Chapter 1

INTRODUCTION

AND

REVIEW OF LITERATURE
1.1 INTRODUCTION

Cancer is disease entity, in which the fundamental rules of cell behavior break down, leading to uncontrolled cell division, causing formation of undesired growth of cells and tissues. Cancer cells usually have abnormal number or arrangements of chromosomes (aneuploidy), indicating a genetic instability that plays an important role in tumor development. This transformation may be triggered in a number of ways, including exposure to chemicals, certain viruses and radiation. In most cases the basis of transformation is probably a mutation event. Cancer cells contain many genetic alterations that accumulate gradually and ultimately give rise to tumor formation.

Cells in the human body perform well organized functions. The cells interact with food-born mutagens, drugs, cosmetics, insect and pest killers etc. These substances can sometimes cause tissue and or cellular damage resulting in the alteration to DNA structure. There is repair system of certain damages. Cell cycle halts cell division until repair of the DNA and avoids inheritance to next generation. Repair process can fix certain types of the damages. Single strand breaks repair can fix adducts depurination / depyrimidination, damage due to UV radiation etc. Double strand breaks can be repaired by non-homologous ends joining and sister chromatid homology. Here, in first case some information loss is there, on the contrary, in second case information cannot be lost. Due to such mutations it enables the cell to miss the checkpoints and cell become immortal. This allows cell to grow without any governance and regulation. Unregulated growth and proliferation of these cells result into either benign or malignant tumors.
Currently, natural plant products are being favored against the synthetic drugs for the treatment of cancer due to fewer side effects. It is also believed that the mixture of herbs is better alternative than single herbal drug for the treatment of cancers as they have the advantage of synergy & potentiation. Vitae Elixxir, a combination of nine herbs is being used under “alternate medicine therapy” as an anticancer agent against different types of human cancers.

Approximately 25,000 plant species are used for medicinal purpose around the globe (Phillipson, 1994). The practice of traditional herbal medicine is widely spread in India, China, Shrilanka, Japan and Pakistan. Very limited scientific studies were done regarding toxicity and efficacy of herbal medicine to support the exact use against disease. Today, there is a need of thorough scientific evaluation of all the medicinal plant and their therapeutic use (Zhu et. al., 2002)

No herbal medicine is safe. There are some adverse effects such as allergic reactions and/or reactions due to interaction of herbal drugs (Seth and Sharma, 2004). Adverse effects are nothing but abnormal, undesirable or harmful change after exposure to the herbal preparation. These adverse effects include alterations in food consumption, body weight, organ weight, gross pathological anomalies, clinical, biochemical, haematological disturbances, microscopic changes at the cellular levels.

The use of medicinal plants all over the world formed an integral part of primary health care (Akinyemci et al., 2005). Early humans were using herbal remedies come from Hippocrates (Amusan et al., 2002; Rivlin, 2001). Now a days
though there is modernization in allopathic drugs, the use of medicinal plants is progressively increased (Jadeja et al., 2011; Abere et al., 2010). More than 25% of modern pharmaceutical drugs have botanical origin. The herb foxglove is the source of digitalis and herb salicin is the source for aspirin. The breast cancer finding drug taxol (Tamoxifen) is extracted from yew tree (Eisenberg et al., 1998). It is believed that the herbal medicines are safe because they are natural, but they may cause toxicity to the patient (Ernst, 2007). Before treatment to human, the natural products should be assessed for their efficacy. Many of the herbs and spices used by human as a food ingredients (Tapsell et al., 2006). Now a days in India the trend is changed toward the traditional medicine. Therefore it has become a need to determine the safety and efficacy of plant products. The lifestyle of human almost all over the world is changed than the earlier time. In the past, humans were dying due to infections at early age. So they did not display a current scenario. The lifespan is increased. People live long life and expressed the symptoms of chronic diseases of old age such as obesity, diabetes, heart diseases and cancer (Orzechowski et al., 2002). In late 90s herbal medicines were in demand. In developed and developing countries herbal medicines had increasing popularity. Lots of research had been done to validate the herbal drugs (Soni et al., 2008). During standardization, the doses were adjusted to the active constituents of the drug. Herbal drugs are considered as a backbone of traditional medicine and used to treat all types of diseases.

Vitae Elixir is the extract of following plants and plant materials:-

Chlorophyllins -

Product of chlorophyll and alkali salts of metal ions after saponification along with plant material is called chlorophyllins. It can be obtained from grass, lucerne, nettle and other plant material is called chlorophyllins.

*Sanguinaria canadenisis*

It is commonly called as bloodroot. It is herbaceous perennial plant mostly found in hilly regions of Eastern United States (Ellingwood, 1919). In America it
is used by the herbal practitioners. The juice of *S. canadenisis* is in practice for the treatment of wide range of diseases from skin cancers to sore throats. It is used against ring worms, warts and fungal diseases. *S. canadenisis* is also used against asthma, bronchitis, cardiac problems such as palpitation of heart and lowering high pulse rate. Due to its nauseating taste it is used as expectorant. High consumption causes burning in the stomach, intestine, thirst, vomiting, faintness, vertigo etc.

The red sap of *S. canadenisis* has strong antibiotic and anti-inflammatory properties (Godowski, 1989). It also has antiseptic, diuretic, expectorant, sedative, stimulant and tonic properties. The herb can be used against emphysema, laryngitis, bronchitis, pharyngitis, sore throats, and croup (Haughton, 2001).

*S. canadenisis* also inhibit the formation of plaque and bleeding of gum (Allen et al., 2001). It is also used in the preparations of mouth wash/rinse and tooth pastes (Damm et al., 1999).

Researchers are currently finding out the selective property of blood root against cancer cells without harming normal cells. Primarily it has shown antiproliferative activity against human carcinoma cells (Ahmad et al., 2000; Foster and Duke, 2000). *Sanguinaria Canadensis* is also practice in veterinary medicine as deworming agent for cattle and sheep (Duval, 1997). In Germany blood root is used to prepare livestock feed.

**Hydrastis canadensis**

It is commonly called golden seal. It is used against cancer and to increase appetite (Foster, 2000). The plant is used in treatments for nasal catarrh, sore throat, tonsillitis, ear diseases, diphtheria, gonorrhea, vaginal and uterine leucorrhea, ulcers, skin disorders, breast cancer, intestinal catarrh, constipation, hepatic congestion, gastritis, acne, lupus, boils, nervous prostration, night sweats, etc. (Bergner, 1997). Currently it has been used as an antibiotic, immune-stimulatory, anticonvulsant, tonic and sedative, infections of eyes, ears, nose, throat, stomach, intestines, uterus and vagina etc. (Bradshaw, 1997; Foster and Duke, 2000). Physiologically it increases the blood supply to spleen, activates
macrophages and white blood cells, stimulates secretion of bile and bilirubin (Anon, 2000). The toxicity of _Hydrastis_ can cause nausea, diarrhoea, vomiting and depression (Russell, 1997). The accumulation of alkaloids causes adverse effects on the digestive system (Bergner, 1997).

It is medicinally used by Americans and Indians as herbal antibiotic and used in washing the inflammation of eyes, throat and alimentary canal (Lloyd & Lloyd, 1984-85). Root extract improves digestion, appetite and acts as tonic and laxative (Foster S. 1991).

**Hypericum perforatum**

The flowers and leaves are analgesic, antiseptic, antispasmodic, digestive, diuretic, nervine, sedative. It is used as tea substitute. Its flowers and leaves are used against nervous disorders such as depression (McIntyre, 2000). It was used in abortion, external ulcers, burns, wounds, sores, cramps, pulmonary complaints, bladder troubles, dysentery, hysteria, haemoptysis and other haemorrhages, jaundice, bowels of urinary passage, spinal injuries, sciatica, diarrhea, menorrhagia and bed wetting (Boobis et al., 1996). Externally it is used to dispel hard tumors, caked breast, ecchmosis (Diwu, 1995).

The biological active ingredients of _Hypericum perforatum_ such as pectin, choline, hypericin and pseudohypericin possess antiretroviral activity and used against AIDS (Daniel et al., 1988).

**Fumaria officinalis**

The species of _Fumaria_ plants are used in Algerian traditional medicine, in the cases of hepatobiliary and gastrointestinal disorders. It was reported that the plant has local reputation in India and Pakistan as anthelmintic, antidyseptic, blood purifier, diuretic, laxative, sedative and tonic. It is also considered as useful for the treatment of abdominal cramps, fever, diarrhoea, syphilis, and leprosy (Suau, et al. 2002). It maintains tonicity of the blood and cleans the blood.

It is mainly used in the liver obstructions. It is used in the treatment of irruptive skin such as eczema. The herb has antispasmodic and diaphoretic
properties. Toxic dose may cause hypnotic and sedative effects (Chopra et al., 1986).

**Rubus villosus**

The parts of plant such as leaves, fruits and bark of the roots are used in medicinal preparations. It has astringent and tonic property. It is also used against acute diarrhoea and mild infection of mucus membrane of mouth and throat (Hedrick, 1972).

**Ferula galbaniflua**

It was known for different medicinal properties such as stimulant, expectorant and antispasmodic, infections of the bronchial mucous membrane, amenorrhea and chronic rheumatism. It is applied externally like a plaster to indolent swelling. The oil has a very pleasant fragrance and used as an important scent for certain psychosomatic problems such as panic attacks caused by stress.

*Ferula galbaniflaua* has tumoricidal potency against in vitro malignant neuroblastoma (Blot, 1997). Hippocrates described its extraordinary curative powers and its earliest use as a traditional medicine known to man as a diuretic, carminative, antiseptic and anti-inflammatory herbal medicine. It was commonly used to treat bronchial afflictions and arthritis (Lin, 1998; Boon et al., 2000). Some species of genus has cytotoxic effect in human MCF7 breast cancer cell, lung and prostate carcinoma (Barthomeuf et al., 2008). Preexisting reports of *F. galbaniflaua* described antitumor properties (Elizabeth and Karam, 2010).

**Frasera caroliniensis**

The roots of *Frasera caroliniensis* are used in tonic preparations. It helps in improving appetite and resolves digestion problems. The root extract also helps to resolve the problems of constipation, colitis pains. It has peculiar characteristic features of helping in prolapsus and leucorrhea and similar problems in females. The extract improves appetite, digestion and also strengthens the biliary apparatus.
**Impatiens pallida**

It is also called jewelweed. The juice from the stems was used against hemorrhoids, warts, corns, jaundice and asthma (Britton, 1970). The juice also gives pain relief from insect bites, nettle stings, burns, sprains, ringworm, and various skin diseases (Keville, 1991).

**Allicin**

Garlic is the rich source of allicin. It acts as antimicrobial agent. Bacteria, fungi, protozoa and viruses are sensitive to crushed garlic preparations. Allicin is the by-product of garlic. Allin is stable precursor which converts allicin in presence of allinase (Ellmore and Feldberg, 1994). It acts as an antioxidant. It acts on sulphur groups in enzymes, proteins results and modify their activities. Allicin penetrates rapidly into cells through the cell membranes.

Allicin enhances the activity of phagocytic cells, natural killer cells and inhibits growth of pathogenic micro-organisms and certain cancer cells (Cavallito and Bailey, 1944).

**Garlic (Allium sativum)**

For centuries, the garlic is used as a medicinal herb. It is having wide range of medicinally important properties like, antimutagenic, anticancer, anti-inflammatory, antihypertensive, antimicrobial, antifungal, antidote, hepatoprotective, hyperglycemic, immunomodulation etc. (Agarwal, 1996; Das et al., 1995; German et al., 1995; Ip C. & Lisk 1995; Wen et al., 1995). Several studies in the recent years have shown the antigenotoxic and antimutagenic effects of garlic for various drugs and chemicals (Shukla and Taneja, 2002).

Regular intake of garlic reduces the risk of oesophageal, stomach and colon cancer. This was thought to be due to the antioxidant effect of allicin in reducing the formation of carcinogenic compounds in the gastro-intestinal tract (Josling, 2001). Garlic consumption of a garlic clove a day could be beneficial in preventing thrombosis. Anti-diabetic effects of garlic (Sheela et al., 1995) are well known. The oral administration of raw garlic clove a day showed glucose
intolerance, weight loss, depletion of liver glycogen in different studies in rats.

Most of the sulfur found in whole garlic cloves is of two types i.e. $S$-alkylcysteine sulfoxides and $\gamma$-glutamyl-$S$-alkylcysteines. The most abundant sulfur compound in garlic is alliin ($S$-allylcysteine sulfoxide), which is present at 10 mg/g in fresh or 30 mg/g dry garlic (Lawson, 1998). Garlic has been in use as medicine to treat leprosy, deafness, severe diarrhoea, constipation, parasitic infections, antipyretic agent, lowering blood pressure, food poisoning, tumors and as a mild anticoagulant (Bensky et al., 1993; Huang, 1999; Minyi, 1992).


In several studies in rabbits fed a high cholesterol diet, garlic or allicin supplementation significantly inhibited hypercholesterolemia, decreased tissue cholesterol, lowered low density lipoprotein (LDL) concentrations, raised high density lipoprotein (HDL) concentrations. Fresh garlic, garlic powder, aged garlic and garlic oil have demonstrated antiplatelet/anticoagulant effects (Ariga et al., 1981; Gaffen et al., 1984).
Sanguinaria canadensis  Impatiens pallida

Hydrastis canadensis  Ferula galbaniflua

Hypericum perforatum  Rubus villosus
In spite of clinical use of Vitae Elixxir no information is available on its toxicity and mutagenicity. The goal of this thesis is to check for the following aspects-
1. Acute toxicity of Vitae Elixxir by single treatment to find out its LD$_{50}$ in mouse
2. The mutagenic potential of Vitae Elixxir by itself using short term tests
3. The antimutagenic/ anticarcinogenic potential of Vitae Elixxir
4. Systemic toxicity of Vitae Elixxir with 28 days repeated treatment in mouse model
5. Antitumorogenic potential of Vitae Elixxir in mouse model

Figure 1.1: Herbs used in Vitae Elixxir
To fulfill above objectives, studies were designed on the basis of National and International guidelines such as OECD, Gaitonde Committee Recommendations (GCR) and DCGI.

1. The acute oral toxicity (LD$_{50}$) levels of Vitae Elixxir in mouse model.
4. In vivo micronucleus test in mouse bone marrow cells.
5. In vivo chromosomal test in mouse bone marrow cells.
6. Subacute oral (28 days) toxicity of Vitae Elixxir in mouse model.
7. Antitumor activity of Vitae Elixxir in mouse model.

Quantitative evaluation of the chemicals with respect to its toxicity after exposure to the biological system is the main goal of the study. All the chemicals are toxic under certain conditions of exposure. Human life is modernized that everyone is always in contact with the chemicals throughout the day and night. Starting from the morning i.e. use of toothpaste till night with mosquito repellants he gets exposed to the chemicals.

Short term toxicity began nearly a century ago when physician and pharmacologist were concerned with potent poison and drugs. The concept of the median lethal dose (LD$_{50}$) was first time introduced by Trevan in 1927 during the standardization of the Digitalis extracts, insulin and diphtheria toxins. He reported that the accuracy of LD$_{50}$ value was dependent on the factors, such as seasonal variation and number of animals used in a test. LD$_{50}$ is a statistically derived expression of single treatment of a test compound that can be expected to kill 50% of the animals (Gehring et al., 1973; Chan et al., 1982).

Toxicity of any chemical studied for a short duration with repeated treatment of 14 days, 28 days is called sub-acute test of that chemical. Such studies provide results of systemic toxicity such as absorption, distribution and excretion of the test compound. Such studies will conclude on physicochemical responses of the test compound, response on the target organs and finally the
deleterious consideration on biological system.

The objective of these studies is to determine the efficacy of the Vitae Elixxir as anticancerous agent. It is also to determine the dose response on biological systems, degree of hazards to the man.

Last few decades the scientists were working on the damage of genetic material. This damage through chemicals was studied under Genetic Toxicology. It is the study of interaction of chemical and physical agents with the process of heredity (Thilly and Howard, 1975). Human always exposed to thousands of organic and inorganic chemicals, pharmaceutical products, cosmetics, preservatives, ayurvedic preparations, pesticides and petroleum products that if not used with adequate dose may prove disastrous. Many of them are capable of altering the integrity of genetic material (Muller, 1927). Similarly there are number of naturally occurring compounds which are capable of altering the DNA, such chemical / substances are called mutagens since they bring change in the genetic constitution of the organism. Such chemicals enforced changes in the further generation leading to what are called mutation. There are many naturally occurring compounds which are known to be mutagenic and / or carcinogenic (e.g. mycotoxins in foods). It is utmost important therefore those chemicals to which people are exposed either intentionally (e.g. therapeutically) or inadvertently (i.e. pesticides) are to be studied for their potential to produce cancer and genetic damage (National Academy of Sciences, 1975; ICPEMC, 1983; EHC 51, 1985).

Some chemicals such as insecticide, pesticides, industrial intermediates, drugs and herbal preparations to which the population is exposed daily by any route are at times dangerous to human health. All these chemicals therefore need to be tested in the laboratories; with the routes to which human are exposed. The toxic levels further extrapolated to the result of human beings.

Rodents are the best models for laboratory studies due to the requirement of less space, short gestation period, large litter size and small life span. Large number of population can be available in short period of time and statistical evaluation can be made using reasonable number of animals. It was therefore
designed that the tests, which can give result in shortest possible time and also economic. Based on this, number of scientist evolved various tests using different animal and plant species to judge effect on the laboratory animals and possible effect on the human population (Frazer A.C. & Sharratt M., 1969).

Muller (1927) studied genetic mutability using radiations in the beginning of Genetic Toxicology. He was awarded Nobel prize in 1946 for his new approach in mutation studies. The most fruitful addition that Miller introduced into the mutation research was the concept of ‘mutation rate’. The term ‘mutation’ in genetically sense was used first by De Vries in 1901 (Adler, 1984). This term was used to sudden hereditary changes which were observed in the evening primrose ‘*Oenothera lamarikiana*’. Experimentally, mutagenesis by chemical agents was first achieved by Auerbach and Robson in 1944 (Adler, 1984). Significant increase in mutation by nitrogen mustard using sub mammalian species was reported in Drosophila which observed increase in induction of sex linked mutations from about 0.2% in the controls to as high as 24% in treated flies (Mitchell and Combes, 1984). After 20 years, Auerbach (1962) demonstrated genetic changes in animals, induced by radiation and chemicals. The group of several scientists showed co-relationship between mammalian carcinogens and mutagens (Bridges, 1973; Ames et al., 1975; Brusick, 1978).

Carcinogenic activity is determined on the basis of tumor formation ability of chemical to study in laboratory animals, may last for a year or two which is highly expensive. Researchers developed relatively inexpensive tests using biological system with less time in rodents which are referred as “**Short Term Tests**”. These are bacterial mutation test, genotoxicity studies using yeast cultures, unscheduled DNA synthesis in cultured mammalian cells, in vitro cytogenetic and sister–chromatid exchange, in vitro cell mutation assays, use of higher plants to detects mutagenic chemicals, Sex–Linked-Recessive Lethal assay (SLRL) in Drosophila; In vivo cytogenetic, dominant lethal assay etc (EHC 51, 1985). However considering all tests with their merits and demerits, for relevance as an indicator of mutagenicity and carcinogenicity in man a battery of tests need to be conducted and evolved (Brusick, 1982).
Anticancer agents are generally toxic and more precisely mutagenic. Considerable attention has been focused on identifying the anticancer and antitumor properties of the plant products. The efficiency of diet supplement in the prevention and treatment of cancer though controversial but hot topic in the research field. In the survey of some research it has proved that these supplements show some anticancerous properties. It is the challenge to design the research protocols and therapy as well. The treatments should be targeted cell specific. Till today none of the drug fulfills the criteria (Mazumder et al., 1997).

Several government agencies such as Food and Drug Administration (FDA, U.S.A.), Environmental Protection Administration (EPA), Occupational Safety and Health Administration (OSHA), Organization for Economic Co-operation Development (OECD, Paris), have outlined testing programmes for specific purpose (WHO, 1958).
1.3 BATTERY OF THE TEST SYSTEMS

To determine the effect of Vitae Elixxir on biological systems and to obtain data on the dose–response characteristics, a variety of test systems were used. The battery of the test system is used which provides the information on the degree of hazard to human and environment associated with its exposure.

Toxicity testing procedures have been developed by some scientists and government authorities such as WHO, OECD, ICH, FDA which included acute, subacute, sub chronic and chronic studies. Some short term tests (STTs) such as in vivo micronucleus test, in vivo/in vitro chromosomal aberration assay, sperm head assay and bacterial mutation test (Ames test), are also employed in such testing.

AMES SALMONELLA / MICROSOME ASSAY:

The data on more than 5000 chemicals have been published along with original methods of Ames test. Ames test has also been used to determine the mutagenicity of complex environmental and biological mixtures. The data generated by Ames Salmonella test have been shown subsequently to be carcinogenic in animal test.

The Ames Salmonella test was first validated in a study of 300 chemicals most of which were known carcinogens (Mc Cann et al., 1975a; Mc Cann & Ames, 1976; Mc Cann & Ames, 1977). It was subsequently validated in studies by the Imperial Chemical Industries, the National Research Agency in Tokyo (Sugimura et al., 1976) & International Agency for Research on Cancer (Bartsch et al., 1980). Nearly 90% of the carcinogens tested were mutagenic in the studies.
Ames test is very short duration test as far as time span is considered. Within 48 hours the test compound can be tested for its mutagenicity. The space required is very limited, just a laminar flow unit and preparation room. By considering its quickness, instant results, reliability of the data generated and economy, the test was selected as the primary screen. This test is now widely accepted for testing the suspected mutagens or carcinogens.

**ACUTE TEST**

Acute test is short duration test and a single treatment dose with 14 days observation period. It is also valuable test and confirms the dose levels for further testing such as subacute and chronic tests. Thus acute studies are often called “first line of defense” in the absence of data from long term studies. Acute tests are classified on the basis of route of administration of the test article. The acute tests are oral, intra-peritoneal, inhalation, dermal, intra-muscular, subcutaneous, primary skin and mucous membrane irritation. The implementation of the route is depending on the administration of the drug in the human and/or animal use or accidental route of exposure. Vitae Elixxir is the oral supplementary drug for breast cancer patient; therefore, the route chosen is oral intubation. Acute oral toxicity study is the initial step in identifying the toxicological properties of a substance. It provides information on health risks resulting from a single treatment dose through a defined route and serve as a basis for the classification of the test article. It permits the selection of optimum dose levels for toxicity studies with repeated administration of the test substance (Society of Toxicology of Canada, 1985).

An acute toxicity study determines the median lethal dose ($LD_{50}$) of the compound (Curtis & Doulls, 1975). Acute studies are determining median lethal dose ($LD_{50}$) of a test substance. $LD_{50}$ is not absolute biological constant to be equated. $LD_{50}$ is only one of many indices used in defining acute toxicity. It is statistically derived expression of a single dose of a test material that can be expected to kill 50% of the animals (Gehring et al., 1973; EHC 6, 1978). It is also
defined as the adverse effect occurring in a short span of administration of single
dose or multiple doses given in 24 hours (Hagan, 1959).

When the data is unavailable and compound is new, acute toxicity studies
are indicated to the relative toxicity of the compound, to investigate its mode of
action, signs of ill health due to toxicity of compound, body weights, gross
pathological changes if any due to test compound.

The LD\textsubscript{50} being a calculated value is always accompanied by some
estimation of the error of the value such as the confidence limits. The most
commonly used methods for calculation of the LD\textsubscript{50} are the graphic method of
Litchfield & Wilcoxon (1947), the logarithmic probit graph paper method of
Miller & Tainter (1944) & the method of moving averages of Thompson (1947)
& Weil (1952). LD\textsubscript{50} comes under the heading of acute toxicity.

The deaths which occurs after the first 24 hours, may give some indication
of the effect that, the chemical may have effect at lower levels, when administered
for longer time periods. The doses are selected to provide data for estimating LD\textsubscript{50}
and to obtain information on the slope of the dose response curve. Doses are
selected in logarithmic progression (Weil, 1952).

**SHORT TERM TESTS TO DETECT GENETIC DAMAGE**: -

Genetic damage produced by test compound is mutation to cell death. It is
therefore very important that suitable test procedures should be employed for
detecting all possible damage at different genetic levels (Obe et al., 1982). In
present study genetic damage is considered at two levels, namely gene mutation
and chromosome anomalies for which, test procedures are Ames
*Salmonella*/microsome assay, modulation studies, in vivo chromosome aberration
analysis in mouse bone marrow and in vivo micronucleus test in mouse bone
marrow.

**CYTOGENETIC TESTS IN MAMMALS**: -

Generally Micronucleus test (MNT) and Chromosome Aberration test
(CA) are the two in vivo and in vitro short term tests which are generally chosen
for testing the clastogenicity of test substance. The main drawback of in vitro tests is the metabolic process, distribution and excretion characteristics of mammals are not readily taken into account. In vivo genetic toxicity tests are carried out using small number of animals. This test include all these factors are equally rapid but more costly. Mice, rats and Chinese hamsters are the most commonly used species for in vivo mutagenicity tests.

After several treatments of test compound by oral gavage, the tissues are collected after an appropriate interval. The samples are evaluated by the analysis of metaphases for chromosomal aberrations and the assessment of micronucleus frequencies. Most commonly both techniques are applied to bone marrow, however spleen, kidney, liver, whole embryo, total organs and germ cells have been also used (Adler, 1984; Schmid, 1975).

Screening of Vitae Elixxir for its mutagenicity by the short term tests the criteria considered are time duration, reliable data generation, less space, less expensive and acceptance of the data world wild.

If the results obtained from Ames test are borderline or positive then there is subsequent need to use short-term tests, such as micronucleus test and chromosome aberration test model. The results obtained from these studies could be extrapolated to human with more care. Micronucleus test is fairly quick to determine the clastogenicity of the test article. The other test selected is chromosomal aberration test. This is the short-term test which requires utmost technical expertise and takes more time to screen the metaphases to determine aberrations.

The aberrations observed in metaphase cells are basically of two types i.e. chromosome and chromatid type aberrations. Chromosome type aberrations are those in which alteration results from damage and expressed in both sister chromatids at the same time. Chromatid type aberrations are those in which change is expressed as break in single chromatid break and reunion between chromatids.
A. Chromosomal type aberrations: -

Chromosomal type aberrations are deletion, inversion, acentric and centric rings and interchange

i. **Deletion** is a single break in chromosome during ‘G1’ phase perpetuates in ‘S’ phase and affects both chromatids in following metaphase. This single break may result in a detached portion of the chromosome. Very small fragments are called as minutes.

ii. **Inversion** is the breaking event represents interchange with in a chromosome in which the deleted fragments rejoins the same chromosome in the inverted position.

iii. **Acentric & centric rings** are the breaking events in the same chromosome and fused sticky ends results in the formation of either centric rings (with centromere) and a fragment or an acentric ring (without centromere) and a fragment.

iv. **Interchanges** are of two types, symmetrical interchange and asymmetrical interchange. In symmetrical interchange exchange occur in two chromosomes while in asymmetrical interchange gets exchanged in two chromosomes without loss of any chromosomal material (Savage & Papworth, 1982).

B. Chromatid type of aberrations:-

1. **Chromatid break** is a single break which yields a deleted chromatid and a fragment.

2. **Isochromatid breaks** occur in both the chromatids in the same position resulting in a deleted chromosome and two acentric fragments.

3. **Achromatic lesions or gap** appears as a small unstained and constricted region in the chromatid, which may later develop into an aberration. Gap length is not more than the width of chromatid (Evans, 1962; Natrajan and Zwanenburg, 1982).

These three tests of mutagenicity are interdependent and help in deriving
the conclusion at the end by combining the results of the studies (Lloyd et al., 1980; Ashby, 1983).

**SUBACUTE TOXICITY TESTS :**

These studies are of shorter duration than sub chronic studies. Generally the duration of these studies is either 14 or 28 days with administration of drug on daily basis. The choice of the study duration is dependent on many factors. Generally a 28 days study has greater chances of producing more valuable information than 14 days study (Chan et al., 1982). The longer exposure increases the possibility of detecting effects associated with the test material. Secondly dose selection is somewhat dependent on the purpose of the study. If the short term dosing is designed to produce information to be used in the design of sub chronic study then dosing should ensure that any potential adverse effect would be observed. Higher doses generally are used in short term studies to determine target organs and it may not be as critical so as certain a No Observed Adverse Effect Level (NOAEL) (Paget & Thomson, 1979).

However it is always useful to have a NOAEL in a toxicology study. If no further studies are planned on the compound, the dose range that will include a NOAEL becomes much more important since little information generally is available other than acute toxicity data. It may be necessary to run a more comprehensive range of dose levels in short term repeated dosing studies than in sub chronic studies. The most common routes of administration in shorter term toxicity studies are dietary or oral intubation. If the chemical is a pharmaceutical product, intended for oral administration to humans; the chemicals should be administrated by oral intubation during sub chronic studies (EPA, 1983).

**ANTITUMOR ACTIVITY :**

To screen Vitae Elixxir another in vivo model used is antitumor activity. This study predicts direct activity of the compound to the targeted organ. In antitumor activity the test substance is compared with standard drug of same activity. Activity of Vitae Elixxir was compared with Standard drug
Cyclophosphamide (CP). It is used against variety of human cancers. In animal models it has modulatory activity and chemoprotective too. It is an alkylating agent used in cancer chemotherapy (Garaci, et al., 1990; Dorr, 1991). It is used with different dosage and the response of the individual. It can also be used along with radiations and or other therapies (Indap et al., 1986). There are many references on use of CP as potent anticancer drug. It is used against variety of cancers. Administration of CP at 250 mg/kg body weight in Balb C mice increases the life span causing slight reduction in body weight in leukemic mice.

Though it is time consuming test requires more than a month and more animals are involved, lots of data will be generated to get valuable results. The animals will be subjected to known cancerous cells and treated with test article along with the drug control. Such study gives us dose dependent results and can be extrapolated considering all the parameters such as body weights, food consumption, haematology, organ weights, spleen index, increased life span etc.