INTRODUCTION

Cancer is one of the most dreaded disease of human beings and is a major cause of death all over the globe. More than a million Indians suffer from cancer and a large number of them die from it annually. The mechanisms that underlie development of cancer or oncogenic transformation of cells, its treatment and control have been some of the most intense areas of research in biology and medicine.

In our body, cell growth and differentiation is highly controlled and regulated. In cancer cells, there is a breakdown of these regulatory mechanisms. Normal cells show a property called contact inhibition by virtue of which contact with other cells inhibits their uncontrolled growth. Cancer cells appear to have lost this property. As a result of this, cancerous cells just continue to divide giving rise to masses of cells called tumors. Tumors are of two types: benign and malignant. Benign tumors normally remain confined to their original location and do not spread to other parts of the body and cause little damage. The malignant tumors, on the other hand are a mass of proliferating cells called neoplastic or tumor cells.

These cells grow very rapidly, invading and damaging the surrounding normal tissues. As these cells actively divide and grow they
also starve the normal cells by competing for vital nutrients. Cells sloughed from such tumors reach distant sites through blood and wherever they get lodged in the body, they start a new tumor there. This property called metastasis, is the most feared property of malignant tumors. As a malignant tumor grows, it damages nearby tissue. Some cancers, like leukemia, do not form tumors. Instead, these cancer cells involve the blood and blood-forming organs and circulate through other tissues, where they grow.

Cancers are classified according to the types of cells in which they develop. Most human cancers are carcinomas, which arise from the epithelial cells that form the superficial layer of the skin and some internal organs. Leukemia affect the blood and blood-forming organs such as bone marrow, the lymphatic system and the spleen. Lymphomas affect the immune system. Sarcoma is a general term for any cancer arising from muscle cells or connective tissues.

Cancer is a group of disease with similar characteristics, which can occur in all living cells in the body and different cancer types have different natural history. The myth that cancer affects people mostly in developed countries is being broken by the fact that, of ten million new cancer cases seen each year worldwide, nearly 5.5 million are in the less
developed countries. Cancer is the second most common cause of death in the developed world and a similar trend has emerged in the developing countries too. Cancer is, in general, more common in industrialized nations, but there has been a growth in cancer rates in developing countries, particularly as these nations adopt the diet, lifestyle and habits of industrialized countries. Over one million people in the United States are affected by the cancer every year. Anyone can be affected by the cancer at any age however, about 80 percent of all cancers occur in people over the age of fifty-five. Cancer prevalence in India is estimated to be around 2.5 million, with over 8,00,000 new cases and 5,50,000 deaths occurring every year due to this disease. More than 70% of the cases report for diagnostic and treatment services in the advanced stages of the disease, which has lead to a poor survival and high mortality rate.

Among men, lung, esophagus, stomach, oral and pharyngeal cancers are more prevalent, while among women, cancer of cervix and breast are most common, followed by those of stomach and esophagus. (Stewart et al., 2003; Cancer Registry Abstract, 2001; WHO Report, Geneva, 1997; Murthy NS et al., 2004.) Different cancers occur in different states of our country-
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- Esophageal cancers: Southern states of India like Karnataka and Tamil Nadu and also in Maharashtra and Gujarat.
- Stomach cancers: Southern India with the highest incidences in Chennai.
- Oral cancers: Kerala (South India)
- Pharyngeal cancers: Mumbai (Western India)
- Thyroid cancers among women: Kerala
- Gall bladder cancer: Northern India, particularly in Delhi and West Bengal.

Recent times have seen an increase in the incidence of cancer. This is mainly attributed to urbanization, industrialization, lifestyle changes, population growth and increased life span (in turn leading to an increase in the elderly population). In India, the life expectancy has steadily risen from 45 years in 1971 to 62 years in 1991, indicating a shift in the demographic profile. It is estimated that life expectancy of the Indian population will increase to 70 years by 2021-25. This has caused a paradigm shift in the disease pattern from communicable diseases to non-communicable diseases like cancer, diabetes and hypertension.

There is a marked variation among countries in incidence of different cancers. Most of the variation in cancer risk among populations,
and among individuals, is due to environmental factors, such as cigarette smoking and certain dietary patterns, that can affect one's risk of developing cancer. For example, individuals living in Australia have the highest worldwide lifetime risk of skin cancer, at over 20 percent, due to the high level of exposure to the sun of people in Australia. People in India have twenty-five times the average risk of developing oral cancer sometime during their lives due to the popularity of chewing tobacco in this country. In fact, India has the world's highest incidence of oral cancer, with 75,000 to 80,000 new cases a year. The population of Japan has the highest rates of stomach cancer in the world due to the high consumption of raw fish by the Japanese. (American Cancer society, 2010; Chow et al., 2010).

Overall environmental factors defined broadly to include tobacco use, diet, infectious diseases, chemicals and radiation are believed to cause between 75 to 80 percent of all cancer cases in the United States. Tobacco use, including cigarettes, cigars, chewing tobacco and snuff can cause cancers of the lung, mouth, throat, larynx, bladder, kidney, esophagus and pancreas. Smoking alone causes one-third of all cancer deaths in the United States. Heavy consumption of alcohol has also been
shown to increase the risk of developing cancer of the mouth, pharynx, larynx, esophagus, liver and breast.

The exact cause of cancer is not known. Most cancers result from permanent damage to genes or from mutations which occur either due to internal factors such as hormones, immune conditions, metabolism and the digestion of nutrients within cells or by exposure to environmental or external factors. A chemical or other environmental agent that produces cancer is called a carcinogen.

Several infectious agents have also been implicated in development of cancer. Evidence suggests that chronic viral infections are associated with up to one-fifth of all cancers. These include Hepatitis B virus (HBV), which can lead to cancer of the liver, the Epstein-Barr virus, a type of Herpes virus that causes infectious mononucleosis and has been associated with Hodgkin's disease, non-Hodgkin's lymphomas, and nasopharyngeal cancer, the human immuno-deficiency virus (HIV) and a bacteria known as *Helicobacter* species which is associated with an increased risk of developing several cancers, especially Kaposi's sarcoma and non-Hodgkin's sarcoma.

A carcinogen is any substance, radionuclide or radiation, an agent directly involved in the exacerbation of cancer or in the increase of its
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propagation. This may be due to their ability to damage the genome or to the disruption of cellular metabolic processes. Several radioactive substances are considered to be carcinogens, but their carcinogenic activity is attributed to the radiation, for example gamma rays and alpha particles, which they emit. Common examples of carcinogens are inhaled asbestos, certain dioxins and tobacco smoke.

There are many natural carcinogens such as Aflatoxin B$_1$, which is produced by the fungus *Aspergillus flavus* growing on stored grains, nuts and peanut butter is an example of a potent naturally-occurring microbial carcinogen. Certain viruses such as Hepatitis B and human papilloma viruses have been found to cause cancer in humans. The first one shown to cause cancer in animals is Rous sarcoma virus, discovered in 1910 by Peyton Rous. Co-carcinogens are chemicals that do not necessarily cause cancer on their own part, but promote the activity of other carcinogens to cause cancer. A pro-carcinogen is a precursor of a carcinogen. One example is nitrites when taken in diet. They are not carcinogenic themselves, but turn into nitrosamines in the body, which are carcinogenic.

Much a bane of the modern civilization, the accumulation of natural hazards which include toxic chemicals, carcinogens, nuclear
radiation etc. in environment and their exposure to human leads to cancer. The number of affected populations varies among different countries, regions and others social groups and globally it is estimated to affect more than five percent population. The condition afflicts both men and women in almost every age group and even children are not spared. In absence of restricting the use of such chemicals the only remedy remains is the estimation of least potentiality of carcinogenic compounds and proper bio-monitoring using suitable living animal models. Both genotoxic and non-genotoxic modes of action leading to carcinogenesis have been hypothecated using different biological models.

Genotoxic evaluation of suspect environmental mutagens is done by employing different types of experimental models and the data generated from the basis of the warning system to establish the range of toxicity of mutagens(Wild,1975; Menzel,1994).Currently, a variety of protocols are available for screening the mutagenic potential of commonly used physical and chemical agents(Gillet,1970;Corbett,1974; Sobel,1974; Matsumura,1975; Evans, 1977; Gaulden &Liang,1982; Hsu,1982; Hallenbeck & Cunningham-Burns,1985; Sharma et al.,1985; Menzer,1987; Hayes & laws, 1990; Anderson and Conning, 1993;

Mutation in the genomic DNA generally arise during replication that lead to gene mutations and in later stages, numerical and structural changes also take place at chromosome level. Because of this, the study of induced chromosomal aberrations is now a widely accepted parameter for evaluating the chromo-toxicity level of suspect chemicals or radiations (Kilhman, 1966; Evans, 1977; Kumari & Krishnamurthi, 1986; Adhikari & Grover, 1988; Chaudhry & Grewal, 2000). As polytene chromosomes have giant size, their use for observing the targeting effect of a mutagen has been widely recommended (Rabbani & Kitzmiller, 1974; Sharma et al., 1985; Chaudhry & Grewal, 2000; Chaudhry & Anand, 2005).

The -amide group of chemicals have recently been reported to cause mutations which ultimately are the leading cause of malignant cancers. Different living animal models are being used for bio-monitoring of many toxic chemicals including mammals. But in view of the extreme cost and labor involved, there are only a few laboratories in the world where these investigations can be carried out. In the light of these considerations, the possibilities afforded by Drosophila for detecting
various class of mutagens and evaluation of its effect in producing carcinogenic effects, are considerable. Due to short life span, known genetics, morphology and developmental stages, easy to rear in lab, cost effectiveness and ability of the *Drosophila* to permit the simultaneous assessment of spectrum of changes in comparison to other test system which allows only one class of genetic damage, the *Drosophila melanogaster* provides most advantageous test system. Recently, additional advantage in the capacity of *Drosophila* for metabolic activation in verification of carcinogenicity resembling to mammalian liver system have been reported (Sobel & Vogel, 1976) and hence enhances the potentiality of *Drosophila melanogaster* as a suitable biomarker for such evaluation studies.

In the present study, both genotoxic and non-genotoxic mode of action of three -amide group of chemicals by investigating extensively with three different parameters of developmental, morphological and chromosomal changes have been undertaken for the first time using *Drosophila melanogaster* as living biological model for its carcinogenic potentialities.
Brief review of the work already done in the field:

Extensive use of various chemicals, heavy metals, nuclear radiations, food adulterants and dyes have caused serious concern for human health and wild life. Many of the chemicals used in daily life, when exposed to human, may cause mutagenic changes leading to tumor and malignant cancers. Amide group of chemicals are one of those chemical which have carcinogenic properties and carcinogenic properties of some of them have already been established.

Regulatory measures with all the consequences for society, should not be based on data obtained with a single assay system, but would require the conformation after the application of a battery of different test system including human being. The recent findings treat *Drosophila melanogaster* is capable of carrying out some of the metabolic activation reaction as the mammalian liver (Sobels and Vogel, 1976) ,makes the organism eminently suitable for verifying carcinogenic effect of test chemicals under observation.

Recently -amide group of chemicals have been tested on some animal model for their mutagenic and carcinogenic properties. The results are positive in most of cases and are of great concern due to their ultimate carcinogenic effect on human and may cause tumor and malignant
cancer. Since these group of chemicals are being extensively used all over the world, in absence of restricting their use, the problem arise thereby is in evaluating toxic potential, both mutagenic and carcinogenic and have an idea of their safe limits and restricting carcinogenic effects on human being.

From the point of view of genetic hazards, transmissible point mutations and small deletions deserve probably the highest priority particularly the induction of these changes correlates with the risk of carcinogenesis (Sobels and Vogel, 1976). The evaluation of these carcinogenic and mutagenic chemicals and their bio-monitoring in environment is the real solution of this problem and the work has been done to some extent in past years for evaluation of the toxic potential leading to malignant form of cancer in target and non-target organism including rat, rabbit, some mammals, human, *Chironomus, Daphnia*, Mosquito and some mutant form of *Drosophila* from all over the world including India.
The notable references on the above subject include:

Menzel 1994; Zaman et al., 1994; Batiste et al., 1995; Chapin et al., 1995; Dearfield, et al., 1995; Fail et al., 1995; Schulze et al., 1995; Consuegra et al., 1996; Kardos et al., 1996; Kady et al., 1996; Mirkova, 1996; Ushioda, 1997; WHO, 1997; Schmid et al., 1999; Sumner et al., 1999; Toba et al., 1999; Chaudhry & Grewal, 2000; Oldham et al., 2000; Tareke et al., 2000; Zhang et al., 2000; Bebernitz et al., 2001; Granath et al., 2001; Hagmar et al., 2001; Kennedy, 2001; Environ 2002; Hanioka et al., 2002; Paulsson et al., 2002; Reynolds, 2002; Zhu et al., 2002; Besaratinia et al., 2003; Bolt, 2003; Freidman 2003; Michailova et al., 2003; Munoz & Barnett 2003; Tapadia & Lakhota 2003; Tortora et al., 2003; Tyl et al., 2003; Adler et al., 2004; Besaratinia et al., 2004; Chiu et al., 2004; Chaudhry & Anand, 2004; Murty & Mathew 2004; Chaudhry & Anand, 2005; Baum et al., 2005; Besaratinia et al., 2005; Boon et al., 2005; Ghanayem et al., 2005; Rice 2005; Wang, et al., 2005; Zhang et al., 2005; Fuhr et al., 2006; Henning et al., 2006; Manjamatha et al., 2006; Amrein et al., 2007; Asha et al., 2007; Besaratinia et al., 2007; Dobrzynska, 2007; Reda, et al., 2007; Abramsson et al., 2008; Burhan et al., 2008; Meng, et al., 2008; Takahashi et al., 2008; Bai et al., 2009; FDA 2009; Kogenaru et al., 2009; Wilser, et al., 2009; Yesinem & Kalipci 2009; American Cancer society, 2010; Chow, et al., 2010; Elena et al., 2010.
AMIDES

The-amides are a class of organic compounds which can be regarded as having been derived from either acids or amines. For example, the simple aliphatic amide Acetamide (CH$_3$-CO-NH$_2$) is related to acetic acid in the sense that the -OH group of acetic acid is replaced by an –NH$_2$ group. Conversely, Acetamide can be regarded as being derived from ammonia by replacement of one ammonia hydrogen by an acyl group. Amides can be derived not only from aliphatic or aromatic carboxylic acids but also from other types of acids- for example, sulphur and phosphorus-containing acids.

The term substituted amides may be used to describe those amides having one or both hydrogens on the nitrogen replaced by other groups for example, N,N-dimethylacetamide. This compound could also be regarded as an amine, acetyldimethylamine.

Amides are generally quite neutral in reaction compared to the acid or amine from which they are derived, and they are occasionally somewhat resistant to hydrolysis. The simple amides of aliphatic carboxylic acids (except formamide) are solid at room temperature, while the substituted aliphatic carboxylic acid amides may be liquid with relatively high boiling points. The amides of aromatic carboxylic or
sulphuric acids are usually solid. A wide variety of methods are available for the synthesis of amides.

**Uses**

The unsubstituted aliphatic carboxylic acid amides have wide use as intermediates, stabilizers, release agents for plastics, films, surfactants and soldering fluxes. The substituted amides such as dimethylformamide and dimethylacetamide have powerful solvent properties.

Dimethylformamide is primarily used as a solvent in organic synthesis. It is also used in the preparation of synthetic fibres. It is a selective medium for the extraction of aromatics from crude oil and a solvent for dyes. Both dimethylformamide and dimethylacetamide are ingredients in paint removers. Dimethylacetamide is also used as a solvent for plastics, resins and gums and in many organic reactions.

Acetamide is used for denaturing alcohol and as a solvent for many organic compounds, as a plasticizer and as an additive in paper. It is also found in lacquers, explosives and soldering flux. Formamide is a softener for paper and glues and a solvent in the the plastics and pharmaceutical industries.
Some unsaturated aliphatic amides, such as acrylamide, are reactive monomers used in polymer synthesis. Acrylamide is also used in the synthesis of dyes, adhesives, paper and textile sizing, permanent press fabrics and sewage and waste treatment. It is utilized in the metal industry for ore processing and in civil engineering for the construction of dam foundations and tunnels. The polyacrylamides find extensive use as flocculants in water and sewage treatment and as strengthening agent in paper and pulp industry. Aromatic amide compounds form important dye and medicinal intermediates. Some have insect repellent properties.

**Hazards**

The wide variety of possible chemical structures of-amides is reflected in the diversity of their biological effects. Some appear entirely innocuous—for example, the longer-chain simple fatty acid amides such as stearic or oleic acid amides. On the other hand, several of the members of this family are classified as Group 2A (probable human carcinogens) or Group 2B (possible human carcinogens) by the International Agency for Research on Cancer (IARC). Neurologic effects have been noted in humans and experimental animals with acrylamide. Dimethylformamide and dimethylacetamide have produced liver injury in animals and
formamide and monomethylformamide have been shown experimentally to be teratogens.

Although, a considerable amount of information is available on the metabolism of various amides, the nature of their toxic effects has not yet been explained on a molecular or cellular basis. Many simple amides probably hydrolyzed by non-specific amidases in the liver and the acid produced were excreted or metabolized by normal mechanisms.

Some aromatic amides, for example, N-phenylacetamide (acetanilide) are hydroxylated on the aromatic ring and then conjugated and excreted. The ability of a number of amides to penetrate the intact skin is especially important in considering safety precautions.

**Carcinogenesis**

Acetamide and thioacetamide are prepared by heating ammonium acetate and aluminium sulphide and are used in the laboratory as analytical reagents. Both compounds have been shown to produce hepatomas in rats on prolonged dietary feeding. Thioacetamide is more potent in this respect, is carcinogenic also to mice and can also induce bile duct tumours in rats. While human data on these chemicals are not available, the extent of the experimental animal data is such that both of
these substances are now considered possible human carcinogens. Dimethylformamide is also classified as a Group 2B possible human carcinogen by IARC.

Acrylamide is classified as a probable human carcinogen (Group 2A) by IARC. This decision is supported by the results of bio-assays in mice by several routes and yielding multiple sites of cancer, by data on genotoxicity and by acrylamide’s ability to form adducts. The chemical structure of acrylamide also supports the probability that this chemical is a human carcinogen.