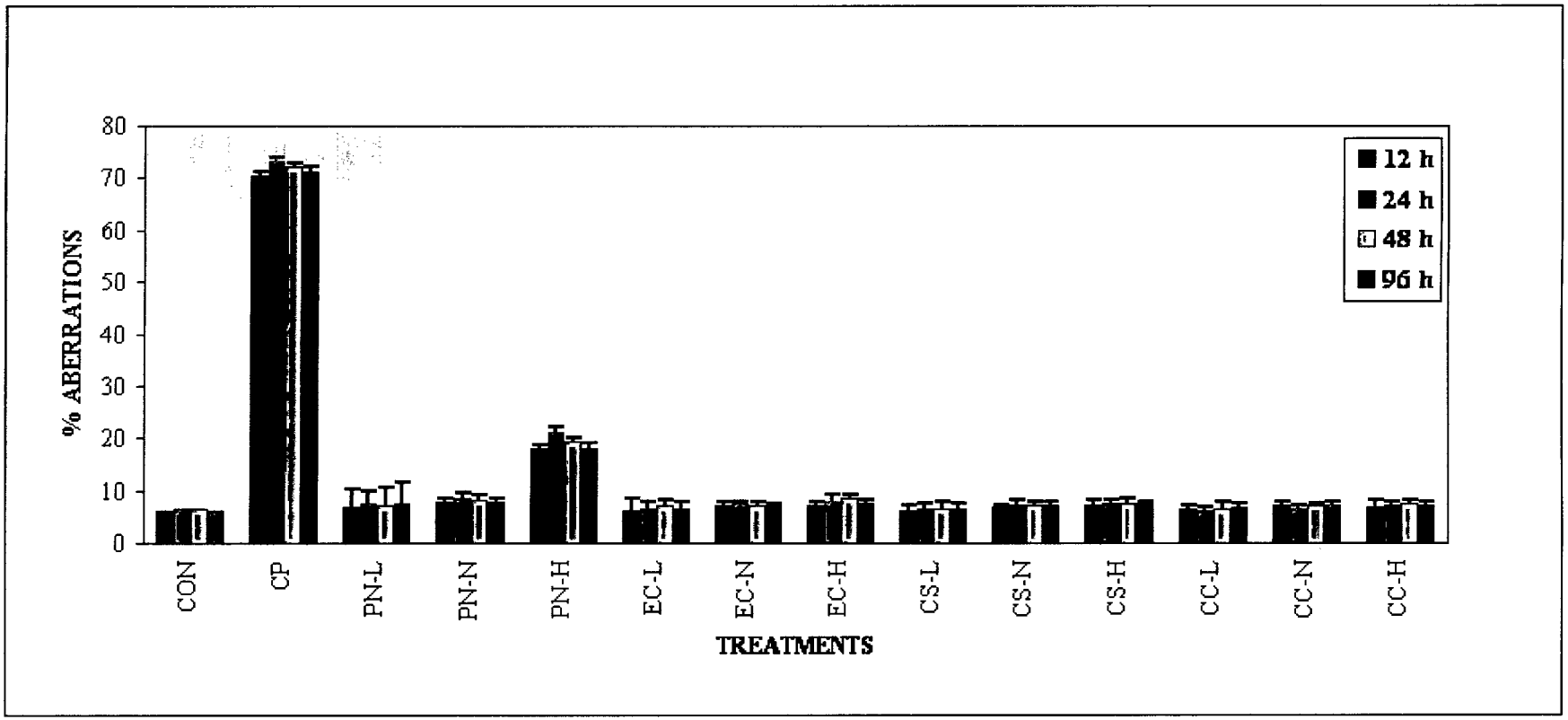
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.5. Chromosomal aberrations (without gap) in bone marrow erythrocytes of *M. musculus* induced by selected spices at different time intervals.



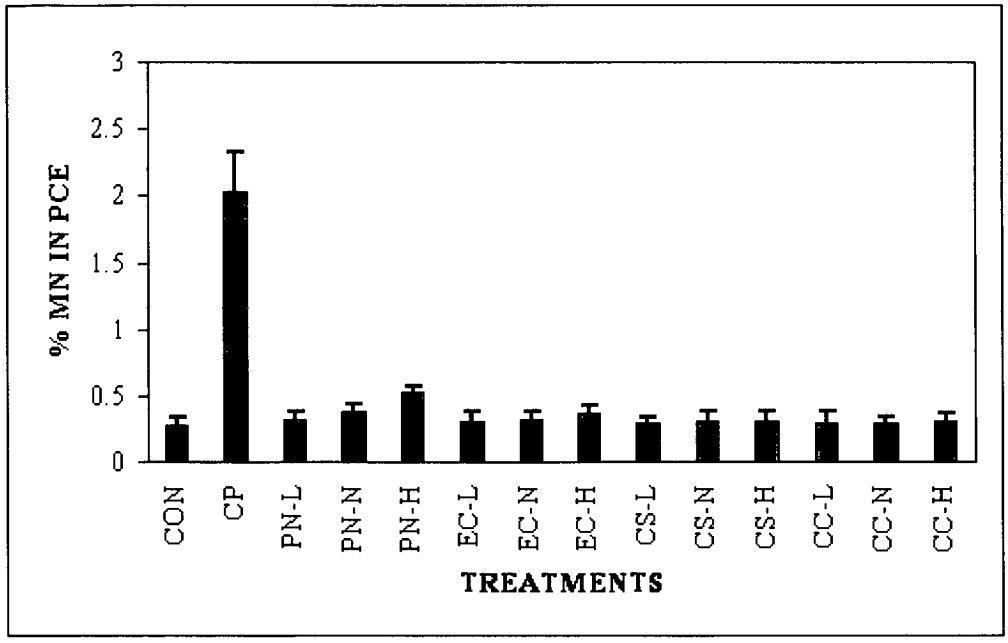
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.6. Chromosomal aberrations (with gap) in bone marrow erythrocytes of *M. musculus* induced by selected spices at different time intervals.



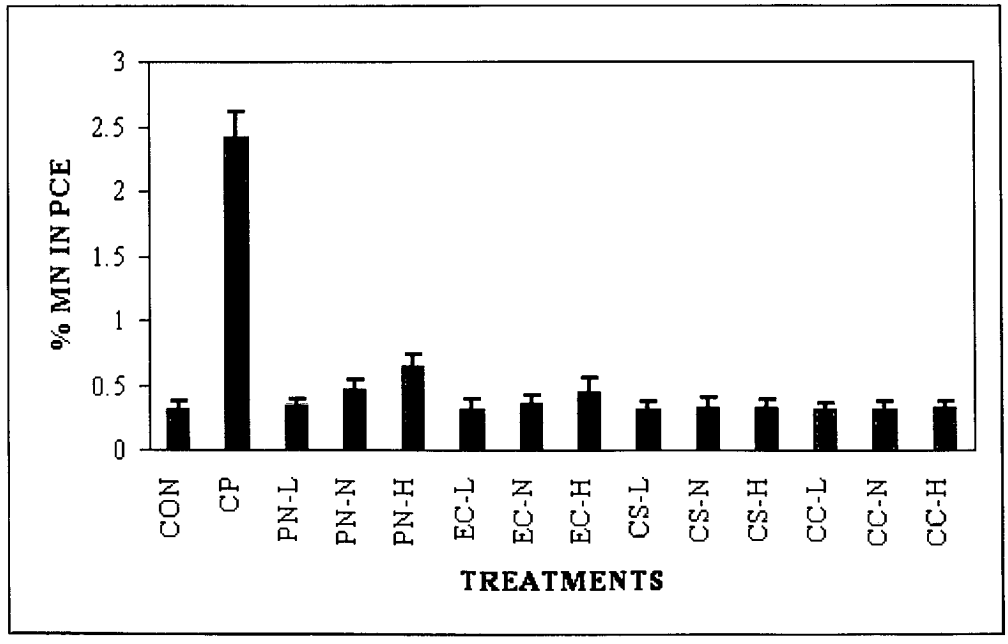
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.7. Percentage frequency of micronuclei (MN) in polychromatic erythrocytes (PCE) of *M. musculus* treated with various doses of selected spices at 12 h.



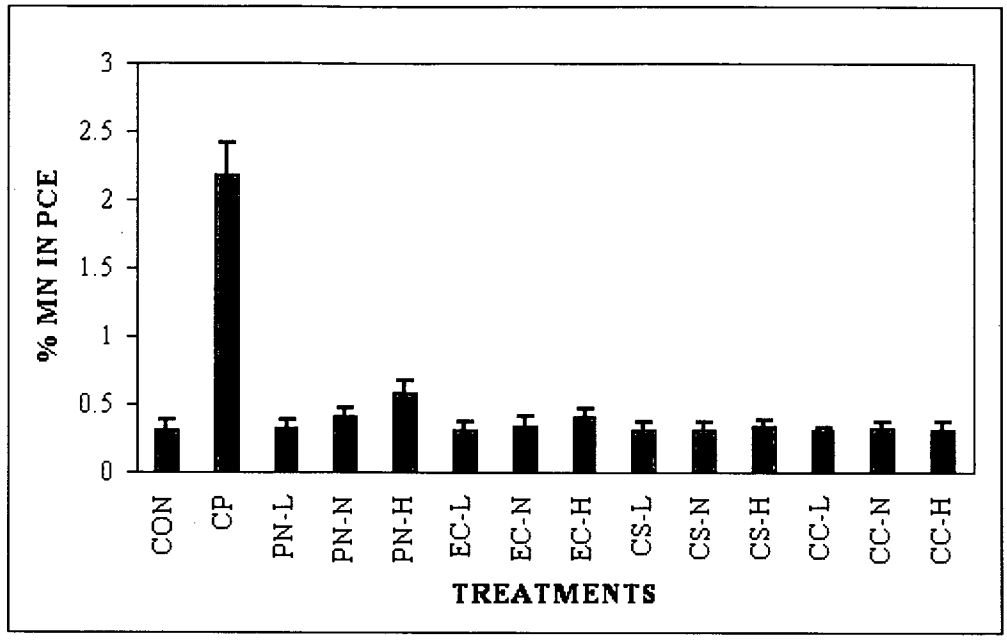
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.8. Percentage frequency of micronuclei (MN) in polychromatic erythrocytes (PCE) of *M. musculus* treated with various doses of selected spices at 24 h.



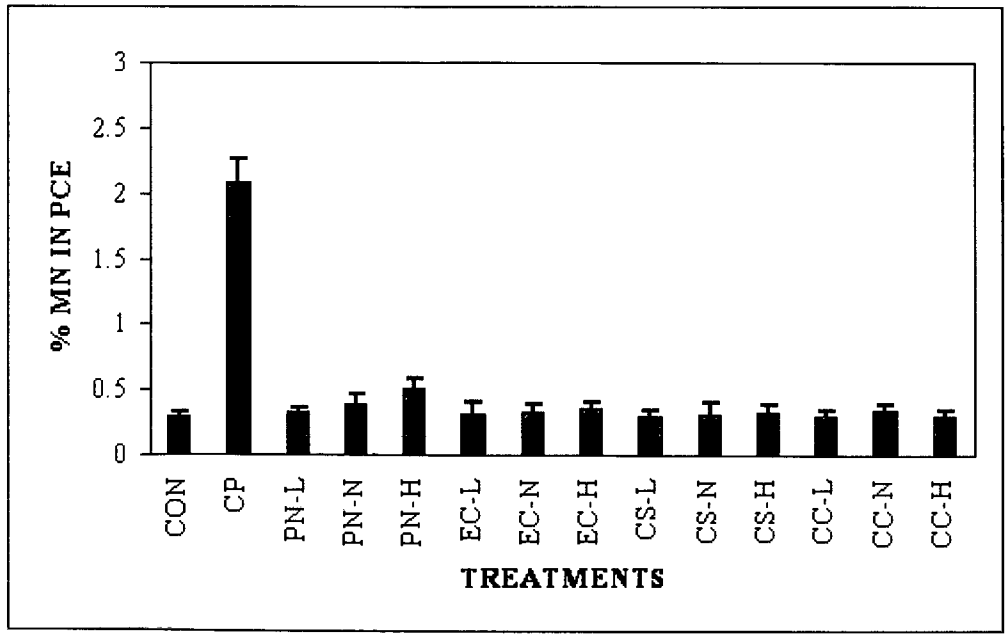
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.9. Percentage frequency of micronuclei (MN) in polychromatic erythrocytes (PCE) of *M. musculus* treated with various doses of selected spices at 48 h.



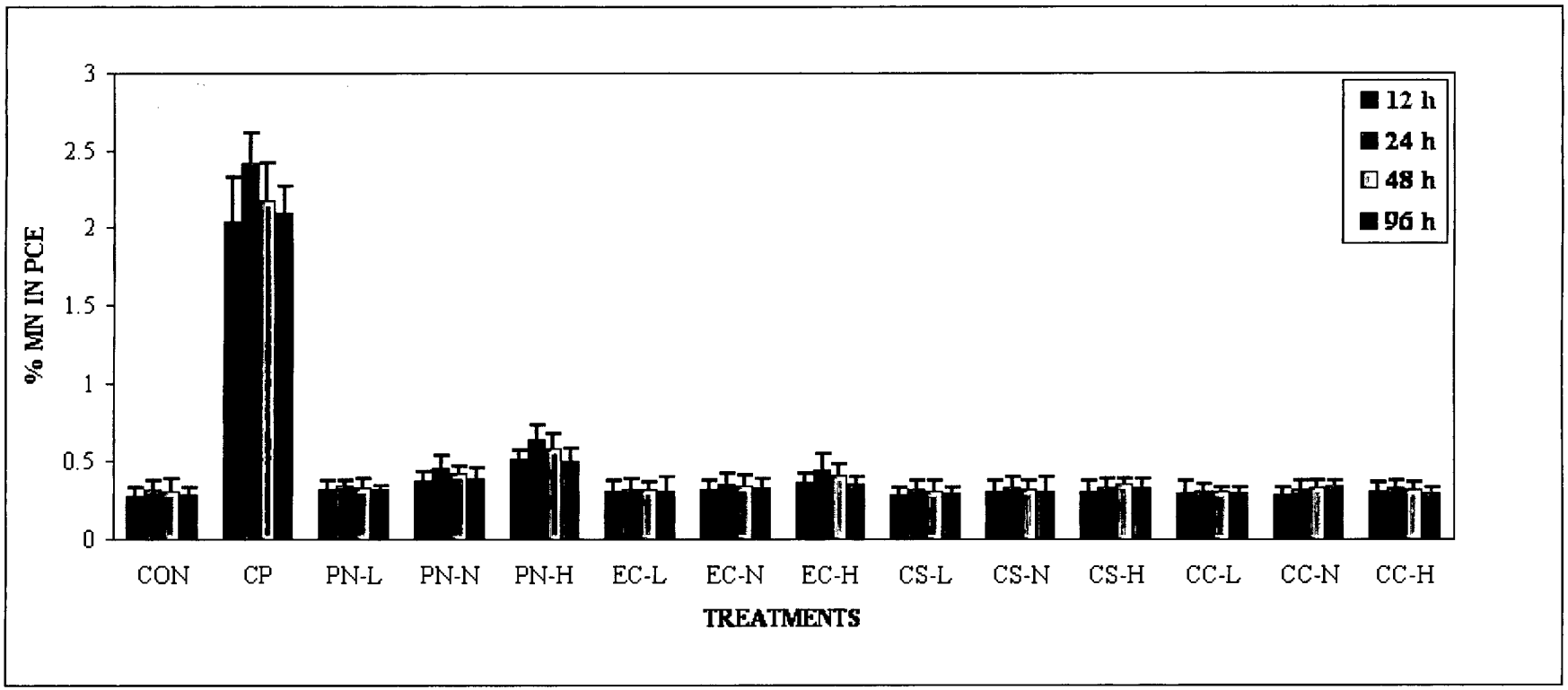
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.10. Percentage frequency of micronuclei (MN) in polychromatic erythrocytes (PCE) of *M. musculus* treated with various doses of selected spices at 96 h.



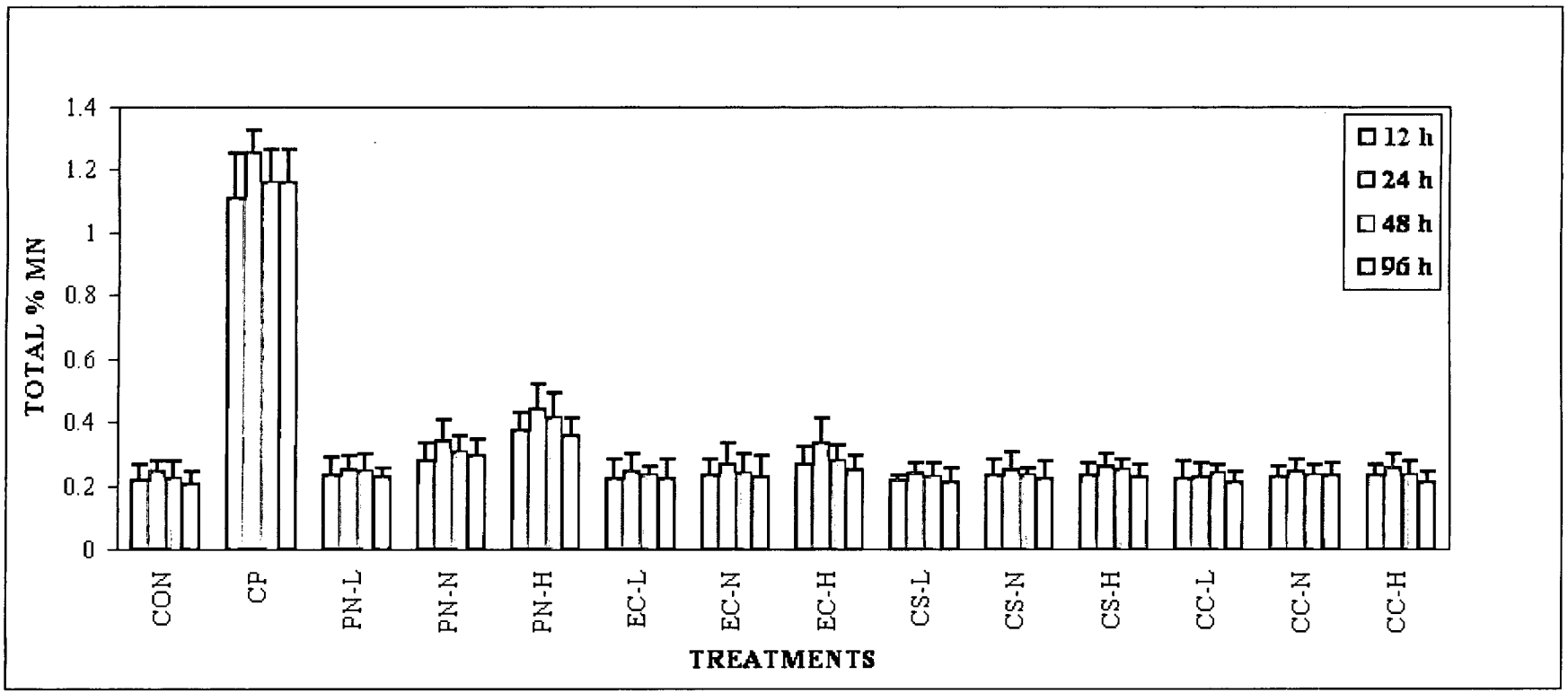
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.11. Effect of different doses of selected spices on induction of MN in bone marrow PCE of *M. musculus* at different time intervals.



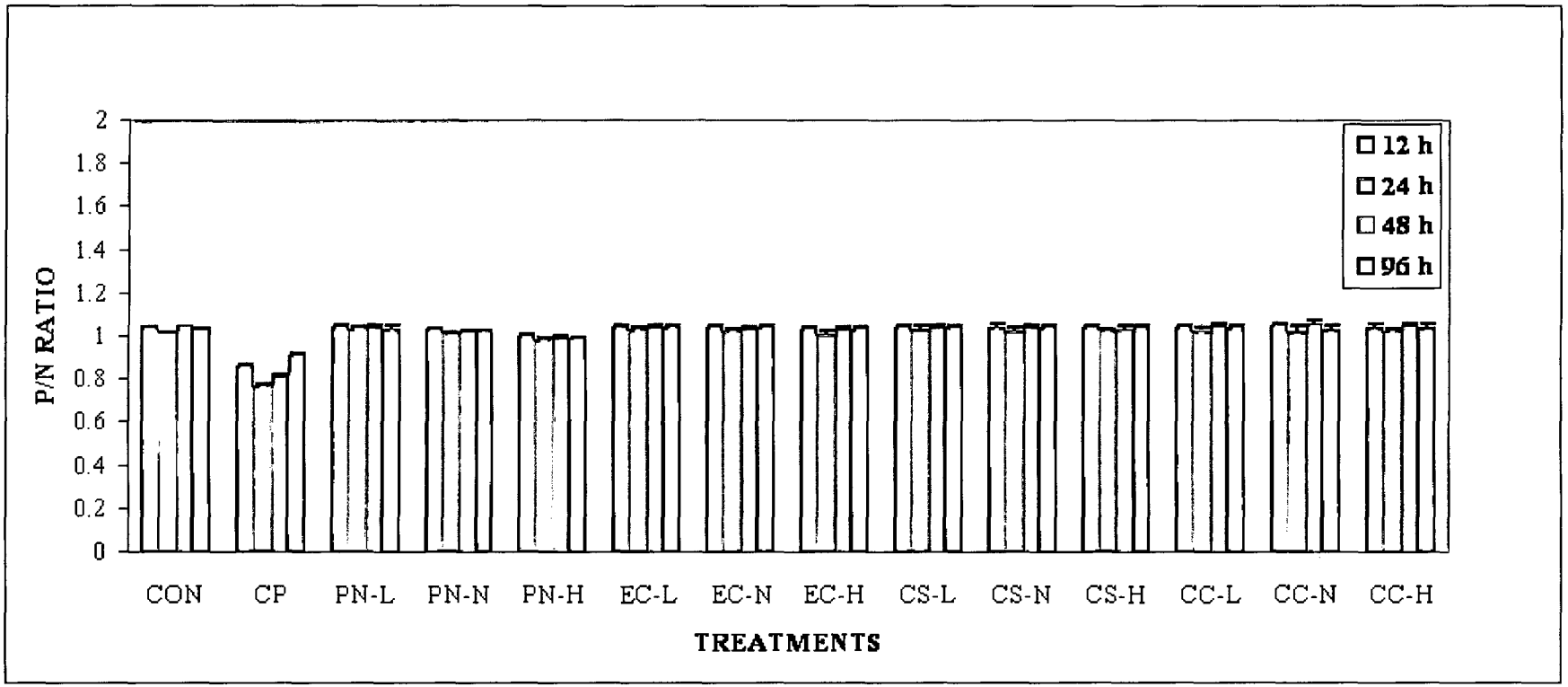
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.12. Effect of different doses of selected spices on induction of MN in *M. musculus* bone marrow erythrocytes (total % MN) at different time intervals.



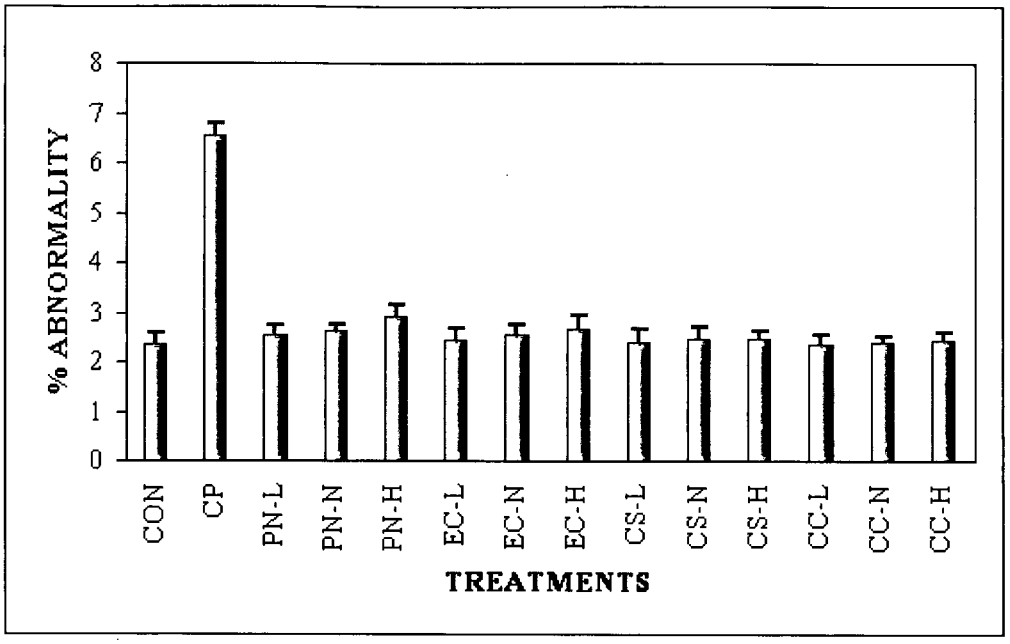
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.13. Illustrates the P/N ratio of bone marrow erythrocytes of *M. musculus* treated with different doses of selected spices at different time intervals.



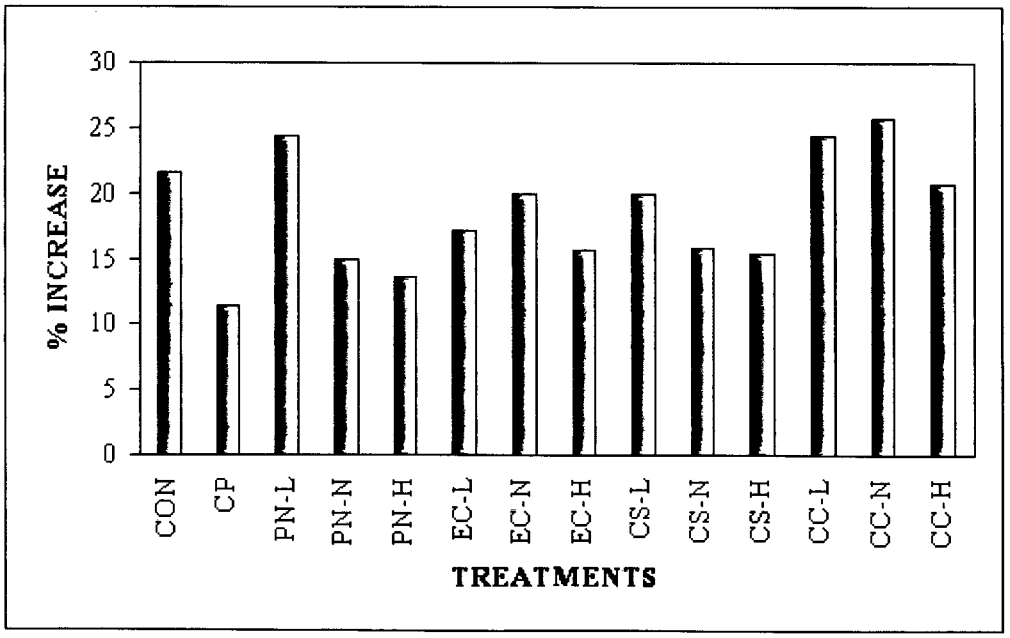
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4. 14. Incidence of sperm-shape abnormality induced by different doses of selected spices in *M. musculus* after 5 weeks.



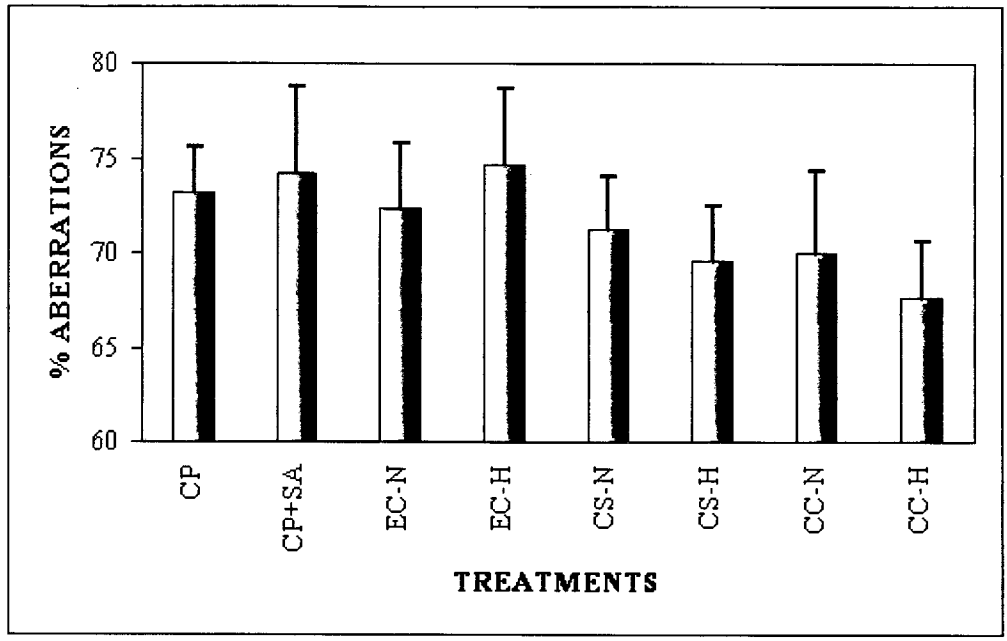
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.15. Graph showing the percentage increase in body weight of *M. musculus* treated with various doses of selected spices and controls after 5 weeks.



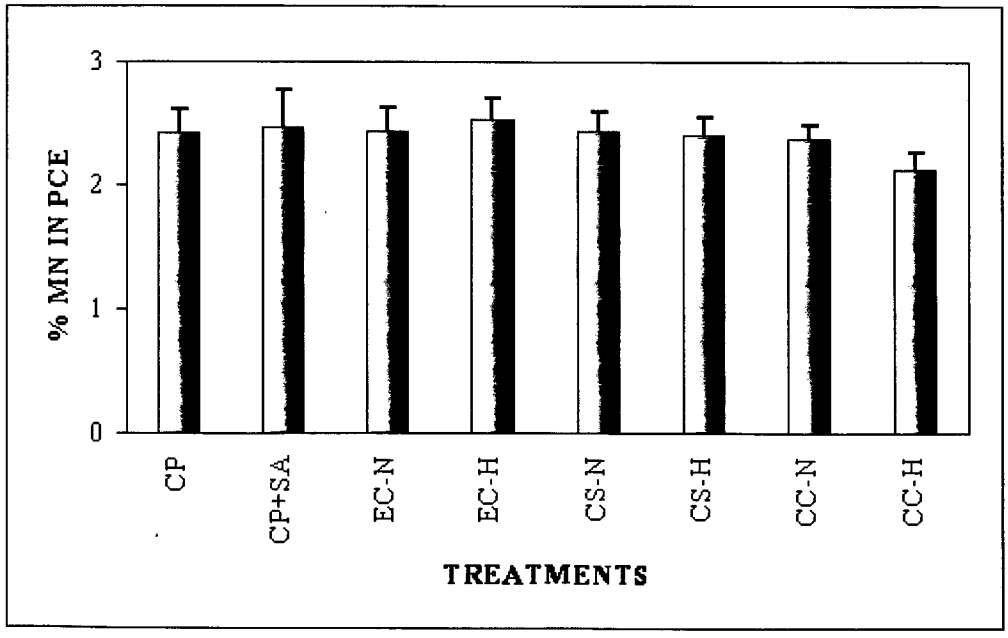
Note:- CON = Control; CP = Cyclophosphamide; PN = *P. nigrum*; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; L= Low; N = Normal; H = High.

Fig. 4.18. Antimutagenic effect of selected spices on bone marrow chromosomal aberrations (with gap) induced by cyclophosphamide (CP) in *M. musculus*.



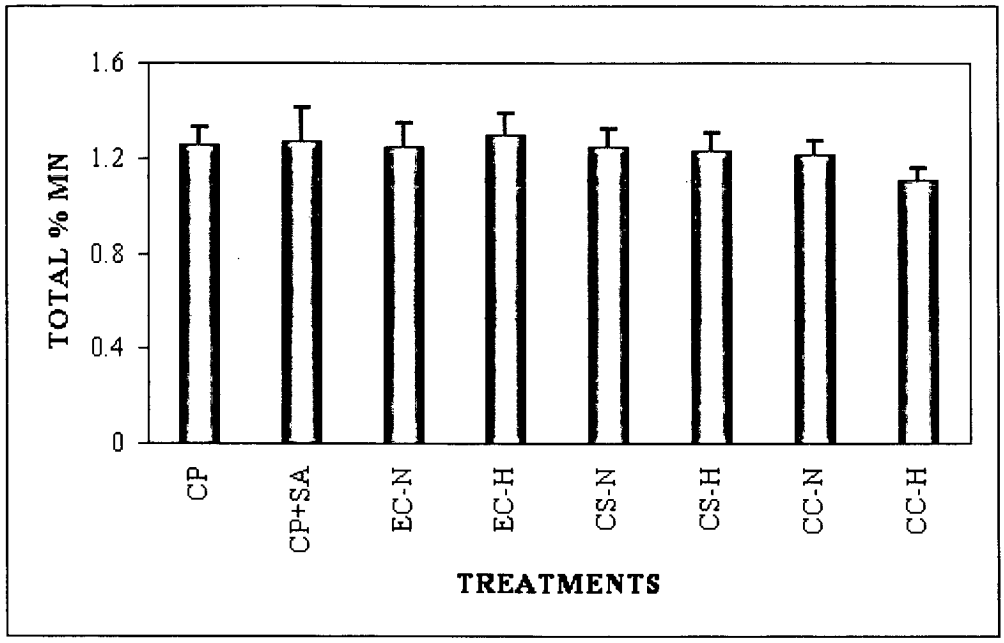
Note:- CP = Cyclophosphamide; SA = Saline; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; N = Normal; H = High.

Fig. 4.19. Inhibitory effect of selected spices on bone marrow (PCE) MN induced by CP in *M. musculus*.



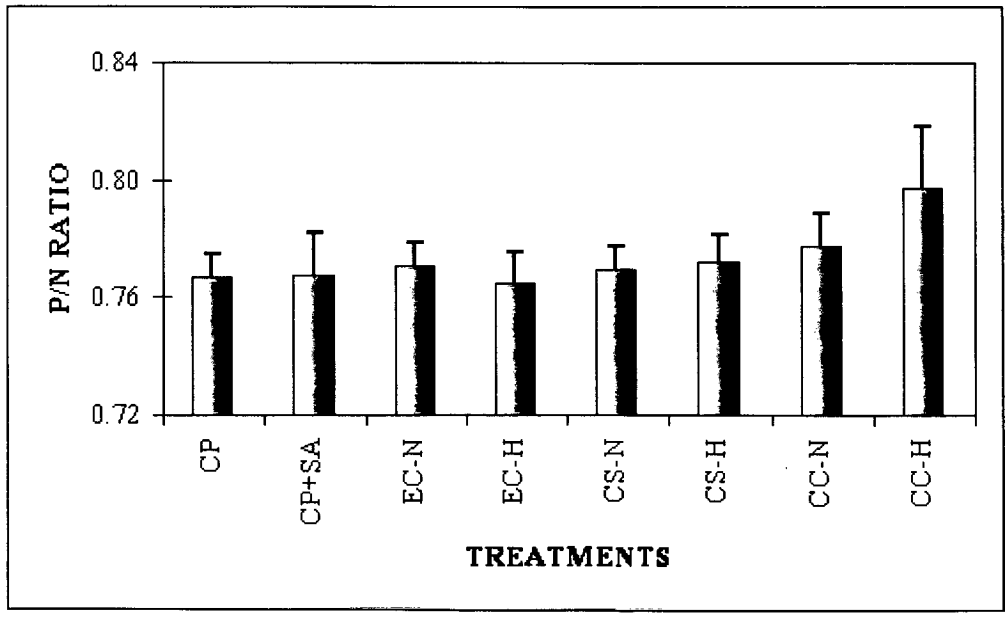
Note:- CP = Cyclophosphamide; SA = Saline; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; N = Normal; H = High.

Fig. 4.20. Inhibitory effect of selected spices on induction of MN in bone marrow erythrocytes (total % MN) by CP in *M. musculus*.



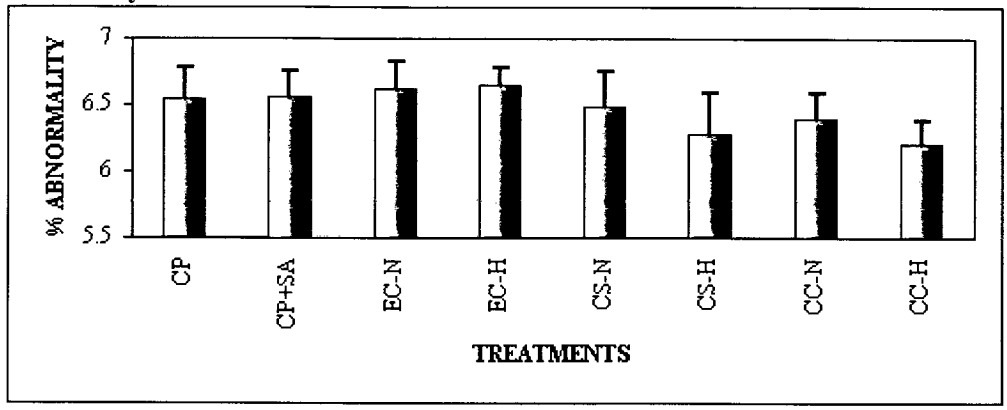
Note:- CP = Cyclophosphamide; SA = Saline; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; N = Normal; H = High.

Fig. 4.21. Effect of pre-treatment with selected spices on P/N ratio of bone marrow erythrocytes treated with CP in *M. musculus*.



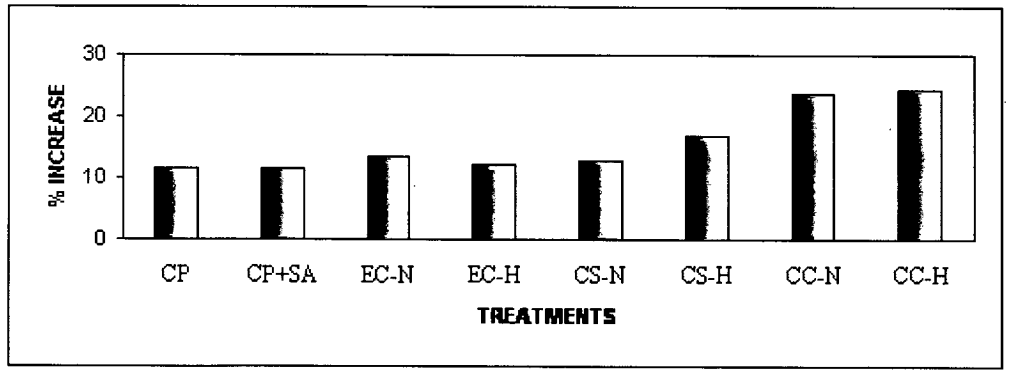
Note:- CP = Cyclophosphamide; SA = Saline; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; N = Normal; H = High.

Fig. 4.22. Effect of post-treatment with selected spices on sperm-shape abnormality induced by CP in *M. musculus* - after 5 weeks.



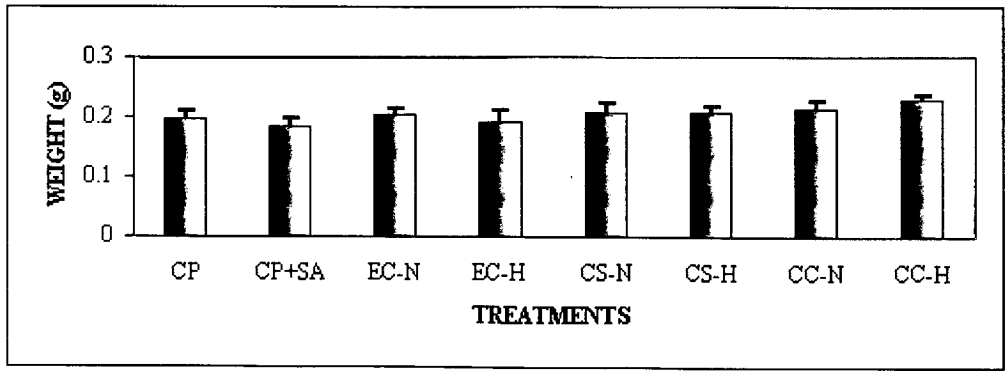
Note:- CP = Cyclophosphamide; SA = Saline; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; N = Normal; H = High.

Fig. 4.23. Effect of two different doses of selected spices on increase in body weight (%) of *M. musculus* treated with cyclophosphamide - after 5 weeks.



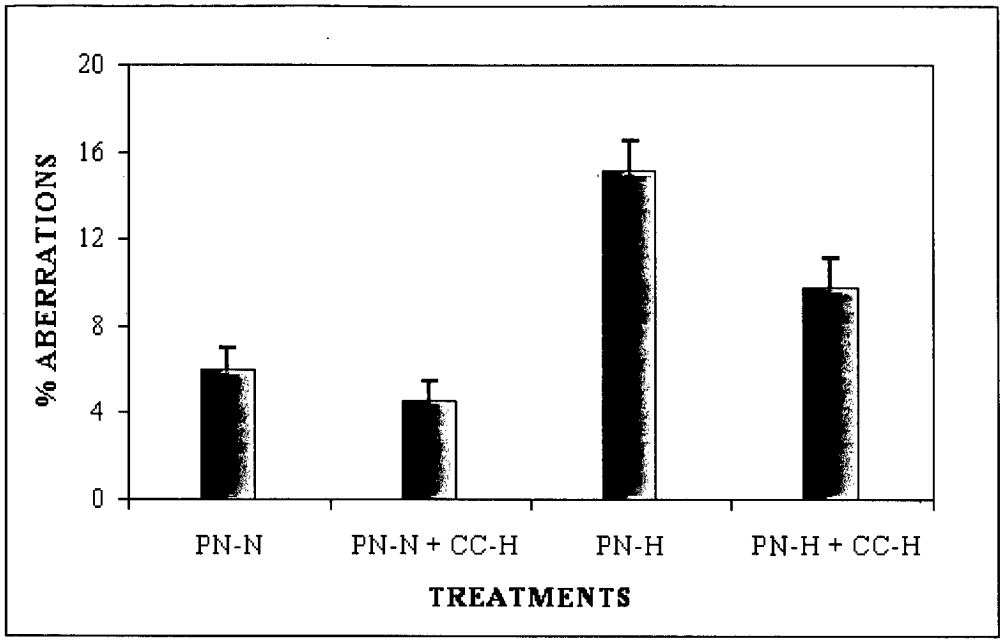
Note:- CP = Cyclophosphamide; SA = Saline; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; N = Normal; H = High.

Fig. 4.24. Effect of two different doses of selected spices on testes weight of *M. musculus* treated with cyclophosphamide - after 5 weeks.



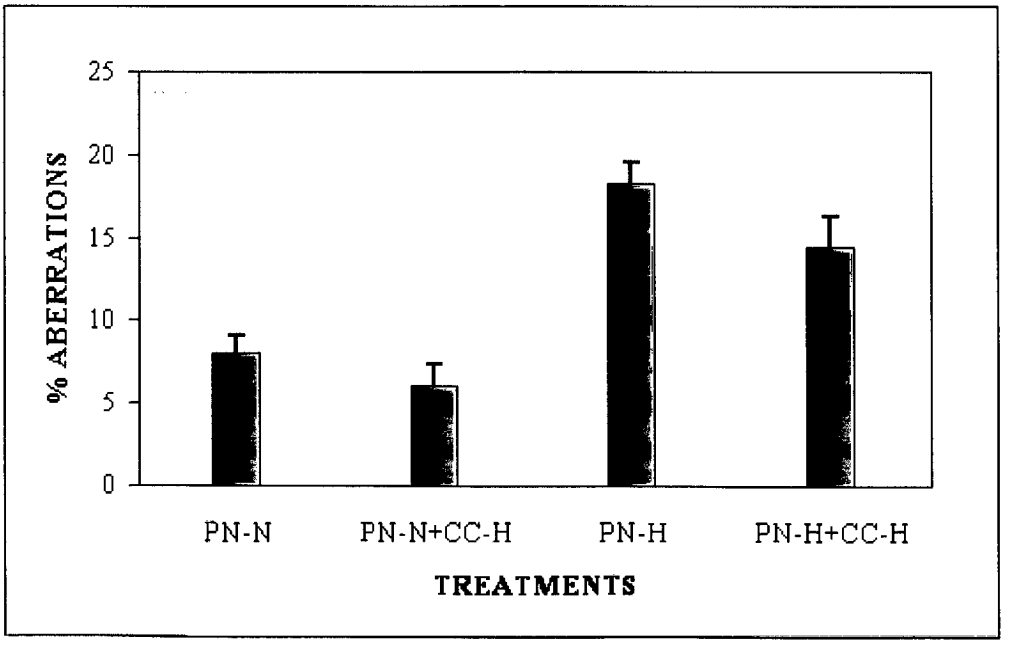
Note:- CP = Cyclophosphamide; SA = Saline; EC = *E. cardamomum*; CS = *C. sativum*; CC = *C. cyminum*; N = Normal; H = High.

Fig. 4.25. Antimutagenic effect of *C. cyminum* on bone marrow chromosomal aberrations (without gap) induced by *P. nigrum* in *M. musculus*.



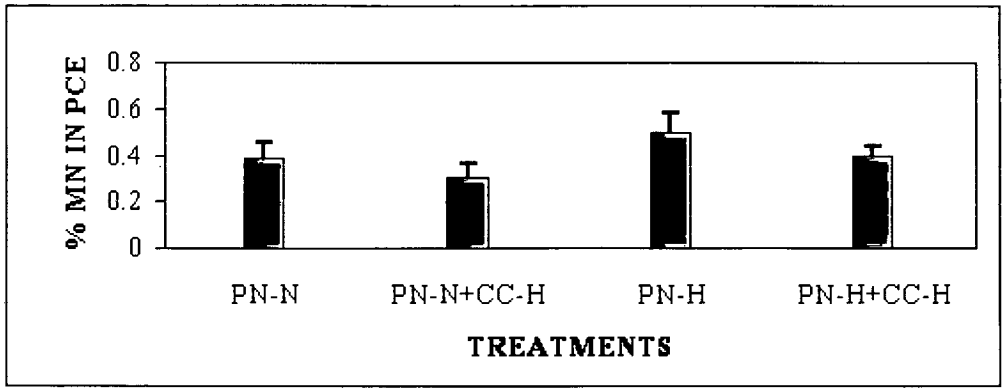
Note:- PN = *P. nigrum; CC* = *C. cyminum;* N = Normal; H = High.

Fig. 4.26. Antimutagenic effect of *C. cyminum* on bone marrow chromosomal aberrations (with gap) induced by *P. nigrum* in *M. musculus*.



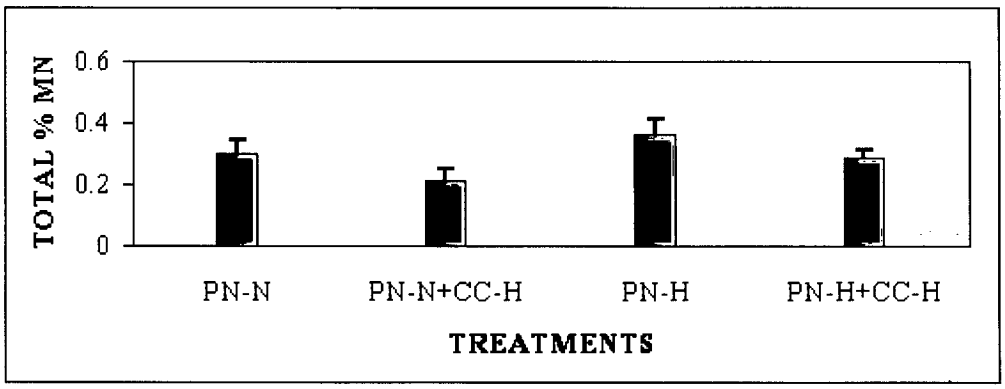
Note:- PN = *P. nigrum; CC* = *C. cyminum;* N = Normal; H = High.

Fig. 4.27. Inhibitory effect of *C. cyminum* on bone marrow (PCE) MN induced by *P. nigrum* in *M. musculus*.



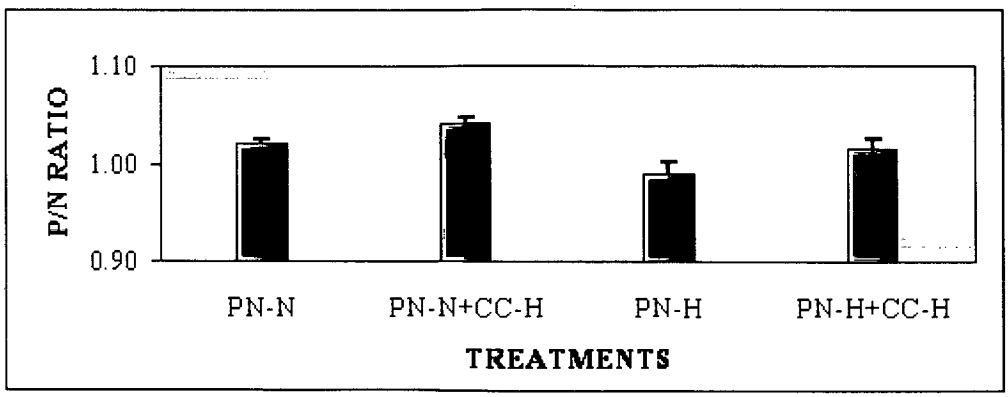
Note:- PN = *P. nigrum; CC = C. cyminum;* N = Normal; H = High.

Fig. 4.28. Inhibitory effect of *C. cyminum* on induction of MN in *M. musculus* bone marrow erythrocytes (total % MN) by *P. nigrum*.



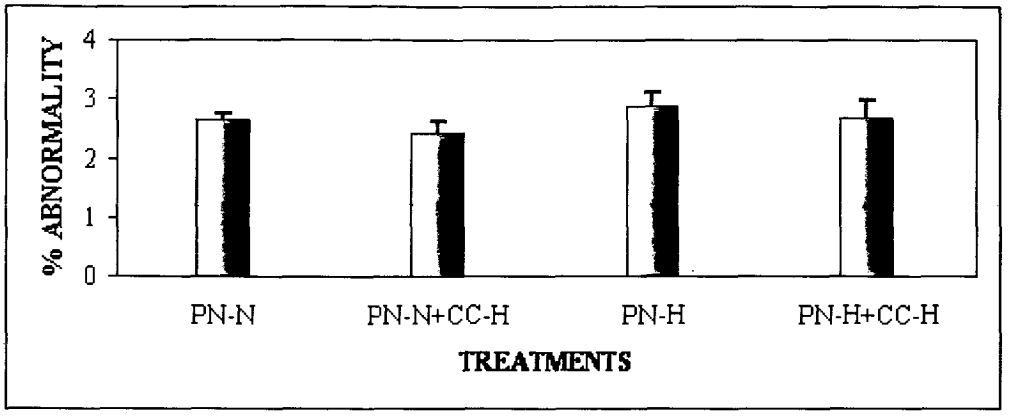
Note:- PN = *P. nigrum; CC = C. cyminum;* N = Normal; H = High.

Fig. 4.29. Graph showing combined effect of *P. nigrum* and *C. cyminum* on P/N ratio of bone marrow erythrocytes of *M. musculus*.



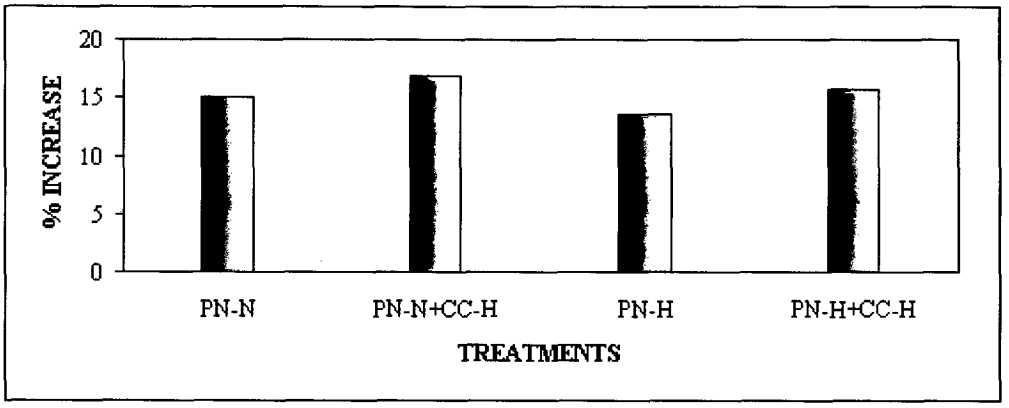
Note:- PN = *P. nigrum; CC = C. cyminum;* N = Normal; H = High.

Fig. 4.30. Effect of *C. cyminum* on sperm-shape abnormality induced by *P. nigrum* in *M. musculus*.



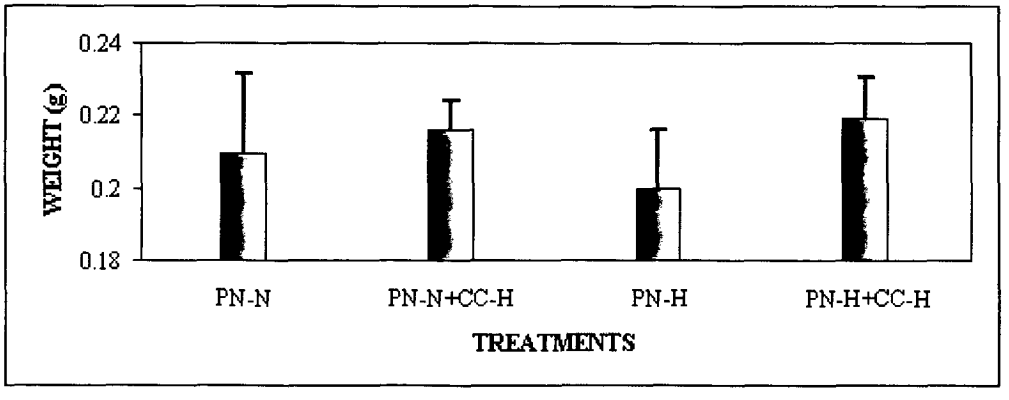
Note:- PN = *P. nigrum*; CC = *C. cyminum*; N = Normal; H = High.

Fig. 4.31. Illustrates effect of combined treatment of *P. nigrum* and *C. cyminum* on body weight of *M. musculus* after 5 weeks.



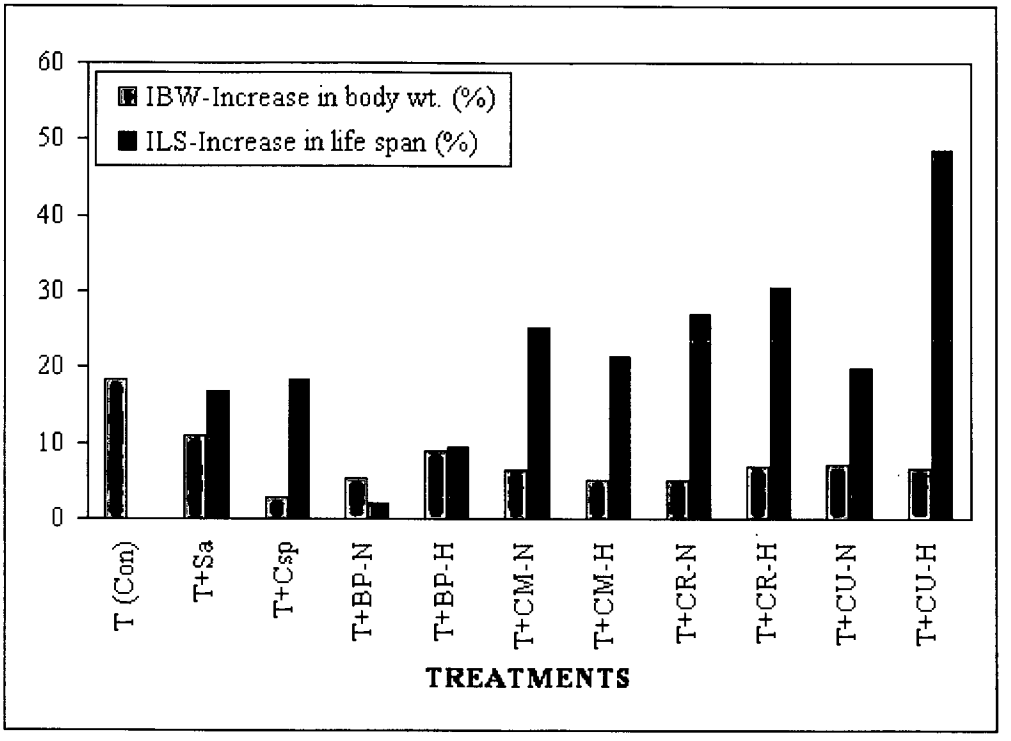
Note:- PN = *P. nigrum*; CC = *C. cyminum*; N = Normal; H = High.

Fig. 4.32. Illustrates effect of combined treatment of *P.nigrum* and *C. cyminum* on testes weight of *M. musculus* after 5 weeks.



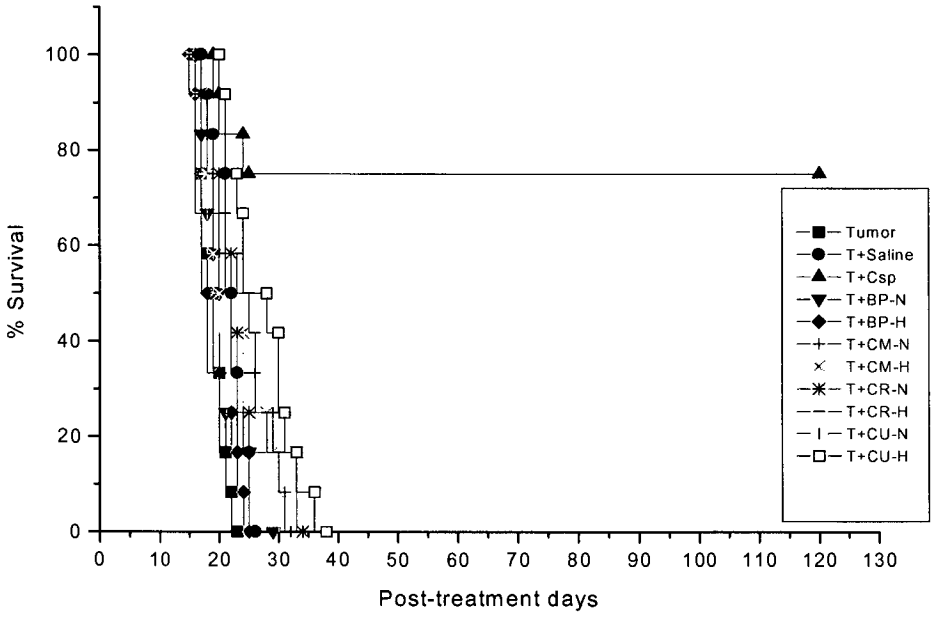
Note:- PN = *P. nigrum*; CC = *C. cyminum*; N = Normal; H = High.

Fig. 4.33. Illustrates effect of selected spices on IBW (after one week of tumour inoculation) and ILS of Ehrlich ascites tumour bearing *M. musculus*.



Note:- T = Tumour; Con = Control; Sa = Saline; Csp = Cisplatin; BP = Black pepper (*P. nigrum*); CM = Cardamom (*E. cardamomum*); CR = Coriander (*C. sativum*); CU = Cumin (*C. cyminum*); N = Normal; H = High.

Fig. 4.34. Kaplan-Meier survival curves for Ehrlich ascites carcinoma in *M. musculus* treated with selected spices and controls. [The values of cisplatin and cumin-H (*C. cyminum*) are significant compared to control (P < 0.001)].



Note:- T = Tumour; Csp = Cisplatin; BP = Black pepper (*P. nigrum*); CM = Cardamom (*E. cardamomum*); CR = Coriander (*C. sativum*); CU = Cumin (*C. cyminum*); N=Normal; H = High.

PLATE - IV

Chromosomal aberrations in bone marrow cells of *Mus musculus*:

(Mitotic chromosomes, 2n = 40) (Magnification: 10 x 100X)

a) Normal Metaphase Chromosomes

b) Chromatid Break (B) and Chromatid Gaps (G)

c) Chromatid Gap

PLATE : IV

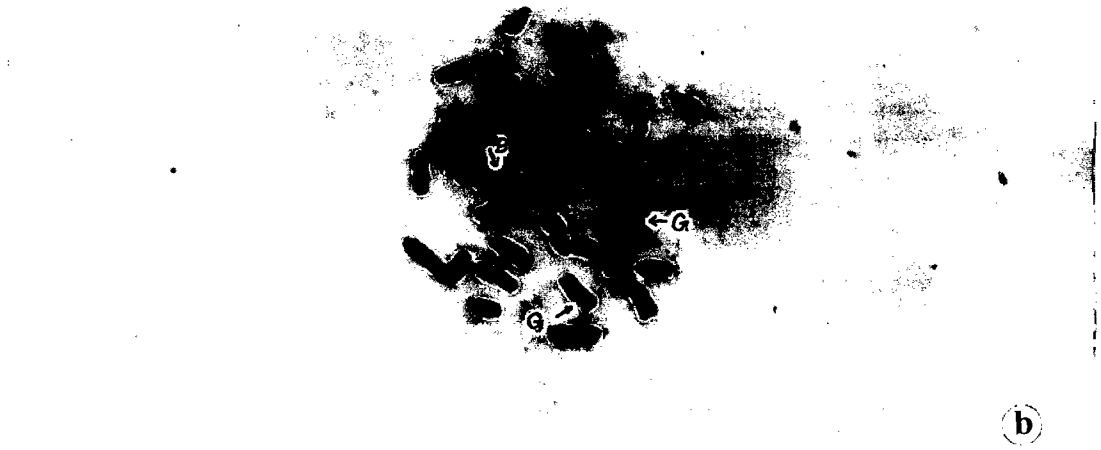


PLATE - V

Chromosomal aberrations in bone marrow cells of *Mus musculus*:

(Mitotic chromosomes, 2n = 40) (Magnification: 10 x 100X)

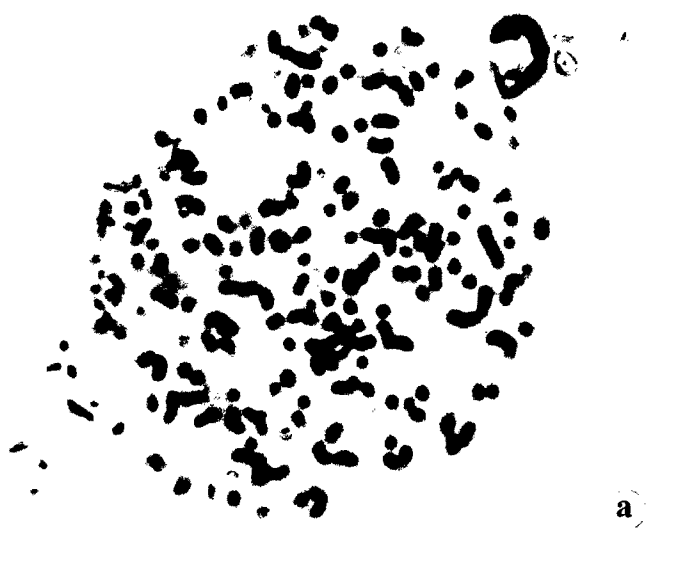
a) Fragments (Endoxan induced)

b) Centric Fusion

c) Ring Chromosome

d) Chromatid Exchange (Translocation)

PLATE : V



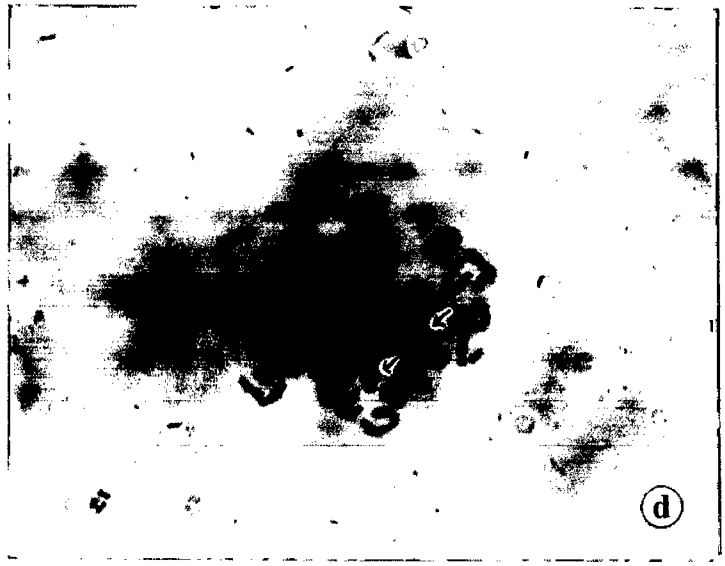


PLATE - VI

Micronucleus (MN) in bone marrow erythrocytes of *Mus musculus*:

(Magnification: 10 x 100X)

a) Normal Polychromatic Erythrocytes - PCE (P) and normal Normochromatic Erythrocytes - NCE (N)

b & c) MN in Polychromatic Erythrocytes

d) MN in PCE (P) and NCE (N)

PLATE : VI

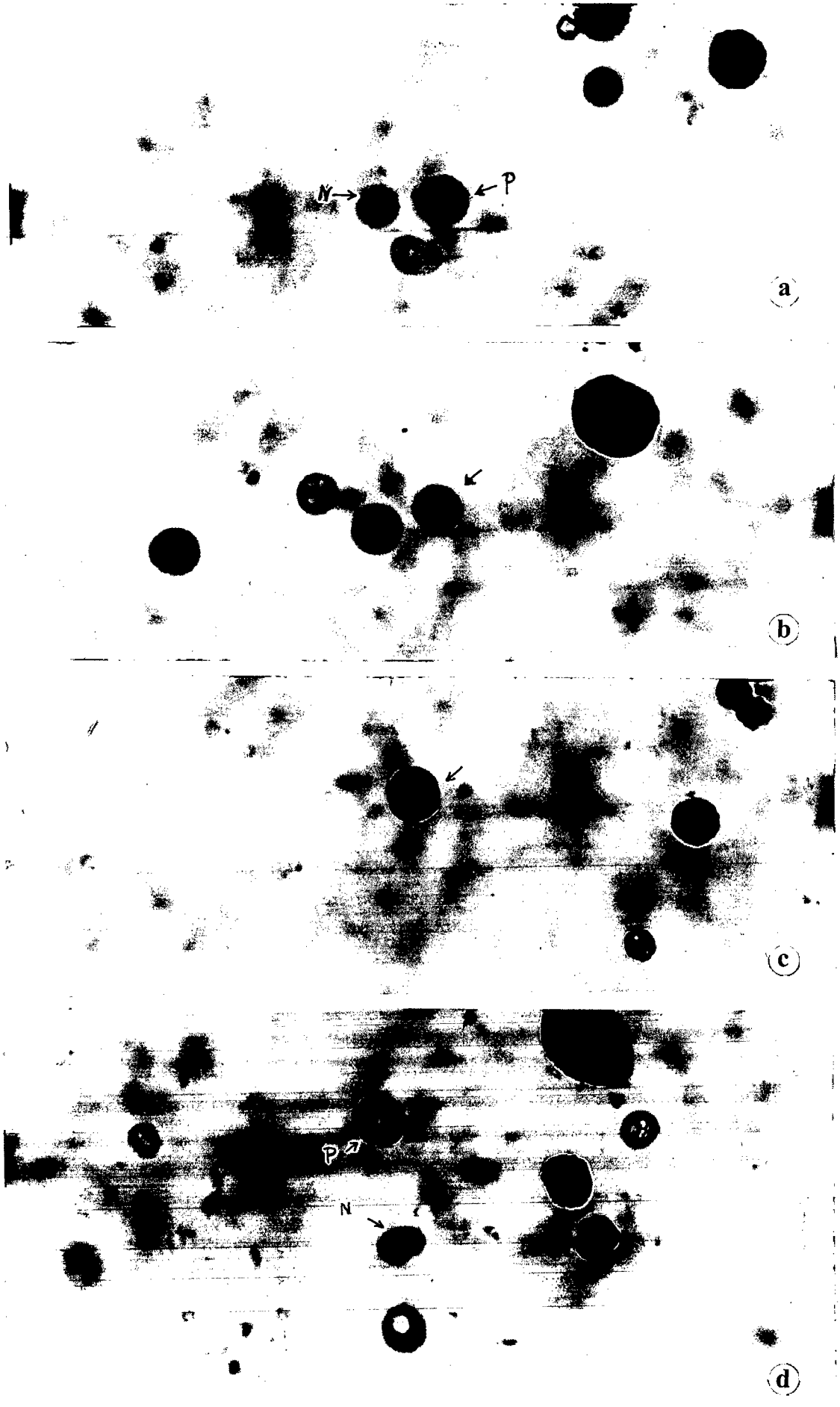


PLATE - VII

Sperm-shape abnormalities in *Mus musculus*:

(Magnification: 10 x 40X)

a) Normal sperms

b) Arrow head (Amorphous) sperm

c & d) Amorphous sperms

PLATE : VII

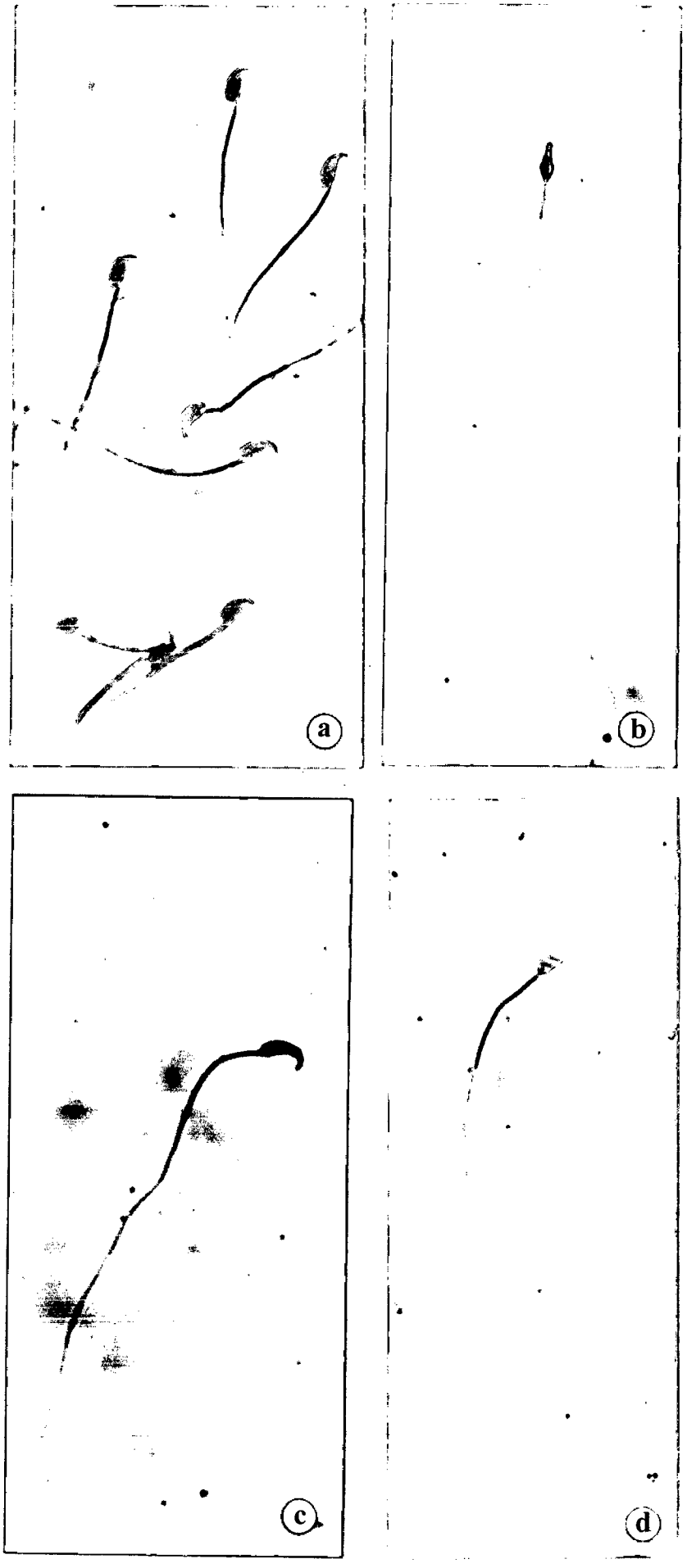


PLATE - VIII

Sperm-shape abnormalities in *Mus musculus*:

(Magnification: 10 x 40X)

a) Hookless sperm

b) Banana shaped sperm

c) Folded sperm

d) Sperm with amorphous head and double tail

PLATE : VIII

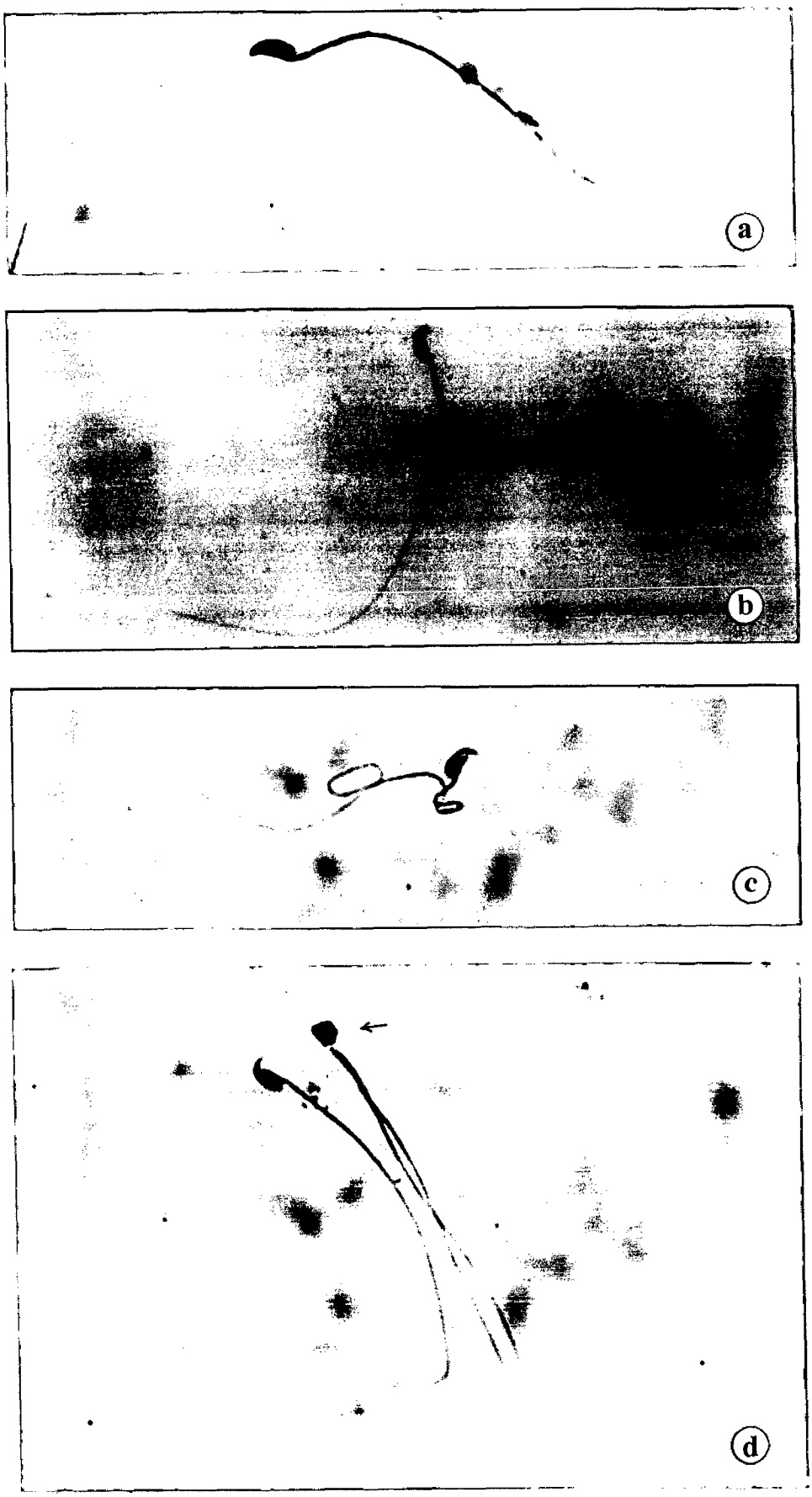


PLATE - IX

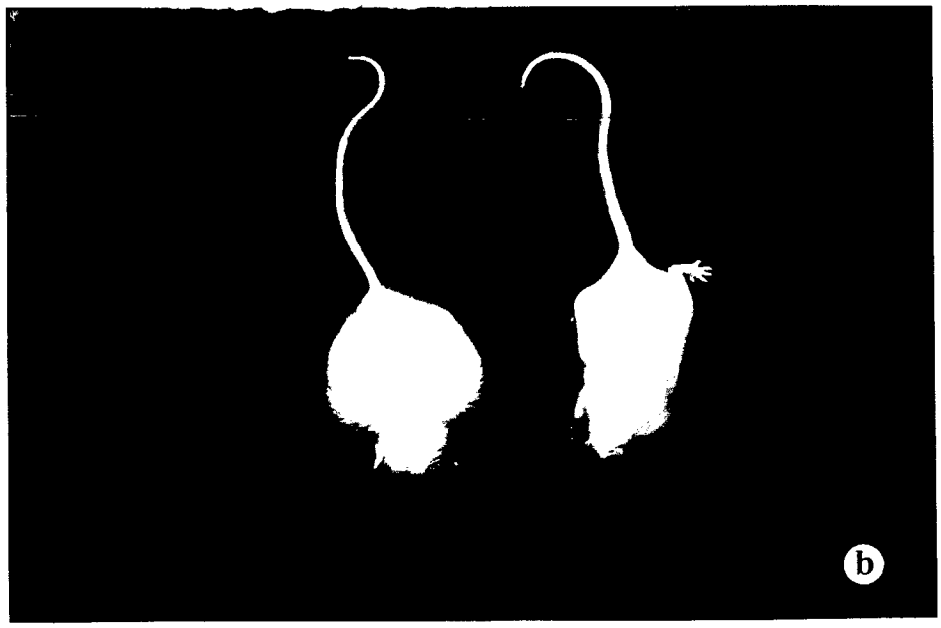
Ehrlich ascites carcinoma in *Mus musculus*:

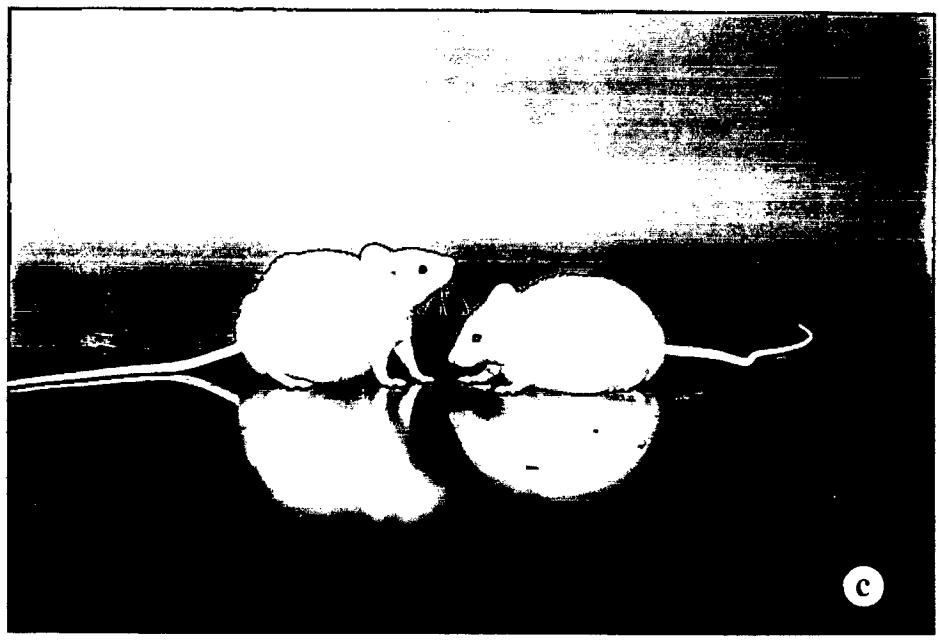
a) Tumoured mouse (Right) and Normal mouse (Left) – Front view

b) Tumoured mouse (Right) and Normal mouse (Left) – Top view

c) Tumoured mice – Side view

PLATE : IX





CHAPTER - V

DISCUSSION

The aim of limiting the frequency of induced mutations in somatic and germ cells deserve considerable attention not only for purely scientific reasons, but also for its possible significance in the protection of human health. This includes for instance, the extension of life expectancy and the improvement of the quality of life, as well as the prevention of many of those diseases which represent the prevalent cause of human mortality.

The present situation demands extensive work in the field of mutagenesis/ antimutagenesis. It has been shown that chemical carcinogenesis and mutagenesis can be inhibited by naturally occurring compounds of plant origin (Ames, 1983; Krishnaswamy and Polasa, 1995; Devasagayam and Tilak, 2002). Some of these natural antimutagens/anticarcinogens are known to be present in dietary vegetables/spices and epidemiological investigations have furnished an evidence for negative correlation between the intake of vegetables/spices and the incidence of cancer (Aruna and Sivaramakrishnan, 1990; Mitscher *et al.*, 1996; Shukla *et al.*, 2002). Ethno medicine is encouraged by the WHO in many countries because it is expanding rapidly and also an alternative solution to health problem (Anonymous, 1985). There is an urgent need to intensify research work in isolating, purification and characterization of the bioactive principle(s) from the traditional herbs/medicinal plants/dietary components which are responsible for curing the various diseases/ailments.

The large diversity of the plant kingdom, variety of dietary spices and the limited studies on this aspect have prompted us to undertake the present work to detect the mutagenic and/or antimutagenic properties of spices. Development of chromosomal aberrations (CA), micronuclei (MN) and sperm-shape abnormality have

been commonly used as sensitive biological indicator in the mutagenic bioassays of a drug (Giri *et al.*, 1998).

Four different spices – black pepper (*Piper nigrum*), cardamom (*Elettaria cardamomum*), coriander (*Coriandrum sativum*) and cumin (*Cuminum cyminum*) of common use in Indian diet were evaluated for mutagenicity, antimutagenicity and tumour reduction activity in this investigation. These spices have not only flavour/aroma but also have reported medicinal properties (Warrier *et al.*, 1995a,b). Among the four spices studied black pepper showed the positive result for mutagenicity whereas cardamom was moderately mutagenic. But in antimutagenic/ antitumour studies only cumin showed positive response.

5.1. MUTAGENICITY STUDIES

Before a substance is labeled as a putative antimutagen or anticarcinogen, it should be tested for mutagenicity or carcinogenicity with the same rigor. Many antimutagenic and anticarcinogenic chemicals also possess mutagenic and carcinogenic activities; some of which appear to be the predominant properties of the chemical and some activities manifest only under restricted test conditions (Zeiger, 2003).

In the present investigation mutagenicity of the alcoholic extracts of black pepper (*P. nigrum*), cardamom (*E. cardamomum*), coriander (*C. sativum*) and cumin (*C. cyminum*) was evaluated *in vivo* using Swiss albino mice *Mus musculus*. Chromosomal aberration assay, micronucleus assay and sperm-shape abnormality assay were used to screen the mutagenicity of these spices. The results obtained clearly indicate the ability of black pepper to induce mutation.

The reason for selecting chromosomal aberrations as the criterion in mutagenicity testing is mainly due to its obvious nature and relative simplicity. The rationale of using aberration is based on the reasons which includes visible change much of which are lethal to cell, which carries it and may also induce invisible changes having high probability of transmission and genetic consequences (Savage, 1975). Some authors regard gaps as non-specific defects. However, Gebhart (1970) showed that the appearance of gaps depends on the concentration of the test agent and the duration of the treatment. Hence, it is untenable to exclude gaps as non-specific defects. Hsu (1983) states that, since the frequencies of both breaks and gaps increase under the influence of mutagens, both should be considered as chromosome lesions without distinguishing them. However, in the literature it has become a common practice to represent the frequency of total aberrations both as 'including gaps' and excluding gaps' (Vijayalaxmi and Rai, 1996).

The results of chromosomal aberration assay showed statistically significant increase in abnormalities, without gap and with gap in mice treated with black pepper in a time and dose-dependent manner. A significant decrease in 'mitotic index' (MI) value indicated decrease in the rate of dividing cells with pepper treatment. This finding is in agreement with the earlier studies (Unnikrishnan and Kuttan, 1988; John and Abraham 1991). In cardamom treated mice statistically significant increase in aberrations were observed only when high dose was used. Coriander and cumin extracts did not show any mutagenic effect in chromosomal aberration assay and mitotic index values.

The micronucleus test studied, is an *in vivo* bone marrow test deviced by Schmid (1975) which measures the mutagenicity/clastogenicity. The formation of micronuclei which originate from chromatin and chromosome lagged behind during

anaphase could be detected easily in erythrocytes. In the present study high frequency of micronuclei were observed in the bone marrow erythrocytes of mice treated with normal and high doses of black pepper. This shows the mutagenic effect of black pepper and supplements to the result of chromosomal aberration test. A statistically significant reduction in P/N ratio confirmed the mutagenic nature of black pepper in mice. There was statistically significant increase in number of micronuclei and moderate decrease in P/N ratio in mice treated with high dose of cardamom. Significant increase either in PCE or in erythrocytes (PCE + NCE) was not observed in coriander and cumin treated animals.

Sperm-shape abnormality assay in mice was carried out in this study as an *in vivo* cytogenetic assay which is important in detection of those agents inducing mutations in germ cells. A number of germ cell mutagens have been reported by this test (Wyrobek *et al.*, 1983). Spermatogenesis in mice produce specific structural sperm cells whose head shapes have been shown to be under genetic control. Induced sperm abnormalities are due to altered function of gene(s) required for normal sperm development (Wyrobek, 1978).

In present study only black pepper showed statistically significant increase in abnormal sperms. However, insignificant increase of abnormal sperms was noticed in mice treated with high dose of cardamom. Other spices tested (coriander and cumin) showed negative results in the present investigation. The mechanism and control of sperm formation are of particular interest since it has been shown that the proportion of sperm abnormalities in individual males can be readily related to dose and time after exposure to various mutagens, carcinogens and teratogens (Wyrobek and Bruce, 1975). Negative results obtained may be either due to the non-availability or less availability of spices or its metabolites at the target sites. Percentage increase in body

weight was very less in high dose pepper treated animals. This indicates toxicity induced weight loss. But drastic increase in body weight was noticed in cumin treated mice. Coriander and cumin treated animals showed increase in their testes weight but in pepper treated mice it was decreasing trend compared to control animals.

Overall the mutagenicity study result indicated that the black pepper is highly mutagenic and cardamom is moderately genotoxic. High doses of all four spices studied induces maximum abnormality compared to other two doses. The different time intervals studied, the mutagenic activity of spices were maximum at 24 h and a decreasing trend was observed at later time intervals. However, this decrease in aberrations during later periods could be due to various possible reasons such as death of damaged cells, the clearance of spice from the body, post-replication repair process, etc. (Giri *et al.*, 1998).

Interaction between time and dose was not statistically significant. The effect of different time intervals did not depend on what level of dose was present. But in P/N ratio the effect of different time intervals depended on level of dose and statistically a significant interaction between time and dose was observed.

5.2. ANTIMUTAGENICITY STUDIES

In the present investigation antimutagenicity of the alcoholic extracts of cardamom (*E. cardamomum*), coriander (*C. sativum*) and cumin (*C. cyminum*) was studied *in vivo* using *M. musculus*. The results showed statistically significant protective effect of cumin against cyclophosphamide (CP) induced mutation.

In chromosomal aberration assay, cardamom enhanced the activity of CP by increasing percentage aberrations. This may indicate the synergic activity of cardamom with CP. Further, the dose-dependent decrease of total aberrations induced

by coriander, although in non-significant quantities may indicate its antimutagenic activity against CP induced mutation. However cumin, especially at high dose, significantly inhibited CP induced aberrations. Increase in mitotic index values of mice suggest a definite protective role of cumin. The comparative analysis of the frequency of MN in PCE, total % MN in erythrocytes and P/N ratio in the mice treated with CP alone and CP plus spices also showed similar result as noted for chromosomal aberration assay, supplements their synergic/antagonistic effect with CP. Study results of genotoxicity assay showed treatment with spice extracts (mixture of cinnamon, pepper, cumin, clove and cardamom) decreased micronuclei frequency in mice induced by urethane (Abraham *et al.* (1998).

Mice treated with CP developed various sperm head abnormalities. This suggests that CP could reach the germ line cells and indicates its potentiality as germ cell mutagen. An analysis of sperm-shape abnormality in mice treated with CP as a single agent and in combination with different spices showed statistically significant decrease in abnormality in the CP plus cumin treated group. Significant increase in body weight and testes weight were observed in cumin treated animals. These indicate the protective effect of cumin against the genotoxic potential of cyclophosphamide.

These results of all the three mutagenic parameters viz., chromosomal aberration test, micronucleus test and sperm-shape abnormality test in present study showing the significant reduction in CP induced genotoxic damage in presence of cumin clearly suggest the protective role of cumin on cyclophosphamide's mutagenic potential.

Cumin (which showed significant antimutagenic ability against CP) studied for its antimutagenicity against pepper (which showed mutagenic potential in mutagenicity screening) showed a decrease of chromosomal aberrations, micronuclei

and abnormal sperms. This proves the ability of cumin to inhibit pepper induced mutagenicity in mice. Our finding is support by the findings of Nagabhushan and Bhide (1986) where they showed antimutagenic action of curcumin against chilli and capsaicin in *Salmonella typhimurium* strains, in which they studied the spice to spice antimutagenic relationship.

5.3. ANTITUMOUR STUDIES

In the present investigation tumour reduction activity of black pepper (*P. nigrum*), cardamom (*E. cardamomum*), coriander (*C. sativum*) and cumin (*C. cyminum*) was studied in mice using Ehrlich ascites carcinoma. High dose cumin treated animals survived maximum number of days (MST 29, ILS 48%) among spice treated groups and was statistically significant compared to controls. But black pepper treated animals survived least number of days among spices (MST 20 & 21; ILS 2% & 9%). This result is contradictory to the findings of Unnikrishnan and Kuttan (1990). It may be because of difference in quantity of spice used for treatment. The spice used in our study is of higher dose and 7 days treatment period instead of their 50 mg/kg spice for 5 days. This indicates that the spice dose is important in tumour induction/reduction. Further in comparative study of oral administration and intraperitoneal injection, they observed that requirement of prior metabolism of the ingredient in spices for their antitumour activity. But our study is in agreement with El-Mofty *et al.* (1988, 1991) and Shwaireb *et al.* (1990). They reported black pepper induced tumour in Egyptian toad, *Bufo regularis*. It is already reported black pepper contains small amounts of safrole and large amounts of the closely related compound piperine (Ames, 1983). Safrole and tannic acid (constituents of black pepper) induced tumour during pre-weaning period in different organs of mice (Wrba *et al.*, 1992).

5.4. GENERAL DISCUSSION

From the above study it is observed that out of four spices-black pepper, cardamom, coriander and cumin which were used for mutagenicity study, black pepper showed mutagenic property. Coriander and hot water extract of black pepper showed non-mutagenicity and mutagenicity respectively in Ames test (Higashimoto *et al.*, 1993) Pepper increased number of revertants in Salmonella strains indicating mutagenic potential (Soudamini *et al.*, 1995). Cumin seeds are not mutagenic in Salmonella strains (Sivaswamy *et al.*, 1991). All these reports are in agreement with our findings that black pepper is mutagenic, coriander and cumin are non-mutagenic although the experimental systems are different. Earlier reports suggest that some spices have a significant chromosome breaking ability in mice bone marrow cells (*in vivo*) and *in vitro* cultured mammalian cells (John and Abraham, 1991; Unnikrishnan and Kuttan, 1988). Accordingly the induction of chromosome breaks by black pepper indicates that extract might have acted directly or indirectly upon DNA.

Maximum frequency of MN in PCE was observed at 24 h time interval for all doses and was decreasing at later time intervals shows that the mutagens or its metabolites may be not active for longer periods. The P/N ratio declined significantly in the case of CP and black pepper treated mice, suggesting suppression of the proliferative activity of the bone marrow. The possible explanation given for the reduction in P/N ratio is that, cell death as a consequence of chromosome breakage in the various nucleated cells creates a void in the marrow canal. The empty space is filled with blood. Freshly produced erythrocytes after maturation remain in the marrow instead of entering the peripheral blood stream. The greater the damage caused by the mutagen, the greater the relative proportion of NCE filling the bone marrow cavity (Schmid and Ledebur, 1973).

Elevated level of abnormal sperms is an indication of mutagenic potency of the test chemical. An agent that induces abnormal sperms can be expected to clearly interfere with the normal differentiation of germ cells (Wyrobek *et al.*, 1984). The percentage frequency of abnormal sperms induced by black pepper showed a dose related increase. The positive result with the sperm-shape abnormality assay indicate that this spice affects the sperm development and suggests its mutagenic potency *in vivo*. The compounds that induce increase in sperm head damage in mice are correlated with known germ cell mutational activity. This suggests that spices yielding positive results in sperm abnormality assay should be regarded as suspect germ cell mutagens in mammals. Chemicals which showed positive results in the sperm head abnormality test proved to be carcinogenic (Wyrobek *et al.*, 1983). Thus this test may also be considered as a prescreening test for carcinogenicity.

Among the three spices-cardamom, coriander and cumin which were selected for antimutagenicity study, cumin found to exhibit antimutagenic property against the genotoxicity induced by chemical agent cyclophosphamide (CP) and black pepper in bone marrow cells/erythrocytes/germ cells of mice at the tested concentration. This suggests that the cumin extract is containing certain component(s), which can exert inhibitory effect on the induction of *in vivo* chromosomal damage by the positive mutagens. Present investigation was confined only to the experiments with crude extract of spices and not with the individual components. Hence, it is not possible to attribute the 'antimutagenic' potency to any particular component. However, it is worthwhile to mention here that some of the components like fibres; vitamins; volatile oil constituents like cumaldehyde, pinene, dipentene, cumene, cuminic alcohol and terpeneol which are present in cumin seeds, may possibly be having important role in modulating the *in vivo* mutagenicity/carcinogenicity/tumour induction. There are

large number of reports on the protective effects of fibres and vitamins like ascorbic acid, riboflavin, etc. (Odin, 1997). Vijayalaxmi and Venu (1999) demonstrated the inhibitory effect of ascorbic acid on cyclophosphamide, mitomycin-C and belomycin induced clastogenicity in the mouse *in vivo* system. Giri *et al.* (1998) reported the antimutagenic potential of ascorbic acid against cisplatin induced mutation.

Out of four spices- black pepper, cardamom, coriander and cumin which were selected for tumour reduction study, cumin found to exhibit antitumour activity against Ehrlich ascites carcinoma. This may be partially due to the rich fibre content of cumin as per the report of Bronzetti *et al.* (1990) that the relationship between cancer and dietary consumption of fibres reduces the risk of cancer. Further, studies on cumin reported to increase the carcinogen-detoxifying enzyme, GST activity in mice, showing a protective effect against carcinogenesis (Aruna and Sivaramakrishnan, 1990). It can be concluded from the present study that cumin is effective in inhibiting the growth of Ehrlich ascites tumour in Swiss albino mice. A high dose treatment was more effective than normal dose treatment.

Present study shows there is no relation between potency (veerya) of spices and mutagenicity. Eventhough cardamom is 'cold spice' showed slight mutagenic effect. But the 'hot spice' cumin was antimutagenic. Hence the assumption that 'hot plant products may cause mutation' becomes contradictory and requires more such studies. This is in consistent with findings of Azizan and Blevins (1995) that there is no relationship between mutagenicity and the pungent properties of spices.

5.5. CONCLUSION AND RECOMMENDATIONS

We could derive following conclusions from the above study.

- Black pepper showed high mutagenic potential in all tests performed.

- Black pepper at 3.8 mg/kg is found to be safe.
- Cardamom is weakly mutagenic.
- Coriander and cumin are not mutagenic.
- Cumin showed antimutagenicity against cyclophosphamide induced mutation.
- Coriander is slightly antimutagenic against cyclophosphamide induced mutation.
- Cumin is antimutagenic against pepper induced mutation.
- Black pepper enhances ascites tumour in mice.
- Cumin increases survival time of mice bearing Ehrlich ascites tumour showing antitumour activity.
- There is no relation between potency (veerya) and mutagenicity/antimutagenicity of spices.

The positive response obtained in the present study indicates the genotoxic potency of black pepper in mouse bone marrow cells. Dietary intake of cumin may be recommended to protect against other mutagenic spices/environmental mutagens/ carcinogens. Further reduction in consumption of mutagenic spices like black pepper will save human beings from dietary mutagen/carcinogen. However Farg and Abo-Zeid (1997) reported that mutagenicity of safrole (a component of pepper) decreases during cooking.

Of all the environmental factors, dietary components appear to play an important role in the initiation/progression of the diseases. Dietary intervention for cancer prevention is needed to control the disease besides avoiding risk factors such as smoking and alcoholism and exposure to genotoxicants. Public education and awareness about the beneficial effects of consuming a healthy diet including plenty of fresh vegetables and fruits with spices such as cumin in adequate amounts to prevent

cancer are required. If the present study holds good for present society, it would be of importance from the view point of mutations and cancer to know more about the role of natural products and dietary agents/spices in modulating the mutagenic effects induced by environmental mutagens/carcinogens. The statement made by Paolini and Nestle (2003) that "providing the other lifestyle factors are taken into account, educational campaigns encouraging the consumption of fruit, fibre and greens can be welcomed and in general, we need to gain a clear understanding of the relationship between the health-promoting effects of certain dietary patterns and behavioural, economic, environmental and cultural determinants as well as of the molecular basis of these relationships" could be fully endorsed.

Our study supports that although there are few mutagenic principles in Indian food, there is still quite a large number of antimutagenic principles in the Indian diet that will nullify/modulate the activity of environmental mutagens. The natural antimutagens/anticarcinogens existing in human foods are probably safe. They are inexpensive and apparently good for people's health. More work is needed to evaluate the usefulness of spices as anti-promoters of human neoplasia.

Mutagenic/Antimutagenic/Antitumour properties of spices were investigated by using chromosomal aberration assay, micronucleus assay, sperm-shape abnormality assay and tumour reduction studies in Swiss albino mice *Mus musculus*. Four different spices - black pepper (*Piper nigrum*), cardamom (*Elettaria cardamomum*), coriander (*Coriandrum sativum*) and cumin (*Cuminum cyminum*) of common use in Indian diet were selected based on economic value and potency for the study.

Three different doses of spices i.e., Low, Normal and High doses were tested at four sampling times i.e., 12, 24, 48 and 96 h for mutagenicity. For sperm-shape abnormality only one sampling time i.e., 5 weeks was used. The different doses of the spices were administered orally to the experimental animals. Cyclophosphamide was used as positive control.

Spices showed non-mutagenicity i.e., cardamom, coriander and cumin were tested for antimutagenicity at 24 h time interval. Cumin which showed antimutagenicity was tested against pepper induced mutation. Tumour reducing activity of all four spices was studied using cisplatin as positive control in mice bearing Ehrlich ascites carcinoma for 120 days.

Among four spices tested black pepper showed mutagenic potential. Cumin showed antimutagenic ability towards cyclophosphamide induced mutation as well as pepper induced mutation. Further, cumin extract enhanced the survival time of mice bearing Ehrlich ascites tumour.

Thus in the present investigation black pepper proved as mutagenic spice and cumin as antimutagenic/anticarcinogenic spice.

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SOURCES OF CHEMICALS*/STAINS

Chemical	Source
Acetic acid glacial (CH_3COOH)	S.d. fine Chemicals Ltd., Mumbai, India.
Bovine serum albumin (BSA) fraction-V	Himedia Laboratories Pvt.Ltd., Mumbai, India.
Cisplatin ($Cl_2H_6N_2Pt$)	Neon Antibiotics Pvt.Ltd., Mumbai, India.
Colchicine ($C_{22}H_{25}NO_6$)	Himedia
Cyclophosphamide/Endoxan (ASTA) ($C_7H_{15}Cl_2N_2O_2P.H_2O$)	German Remedies Ltd., Goa, India.
Disodium hydrogen phosphate (Na_2HPO_4)	E-Merck (India) Ltd., Mumbai.
Eosin yellow (water soluble) ($C_{20}H_6Br_4Na_2O_5$)	Himedia
Ethanol Absolute (CH_3CH_2OH)	S.d. fine
Giemsa stain	S.d. fine
Glycerol ($C_3H_8O_3$)	E-Merck
Immersion oil (Cedar wood oil)	E-Merck
May-Grunwald's Stain	Himedia
Methyl alcohol (CH_3OH)	S.d. fine
Potassium dihydrogen phosphate (KH_2PO_4)	S.d. fine
Potassium chloride (KCl)	S.d. fine
Sodium azide (NaN_3)	S.d. fine
Sodium chloride (NaCl)	E-Merck
Sodium dihydrogen phosphate (NaH_2PO_4)	E-Merck
Trypan blue stain ($C_{34}H_{24}N_6Na_4O_{14}S_4$)	S.d. fine

Note:-* = Chemicals of Analytical Grade.