Chapter 6
SUMMARY

Monitoring the safety of medicine use is of paramount importance since, during the clinical development of medicines, only limited data are generated through clinical trials. Use of medicines outside the specifications described in the licence (e.g. in terms of formulation, indications, contraindications or age) constitutes off-label and off-licence use and these are a major area of concern. There are guidelines which intended to improve awareness of medicine safety issues among public. The following problems occur with the use of medicines.

- Medicines used are often, off-label and unlicensed.
- Over-the-counter, traditional and herbal medicines are readily available, but their use is generally not evidence-based and is often inappropriate.
- Counterfeit and substandard medicines are widespread.
- Abuse by teenagers occurs with non-medical prescription of legal medicines and illegal drugs.
- New and innovative medicines are available with a safety indication, but with no evidence of long-term benefit and risk, e.g. the biological agents.
- Additionally, in resource-poor countries the following may apply:
  - Limited treatment may be available, particularly rural and remote areas.
  - Medicines may be available through illegal street vendors.
  - Medicines are used in public health driven programmes e.g. for the treatment of endemic infectious diseases such as malaria, tuberculosis and for parasitic diseases.
In many of these low-income countries, much of the medicine supply is by-passing the official health care system. Consumers with limited buying power often acquire medicines from acquaintances, relatives and unregistered vendors who have little or no health-care training. In these countries, prescription medicines may often be acquired without a prescription from markets and chemist’s shops. The resulting self-medication, unsupervised by any health professional, also leads to risk of public health. This situation is associated with high risks for adverse consequences because of the risk of poor-quality medicines being taken and the absence of information on how to use medicines in general. Even if serious adverse reactions occur as a result of self-medication with products acquired from street markets, they are often not reported to any health practitioner, since seeing a health professional is often not feasible or is considered too expensive.

The FDA reviews proposals for conducting clinical drug trials, evaluates drug applications and proposed drug labeling, and monitors drugs once they are approved and marketed. However there is a never ending list of marketed drugs with genotoxic potential.

There is significant numbers of pharmaceuticals for which genotoxicity data on in vivo mammalian cytogenetic assays is not at all available. In addition, considerable controversies exist among the genotoxicity reports arising out of different laboratories. Consequently, adequate in vivo cytogenetic data are not available for many of the pharmaceuticals.

The present study was directed towards the same objective, the cytogenetic effects of two commonly used marketed pharmaceuticals namely metronidazole and artesunate was investigated in swiss albino mice in vivo test system using various end points like bone marrow chromosome aberration assay, micronucleus assay, sperm head shape abnormality assay, lipid peroxidation assay (TBARS), reduced glutathione (GSH) and Superoxide dismutase (SOD) assay and comet assay. Further, the pharmaceutical agents may hyper sensitize the cells to another known mutagen (gamma radiation and
X-ray) by way of altering its physiology even though it itself does not directly interact with the cellular DNA; drug–mutagen studies were undertaken. Therefore, considering the above facts and in the view enlighten by the available literature, the present studies were undertaken with the following objectives:

- To study the genotoxic effects of selective pharmaceuticals, currently prescribed by physician, in the somatic cells of mammalian test system.
- To evaluate the effect(s) of these pharmaceuticals in the male germ line cells.
- Study on the effects of dietary antioxidants in modulating the possible genotoxicity potential of pharmaceuticals.
- Study on the drug-mutagen interaction to evaluate the acquired susceptibility of few common pharmaceuticals.

The major findings of the present study are summarized below:

1. Both of the two pharmaceuticals namely metronidazole and artesunate induced significant increase in the frequency of chromosome aberrations in the bone marrow cells of mice.

2. Following the treatment of the test chemicals, significantly higher frequencies of chromatid and isochromatid type of gaps and break were more prevalent. Further decrease in mitotic index was also observed.

3. In the dose ranges tested, both the pharmaceuticals analyzed in the present study, namely metronidazole and artesunate induced significant increase in the frequency of micronucleated cells in the bone marrow cells of mice.

4. The comparison between the frequency of chromosome aberrations and micronucleated bone marrow cells revealed that the frequency of the micronucleus was significantly lower for both the pharmaceuticals tested. As all the broken fragments are not involved in forming visible micronucleus, and highly damaged cells may fail to complete the process of cell division, thus the present observations were as anticipated. This may
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further locate towards a clastogenic rather than aneugenic effect induced by the test chemicals.

5. After 48 hr there was decrease in the chromosomal aberration and micronucleus. This could be due to various possible reasons such as death of damaged cells, the clearance of drug from the body, post-replication repair process etc.

6. Both the pharmaceuticals induced significant increase in the frequency of sperm head abnormality following 24hr of the treatment. The observed abnormalities in the sperm head morphology at 24hr of the treatment may not be attributable to genetic effects. Therefore, it is possible that in the short term, the said pharmaceuticals may interact with the cell membrane components thus resulting in the morphological alterations.

7. Both the pharmaceuticals induced significant increase in the frequency of sperm head abnormality following 35 days of the treatment. These findings indicate that the germ cells may be more sensitive as compared to the somatic cells so far as genotoxicity point is considered. A decrease in sperm count was also observed.

8. Both the test chemicals showed an increased TBARS levels, indicating damage due to lipid peroxidation.

9. There was decrease in hepatic antioxidant enzymes status i.e. reduced glutathione (GSH) and superoxide dismutase (SOD) by the two pharmaceuticals. This could lead to high rate of free radical scavenging.

10. In single cell gel electrophoresis both the pharmaceuticals were found to induce DNA damage.

11. Pretreatment with antioxidants vitamin C and curcumin, significantly reduced the genotoxic effects observed in the present study. Further vitamin C was found be more potent mostly in different genotoxic assay carried out.
12. In the drug mutagen interaction studies, radiation and X-rays showed significant increase in the frequency of chromosome aberrations, micronucleus, abnormal sperm head population, increased levels of TBARS indicating lipid peroxidation and depressed level of GSH and SOD due to more free radical scavenging. Radiation exposure following the treatment of the test agent, showed more pronounced effects the in the animals. Furthermore antioxidant vitamin C and curcumin pretreatment, could ablate these effects.

13. The present findings provide further evidence that genotoxicity testing using multiple cytogenetic end points marginally clarify the true potential of a genotoxic agent, which is otherwise not possible in test models that use single end points for hazard assessment.

Based on the above findings, it can be concluded that metronidazole and artesunate causes significant genetic damage to the exposed population and hence exposure to these chemicals should be restricted. Vitamin C and Curcumin pretreatment may reduce the genotoxic effects of the tested pharmaceuticals to an extent. Since both of the tested pharmaceuticals showed additive effects when treated with radiation as well as X-ray, exposure to low levels of radiation during medication should be avoided. Further, the health risks are sufficient to require the continuous testing of these pharmaceuticals using specific genetic locus to make certain that the public health remains protected.