2.1 DEFINITION AND HISTORY OF DRUGS

The word "drug" is etymologically derived from the Dutch/Low German word "droog", which means, "dry", since in the past, most drugs were dried plant parts. A drug is any biological substance, synthetic or non-synthetic, that when taken into the organism's body, will in some way alter the biological functions of that organism. The word "drug" is usually used to refer specifically to medicine, vitamins, entheogenic sacraments, consciousness expanding and recreational drugs. Drugs are molecules used as medicines or as components in medicines to diagnose, treat, cure, mitigate or prevent diseases.

Some countries also defined what a drug is by law. In the United States, the Federal Food, Drug, and Cosmetic Act defines a drug as being an article "intended for use in the diagnosis, cure, mitigation, treatment, or prevention of
disease in man or other animals" or an article, other than food, intended to affect the structure or any function of the body of man or other animals.

Dispensing of medication is often regulated by the government into three categories — over the counter (OTC) medications, which are available in pharmacies and supermarkets without special restrictions, behind the counter (BTC), which are dispensed by a pharmacist without needing a doctor's prescription, and Prescription only medicines (POM), which must be prescribed by a licensed medical professional, usually a physician.

Medicinal Chemistry in its crudest sense has been practiced for several thousand years. Man has searched for cures for illness by chewing herbs, berries, roots and barks. Some of these early clinical trials were quite successful however not until last 100-150 years has the knowledge of the active constituents of these natural sources been known. The earliest written records of the Chinese, Indian, South American and Mediterranean countries described the therapeutic effects of various plant concoctions (Withering, 1937; Sneader, 1985). The Assyrians described on 660 clay tablets 1000 medicinal plants used from 1900-400 B.C.

Two of the earliest medicines were described about 5100 years ago by the Chinese emperor Shen Nung in his book of herbs called Pen Ts'ao. One of these is the Ch'ang Shan, the root *Dichroa febrifuga*, which was prescribed for
fevers (Silverman, 2004). Therefore, it is impossible to trace the roots of drug
discovery to their true origin.

Many ancient populations made reports on the medicinal properties of
various plant extracts and elixirs, all as result of a necessary trial and error
search for remedies of specific ailments (Sneader, 1985). Nature has been and
still is the single most important source of drugs or drug precursors (Verpoorte,
1998). Although natural products such as morphine, cocaine, salicylates,
atropine, quinine and digitalis are all considered “ancient” in the 21st century,
these natural products and their derivatives remain as useful therapeutics even
today, in some cases, thousands of years after their original “discovery”. So
from early civilizations, man has used nature to heal or soothe specific
ailments. Unwittingly, the use of extracts and whole plants as remedies
amounted to the administration of several chemical entities at once, whose
constitution and synergism was wholly unknown. It was not until the 19th
century when techniques for partitioning some of these extracts into individual
components did single entity drugs become available.

Since the early 20th century, thoughts about drug action and mechanism
expanded as the analytical techniques of biology, chemistry and pharmacology
progressed. Discoveries of different families of therapeutics followed the
seminal observations of Ehrlich, and after 1910, a new era in drug discovery
emerged. Science saw the development of many drugs discovered hundreds of
years earlier. Although quinine was found by early explorers to be used by Indians of South America, it was not isolated until 1823 and development of analogues as antimalarials began in the early 1900's (Burger, 1970). New medicines such as antihistamines, trypanocidals and several important alkaloids, many extracted in the 19th century, were being synthesized and developed into commercial entities. The age of antibiotics began just prior to that famed serendipititious discovery of a crude penicillin culture by Fleming, with the discovery that a dyestuff (Prontosil) could cure gram-positive bacterial infections in man (Silverman, 2004). The active component, sulfanilamide, paved the way for the development of sulfa drugs. The intensive study of Fleming's original molds by Florey and Chain in the early 1940s showed that there was a mixture of several components in the penicillin preparation and these were separated, tested and more active constituents were found and developed into the first anti-infectious agent. Around this same time, more extensive development of antihistamines, analgesics, barbiturates, hormones (e.g., epinephrine), sedatives, hypnotics and antidepressants was seen in the 1940s-1950s. The improvement in chromatographic and diagnostic (detection) techniques as well as advances in synthesis and understanding of chemical principles accelerated the discovery of new drug entities in the second half of the 20th century. Another case of serendipity led to the discovery of Librium in 1957 (Sternbach, 1979) and later to the benzodiazepines class of antianxiety medications, which include Valium and Xanax. Valium was once the best-
selling prescription drug in America. In addition to small molecule therapeutics, the 20th century saw the rise and success of vaccines to cure several bacterial diseases such as tetanus, diphtheria, yellow fever, measles, mumps, rubella and polio. Diagnostic techniques such as X-rays, electrocardiograms, CT and PET scans, ultrasound and MRIs were all products of the last 40-50 years, and each technique played its own role in the design and development of new drug entities.

Nature is still an excellent source of new drugs or more commonly, of precursors of drugs. Of the 20 leading drugs in 1999, nine of them were derived from natural products. Almost 40% of the 520 new drugs approved for the drug market between 1983 and 1994 were natural products. Greater than 60% of the anticancer and anti infective agents that are on the market or in the clinical trials are of natural product origin (Silverman, 2004).

Drug discovery is a very lengthy and time taking process. The average time required to bring a drug to the market range is from 12-15 years at an average cost of $600-800 million. Of the 10,000 compounds evaluated in animal studies, an average 10 will make it to human clinical trials in order to get one compound in the market. Prior to drug approval, the clinical trial consists of three phases namely phase–I, phase–II and phase–III trials. In phase–I trial, which takes about a few months to a year and a half, the safety, tolerability, pharmacokinetic properties and pharmacological effects in 20-100 healthy
volunteers are evaluated. In phase–II trial, which takes about 1–3 years, assessment of the effectiveness of the drugs, its side effects and other safety aspects are evaluated in a few hundred diseased patients apart from clarifying the dosing regiments. In phase–III trials, which spreads over 2 – 6 years, a larger clinical trial with thousands of patients in hospitals that establishes the efficacy of the drug and monitors adverse reactions from long term use is carried out. Apart from these phase–IV studies are considered to be the results found with the already marketed drug which is in general use.

2.2 EFFECTS ON LIVING ORGANISMS

Pharmacodynamics is the study of the biochemical and physiological effects of drugs and the mechanisms of drug action and the relationship between drug concentration and effect. It is often summarily stated that pharmacodynamics is the study of what a drug does to the body, whereas pharmacokinetics is the study of what the body does to a drug (Jackson, 2003; Werner, 2003).

Drug interactions can complicate therapy by adversely increasing or decreasing the action of a drug: interactions may be based on changes in drug levels (Kasper, 2005). It is important to distinguish between actions of drugs and their effects. Actions of drugs are the biochemical physiological mechanisms by which the chemical produces a response in living organisms.
The effect is the observable consequence of a drug action. One major problem of pharmacology is that no drug produces a single effect. The primary effect is the desired therapeutic effect. Secondary effects are all other effects beside the desired effect, which may be either beneficial or harmful. Drugs are chosen to exploit differences between normal metabolic processes and any abnormalities, which may be present. Since the differences may not be very great, drugs may be nonspecific in action and alter normal functions as well as the undesirable ones. This leads to undesirable side effects.

The biological effects observed after a drug has been administered are the result of an interaction between that chemical and some part of the organism. Mechanisms of drug action can be viewed from different perspectives, namely, the site of action and the general nature of the drug-cell interaction. Drugs act on the cell membrane by physical and/or chemical interactions. This is usually through specific drug receptor sites known to be located on the membrane. A receptor is the specific chemical constituents of the cell with which a drug interacts to produce its pharmacological effects. Some receptor sites have been identified with specific parts of proteins and nucleic acids. In most cases, the chemical nature of the receptor site remains obscure.

In general the bonds formed between a drug and a receptor are weak noncovalent interactions: consequently the effects produced are reversible. Because of this a drug becomes inactive, as soon as its concentration in the
extracellular fluids decreases. Often it is desirable for the drug effect to last only a limited time so that the pharmacological action is terminated. Sometimes a prolonged effect of the drugs is desirable for a chemotherapeutic agent, a drug that acts selectively on a foreign organism or cell to form an irreversible complex with its receptor so that the toxic effect of the drug persists for a longer duration. In this case a covalent bond is required (Silverman, 2004).

The undesirable effects of a drug include:

- Increased probability of cell mutation (carcinogenic activity).
- A multitude of simultaneous assorted actions which may be deleterious.
- Interactions (additive, multiplicative, or metabolic).
- Induced physiological damage, or abnormal chronic conditions

### 2.3 EFFECTS ON PUBLIC HEALTH

The beneficial effects of drugs are coupled with the inescapable risk of untoward effects. Although adverse drug reactions are common, it is difficult to ascertain their incidence, seriousness and ultimate health effects. Available information comes from evaluations of hospitalized patients, epidemiological surveys, premarketing studies and voluntary reporting most notably to the U.S. Food and Drug Administration’s Medwatch System. In a systematic literature review of cutaneous reactions to drugs, the reaction rates varied from 0 to 8% and were highest for antibiotics. In a series of 48,005 patients over a 20-year
period, morbilliform rash (91%) and urticaria (61%) were the most frequent skin reactions (Kasper, 2005).

Just as there is no health benefit without potential toxicity, there is no absolute goodness about drugs. However, their enormous health benefits outweigh the drawbacks in individual cases. The word drug has acquired bad connotations in recent years because the widespread abuse of a few chemicals that affect the central nervous system has become a serious sociological problem. Nevertheless, drugs act on many other organs in the body, can benefit as well as harm the nervous system.

Drugs are chemical compounds that modify the way the body and mind work. Most people think that these biological activities should help or heal sick people or animals. There is, however, no known drug that is not harmful or even poisonous at high doses, and much of the scientific work on drugs has attempted to widen the gap between effective and toxic doses (Burger, 1970).

In most of the developing countries including that of India, with generally poor economic conditions of the population, have few trained medical personnel. Therefore, all medical ailments are not brought under direct medical supervision. As a result, a large percentage of the population resorts to self – medication with complete ignorance of the correct prescriptions. Further, the drugs are not strictly obtainable by prescriptions, but are readily available as over the counter products for the general public which pose an added threat of
indiscriminate use and abuse. Therefore, it is highly essential that detailed investigations are carried out on the genotoxicity of the most commonly used drugs using multiple cytogenetic endpoints in multiple laboratories in a global basis.

2.4 DRUGS AND GENOTOXICITY

Natural selection is the force that ensures that useful genes are retained in the genome. Mutations occasionally do occur spontaneously during DNA replication, causing changes in the sequence of nucleotides. Such changes or mutations also can arise from radiation that causes damage to the nucleotide chain or from chemicals that lead to errors during the DNA-copying process. Mutations come in various forms: a simple swap of one nucleotide for another; the deletion, insertion, or inversion of one to millions of nucleotides in the DNA of one chromosome and translocation of a stretch of DNA from one chromosome to another (Lodish et. al., 2004).

A drug is considered to be genotoxic if it tested positive or equivocal in at least one of the standard battery tests. A drug is considered to be a rodent carcinogen if it increased incidence in at least one of the genders of one species at least at one site, which was not expected (based on known pharmacologic mechanism) for that particular drug class (Synder and Green, 2001).
Some of the frequently prescribed drugs include the quinolone group of antibacterial agents like ciprofloxacin, enrofloxacin, norfloxacin, lomefloxacin, oxofloxacin and sitafloxacin; the nitroimidazole antiprotozoan agent metronidazole; the bis-triazole antifungal agent fluconazole, and amphotericin B; the antimalarial agents chloroquine, primaquine and amodiaquine; the antihelminthic agents albendazole and piperazine; the anti TB agents rifampin, isoniazid and pyrazinamide; the antiepileptic agents lamotrigine and valproic acid; the anti-HIV agents navirapine and zidovudine; and others like ranitidine, esomeprazole etc.

Some of the other very frequently used over the counter drugs include the non-steroidal anti-inflammatory (NSAID) drugs like paracetamol, which has negligible anti-inflammatory activity, and is strictly speaking not a NSAID but commonly used as analgesic and antipyretic drug.

However, from a detailed electronic search in the databases of Medline, PubMed, Toxline and United Sates Physical Desk Report (PDR), it has been found that not much of independent investigations have been made on the genotoxicity of most of the marketed pharmaceuticals. In fact, for a quite significant number of pharmaceuticals, no genotoxicity data is at all available in the in vivo mammalian cytogenetic assays. In addition, considerable controversies exist among the genotoxicity reports arising out of different laboratories.
In a survey of the PDR database, Synder and Green (2001) observed that marketed pharmaceuticals are comprised of a high fraction of putative rodent carcinogens. This study had excluded the nucleoside analogues, anticancer cytotoxic agents and topoisomerase inhibitors.

A brief survey of the published report in referred journals is presented as under. The genotoxicity potential of metronidazole has been reviewed by Bendesky et. al. (2002). Martelli et. al. (1990) reported that in primary cultures of both rat and human hepatocytes, metronidazole produced DNA fragmentation and unscheduled DNA synthesis. However, under similar experimental conditions, human hepatocytes were more resistant to genotoxicity than those of the rat. Menendez and coworkers (2001) using the alkaline version of the single cell gel electrophoreses (SCG, ‘comet’) assay, evaluated the amount of single strand breaks and ‘alkali labile‘- sites of the DNA in 10 healthy subjects treated with metronidazole per day for 10 days. The results showed a significant increase in DNA damage in nine out of 10 individuals. However, Fahrig et. al. (1997) arrived at different results with the comet assay under alkaline conditions. They found no differences in DNA damage before and after the treatment.

Herbold et. al. (2001) in a study reported that ciprofloxacin produce neither any mutagenicity nor carcinogenicity in rodent test system. However a survey of the 1999 PDR revealed that although ciprofloxacin is found negative
in mutagenic studies, but it was positive in mouse lymphoma assay (Synder and Green, 2001). Further, in contrast to the above two reports Goria et al. (1999) reported that ciprofloxacin induced significant increase in chromosome aberration detected as chromatid and chromosome breaks and gaps. The genotoxicity of fleroxacin, lomofloxacin and norfloxacin has also been reported (Chetelat et al., 1996; Polianskaia and Sizova, 1996; Maura et al., 1994).

Apart from the quinolone antibiotics, significant controversies exist regarding the genotoxicity of antimalarial drugs (Tunca et al., 2002; Azas et al., 2002; Riccio et al., 2001; Chatterjee et al., 1998). Among the anti-HIV drugs while no adverse genetic effects have been observed against zidovudine and the amino sugar N-butyldeoxynojirimycin; but mild genotoxicity for fullerene and significant genotoxicity for the acyclic nucleotide analogue HPMPC and PMEA has been reported (Zakharenko et al., 1997; Greene et al., 1996; Bila et al., 1993; Oshiro et al., 1992).

The safety of NSAIDs is at stake as cited by Rainsford et al. (2001). The FDA also concluded that adequate data on the risks associated with the use of most NSAIDs, especially at high doses over long periods, was largely lacking. Given this gap in knowledge, and in light of recent studies on a few older NSAIDs and the newer COX-2 drugs, the agency decided to ask manufacturers of all NSAIDs to put a warning on drug labels, inserts and boxes
about potential risks to the heart with long-term use (Consumer Reports Best Buy Drugs, 2005).

From a drug development standpoint, it is important to have a thorough understanding of the mechanism of any 'positive' genetic toxicology findings so that informed decisions may be made with respect to risk. Further, in the requirements of most of the regulatory agencies, in vivo cytogenetic assays (micronucleus/chromosome aberrations/sister chromatid exchanges) are not made essential which is substituted by the equivalent in vitro assays. Consequently, adequate in vivo cytogenetic data are not available for many of the pharmaceuticals. However, the in vivo test is especially relevant to assess genotoxicity hazard because it allows consideration of factors of in vivo metabolism, pharmacokinetics and DNA-repair processes and is also useful in further investigation of a mutagenic effect detected by an in vitro genotoxicity test (Krishna and Hayashi, 2000).

Genotoxicity data of pharmaceuticals on germ line cells are extremely scanty. Chemically induced increase in sperm head damage is highly correlated to known germ cell mutagens (Wyrobek and Bruce, 1975). Since mutations in the germ line cells are the only cells capable of transferring a mutation to the next generation, therefore, more studies are required on the effect of pharmaceuticals in these cells.
The drugs selected for our study are metronidazole, ciprofloxacin, chloroquine, acetaminophen, and aspirin. These drugs are the most widely used in India and in addition, considerable controversies exist among the genotoxicity reports arising out of different laboratories and moreover, there is very less available reports on the safety of these mostly marketed Pharmaceuticals using the cytogenetic endpoints.

2.4.1 METRONIDAZOLE

Metronidazole (1-[2-hydroxyethyl]-2-methyl-5-nitroimidazole), CAS Registry No. 443-48-1, chemical formula C₆H₉N₃O₃, and has the molecular weight of 171.16, is a nitroimidazole anti-infective drug used mainly in the treatment of infections caused by susceptible organisms, particularly anaerobic bacteria and protozoa. Nowadays, it is one of the most used drugs worldwide and is among the top 100 most prescribed drugs in the U.S.A. (data by IMS Health, Rxlist www.rxlist.com/top200.htm, 1999) and one of the 10 most used drugs during pregnancy (Thapa et. al., 1998). It appears in the essential drug list of the World Health Organization (WHO, 1999a).

This drug has activity against protozoans like Entamoeba histolytica, Giardia lamblia, Trichomonas vaginalis and gram-negative anaerobes such as Helicobacter pylori, for which the drug has been proved an effective treatment (Freeman et. al., 1997). In addition metronidazole has been applied as a
radiosensitizer of hypoxic or cells (Rauth, 1984). Metronidazole is active only against anaerobic organisms. Metronidazole has been found to interact with and bind to DNA of both bacterial and mammalian cells under anaerobic conditions (Ludlum et. al., 1988; La Russo et. al., 1977; Knight et. al., 1978), but data concerning its capability of inducing DNA damage are inconclusive (La Russo et. al., 1977; Probst, 1981; Sina et. al., 1983). Metronidazole is a potent mutagen in bacterial systems (Voogd, 1981); the main DNA lesions being base pair substitutions (Vanelle et. al., 1990). Genotoxicity data on metronidazole in mammalian test system are contradictory (Bendesky et. al., 2002). An increase in the mutagenicity of serum in female mice 8 hr after administration of metronidazole was observed (Dobias, 1980). Furthermore, it has been observed that metronidazole induces DNA single-strand breaks in the lymphocyte of the patients on standard doses of the drug (Bendesky et. al., 2002). Toxicological investigations following long-term administration of high doses of 5-nitroimidazole derivatives to rats and mice showed induction of various tumors (Rustia et. al., 1972). However there is disagreement about the genotoxicity of the drug. No increase in sister chromatid exchanges in human lymphocytes exposed either to metronidazole or to two of its major urinary excretion metabolites at a concentrations of up to 1000mg/l for 75 hr (Lambert et. al., 1979). Study reported negative results in a dominant lethal test in rat and mouse (Bost, 1977) and a negative result in a micronucleus test in mice (Hartley-Asp, 1981). However, experimental data on genotoxic effects of
metronidazole showed a linear dose-response increase of micronuclei in mice bone marrow cells and in human lymphocyte cultures blocked with cytochalasin B (Mudry et. al., 1994). At the same time, a significant increase of chromosomal aberrations in human lymphocyte cultures and abnormal anaphases in CHO (Chinese hamster ovary) cells were observed (Mudry et. al., 1994). Hence, Genotoxicity data on metronidazole in mammalian test system are contradictory (Bendesky et. al., 2002). On the other hand, there is scanty literature on its reproductive effects. There is insufficient data about its effects on sperm morphology.

2.4.2. CIPROFLOXACIN

Ciprofloxacin (1,4-dihydro-1-cyclopropyl-6-fluoro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid), CAS Number is 85721-33-1, chemical formula C17H18FN3O3, and has the molecular weight 331.345; is a synthetic antibiotic having bactericidal activity. Manufactured and sold by Bayer Pharmaceuticals under the brand names Cipro® and Ciproxin® (and other brand names in other markets, e.g. veterinary drugs), belonging to a group called fluoroquinolones.

Ciprofloxacin is a broad-spectrum antibiotic that is active against both Gram-positive and Gram-negative bacteria. The major adverse effect seen with use of is gastrointestinal irritation, common with many antibiotics. Because of
its general safety, potency and broad-spectrum activity, ciprofloxacin was initially reserved as a "last-resort" drug for use on difficult and drug-resistant infections. However, genotoxicity data on ciprofloxacin in mammalian test systems are contradictory.

Photosensitivity is a well-known adverse reaction with ciprofloxacin (Jensen et al., 1987). Although not licensed for use in children under the age of 12 years of age due to reports of arthropathy in young animals, it is often prescribed in this age group, particularly in children with CF; few safety problems have been reported (Chysky et al., 1991). Patients taking ciprofloxacin are usually advised to protect their skin from direct sunlight. It is reported that more attention should be paid to indoor sources of UV light (Adam et al., 1999). There are reports which indicate that in the presence of ultraviolet irradiation (UV) a number of fluoroquinolones exert photochemical toxic and mutagenic effects (Chetelat et al., 1996; Jeffrey et al., 2000; Marrot and Agapakis-Causse, 2000; Martinez et al., 1998; Rosen et al., 1996; Spratt et al., 1999). Ciprofloxacin was also found to inhibit the catalytic DNA strand passage activity (Barrett et al., 1989).

It is reported that Clinicians should be aware of the potential bleeding complications that can occur with the ciprofloxacin–warfarin drug–drug interaction (Robert et al., 2000).
At high concentrations, some fluoroquinolones have been reported to exhibit genotoxic effects in eukaryotic systems as a result of topoisomerase inhibition (Kohlbrenner et. al., 1992; Robinson et. al., 1991). However, there are reports which states that ciprofloxacin is considered to be safe for therapeutic use (Bernd et. al., 2001). Furthermore, levels of reactive oxygen species generated during the photoactivation of fluoroquinolones were not found to correlate to the genotoxic effects (Martinez et. al., 1998; Umezawa et. al., 1997). There are reports which show negative results with ciprofloxacin in the in vivo studies (Herbold et. al., 2001). Studies in humans by Mitelman et. al. (1988) was also negative.

Though contradictory reports are available with ciprofloxacin assessed in the somatic cells, not much availability of reports were observed in the germ cells. There is insufficient data about its effects on sperm morphology.

2.4.3. CHLOROQUINE

Chloroquine (N"-(7-chloroquinolin-4-yl)-N,N-diethyl-pentane-1,4-diamine), CAS number is 54-05-7, chemical formula C_{18}H_{26}N_{3}Cl and has the molecular weight of 319.872, is a commonly used form of medication against malaria. As it also mildly suppresses the immune system, it is used in some autoimmune disorders, such as rheumatoid arthritis and lupus erythematosus. Chloroquine can be used for preventing malaria from *Plasmodium vivax*, *ovale* and
malariae. Many areas of the world have widespread strains of chloroquine-resistant *Plasmodium falciparum*, so other antimalarials like mefloquine or atovaquone may be advisable instead. Combining chloroquine with proguanil may be more effective against chloroquine-resistant *Plasmodium falciparum* than treatment with chloroquine alone, but is no longer recommended by the CDC due to the availability of more effective combinations.

Reports on the cytogenetic toxicity of chloroquine is contradictory. It is reported that at the G2 stage chloroquine in the concentration of 15 μg/ml had no cytogenetic effect and in the concentration of 100 μg/ml – it increased the number of chromosome aberrations significantly (Shalumashvili, 1976). There are reports which suggesting that chloroquine can be added to the long list of drugs and chemicals causing damage to chromosomes (Shaw, 1970). It is also reported by Evans (1970) that chloroquine induced chromosome damage may be associated with genetic change.

There are reports that results of the *in vivo* sister chromatid exchange and chromosome aberration assays indicate that the three drugs chloroquine, primaquine and amodiaquine are genotoxic in bone marrow cells of mice (Chatterjee *et. al.*, 1998). However, Riccio (2001) reported that although both chloroquine and AQ-13 showed weak bacterial mutagenicity, this mutagenic effect was not confirmed in either the mouse lymphoma mutagenesis assay or the micronucleus assay.
Hence chloroquine shows contradictory reports, but there is even scanty literature on the effects of chloroquine in the Germ line cells.

2.4.4. ACETAMINOPHEN

Acetaminophen (N-acetyl-p-aminophenol), CAS No. is 103-90-2, chemical formula is $\text{C}_8\text{H}_9\text{NO}_2$, and has a molecular weight of 151.17, is a non-opiod analgesic without anti-inflammatory effects. It was first used in medicine in 1893, but became very widely used after approval by the FDA in 1950. It is used to treat both acute and chronic pain. Perhaps the most notable property is that, unlike, acetaminophen does not have peripheral anti-inflammatory effects or blood-thinning properties. It is used to relieve mild to moderate pain or to reduce fevers. It is considered a drug of choice in patients who are intolerant, have ulcers, or difficulty in blood clotting. It is available in many dosage forms over the counter and in strengths for children and adults.

Acetaminophen is generally well tolerated. It is used to treat many conditions such as headache, muscle aches, arthritis, toothaches, backache, colds and fevers. Acetaminophen may also be used for purposes other than those listed in this medication guide.

The major side effects are:

Allergic reaction (difficulty breathing; closing of your throat; swelling of your lips, tongue, or face; or hives);
• Liver damage (yellowing of the skin or eyes, nausea, abdominal pain or discomfort, unusual bleeding or bruising, severe fatigue);

• Blood problems (easy or unusual bleeding or bruising).

There are reports saying that acetaminophen-induced hypothermia in mice is mediated by a prostaglandin endoperoxide synthase 1 gene-derived protein (Samir et al., 2004). Paracetamol was tested for carcinogenicity by oral administration in mice and rats. In one strain of mice, a significant increase in the incidence of multiple liver carcinomas and adenomas was observed in animals of each sex at a markedly toxic dose; in two studies on another strain, no increase in the incidence of any tumor was observed at a well tolerated dose that was approximately half that in the preceding study (International Agency for Research on Cancer (IARC)). There is also report about the induction of chromosome aberrations in vivo in bone marrow cells of mice by paracetamol (Severin et al., 1995). Powell et al. (2006) have undertaken a study to substantiate the findings of a gene expression signature for oxidative stress by a subtoxic dose of acetaminophen in rat liver using a panel of sensitive biomarkers of oxidative stress and oxidative DNA damage. In a recent report, Ibrulj et al. (2007) found that paracetamol in overdoses may induce genetic alterations in the cultured human lymphocytes. There are various reports indicating the toxicity of acetaminophen in the hepatic cells but reports indicating genotoxicity in the somatic cells and germ line cells are very scarce. There is insufficient data about its effects on sperm morphology.
2.4.5 NIMESULIDE

Nimesulide (N-(4-nitro-2-phenoxyphenyl) methanesulphonamide), CAS No. 51803-78-2, chemical formula C\textsubscript{13} H\textsubscript{12}N\textsubscript{2}O\textsubscript{5}S, and has the molecular weight of 308.31. There are reports indicating the toxicity of nimesulide (Li et al., 2003).

The genotoxic potentiality of nimesulide was evaluated in vivo in murine bone marrow cells (Khan et al., 2003). The motility of sperm was affected severely after 6h of nimesulide administration that suggested a crucial role of COX-2 towards fertility of mice sperm (Thotakura, 2007). Though in some cases nimesulide is indicative of showing toxicity but it is reported by Wober (1999) that nimesulide is well tolerated among all age groups of patients and even when used concomitantly with other drugs. Various adverse effects of nimesulide are reported (WHO, 1999b; Dourakis, 2001; Cordeiro, 2000; Schattner 2000; Balasubramaniam, 2000).

The reports on the effect of nimesulide in the somatic cells using cytogenetic endpoints are very less available. At the same time, there is scanty literature on its effects in the germ line cells. There is insufficient data about its effects on sperm morphology.
2.4.6 ASPIRIN

Aspirin (2-(acetyloxy)benzoic acid), CAS No. 50-78-2, chemical formula is C₉H₈O₄ C₆H₄ (OCOCH₃) COOH, and has the molecular weight of 180.16. The synonym of this drug is acetylsalicylate acetylsalicylic acid O-acetylsalicylic acid. It is often used as an analgesic (against minor pains and aches), antipyretic (against fever), and anti-inflammatory. It has also an anticoagulant ("blood-thinning") effect and is used in long-term low-doses to prevent heart attacks.

Low-dose long-term aspirin irreversibly blocks the formation of thromboxane A2 in platelets, producing an inhibitory effect on platelet aggregation, and this blood-thinning property makes it useful for reducing the incidence of heart attacks. Aspirin produced for this purpose often comes in 75 or 81 mg dispersible tablets and is sometimes called "Junior aspirin" or "Baby aspirin." High doses of aspirin are also given immediately after an acute heart attack. These doses may also inhibit the synthesis of prothrombin and may therefore produce a second and different anticoagulant effect.

Several hundred fatal overdoses of aspirin occur annually, but the vast majority of its uses are beneficial. Its primary undesirable side effects, especially in stronger doses, are gastrointestinal distress (including ulcers and stomach bleeding) and tinnitus.
The reports on aspirin are very limited and contradictory. There is a report about the genotoxicity of analgesic compounds by an *in vitro* micronucleus assay (Dunn *et al*., 1987). At the same time there are reports about the suppressive effect of aspirin on chromosome aberration induced by mitomycin C in mice (Niikawa *et al*., 2001).

Jenkins *et al*. (2004) reported that aspirin induced asthma in adults. This report was in support with the earlier reports (Marquette *et al*., 1992; Rachelefsky *et al*., 1975; Schuhl *et al*., 1979). At the same time there are reports of chemoprotective role of aspirin in the colorectal cancer which might extend to other gastrointestinal cancers such as esophagus and stomach (Antonio, 2003). There is evidence that supports the similar effects (Thun *et al*., 1993; Li *et al*., 2000; Giovannucci *et al*., 1995). There are reports that NO, one of the metabolites of aspirin has been shown to serve as vasoprotector, including inhibition of platelet aggregation and leukocyte adherence to the site of injury (George, 1999, Kibbe *et al*., 1999, Claudio *et al*., 2002).

However, the genotoxicity reports using cytogenetic endpoints are very rare. There is very limited literature available on the effects of aspirin in the germ line cells.