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GENOTOXIC POTENTIAL OF SOME COMMONLY USED ANTIMALARIALS: A REVIEW

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ABSTRACT

Malaria caused mostly by *P. falciparum* and *P. vivax*, remains one of the most important infectious diseases in the world. The numbers of antimalarial drugs in use are very small. Drug toxicity must be acceptable to patients and should cause less harm than the disease itself. Assessment of hazard and risk varies throughout drug development as more persons are exposed for longer periods of time and more nonclinical information on the hazard is collected and evaluated. Cancer risk for human pharmaceuticals is important because drugs are taken at pharmacologically active doses and often on a chronic basis. Epidemiologic studies on patient populations have limited value because of the long latency period for most cancers and because these studies lack sensitivity. Besides the mutagenicity and genotoxicity testing of antimalarial drugs as a part of pre-clinical trials, there are several literatures confirming the mutagenicity and genotoxicity of marketed antimalarial drugs. Genetic abnormalities may also play a part in the incidence and severity of adverse reactions to drugs. In this paper, a comprehensive review of literature pertaining to the mutagenic and genotoxic properties of some commonly used antimalarial drugs is presented.

INTRODUCTION: Malaria is a mosquito borne infectious disease caused by eukaryotic protist of the genus *Plasmodium*. It is widespread in tropical and subtropical regions, including parts of Asia, America and Africa. Each year there are approximately 250-500 million cases of malaria, killing between one and three million people, the majority of whom are young children [1].

Several drugs are used for the treatment of malaria. Use of prophylactic drugs are seldom practical for full time residents of malarial endemic areas, and their use are usually restricted to short term visitors and travelers to malarial regions. This is due to the cost of purchasing drugs, negative side effects from long term use, and because some effective antimalarial drugs are difficult to obtain outside the wealthy nations. Quinine was used starting in the 17th centuary as a prophylactic against malaria. The development of more effective alternatives such as Chloroquine, Primaquine and Quinacrine in the 20th centuary reduced the reliance on quinine. Today, quinine is still used to treat chloroquine resistant *Plasmodium falciparum*, as well as severe and cerebral stages of malaria but is not generally used for prophylaxis.

There are three potential ways to control malaria: elimination of vector, drug therapy and vaccination. Elimination of the vector currently is the simplest and most cost effective. The current antimalarial drugs, while effective against certain species, also have significant adverse reaction, and resistance is

increased. New antimalarial drugs must be developed constantly, because the protozoa develop resistance by a variety of mechanisms and there are wide variety of adverse reactions. During the drug discovery period, the sponsor may cease development of compounds found to be genotoxic in *in vitro* screens, depending on possible therapeutic indications. If a compound is negative in the *in vitro* screen and in the subsequent *in vivo* study for chromosomal damage, carcinogenicity studies may not need to be reported until submission of the New Drug Application.

Reasons for conducting carcinogenicity studies includes previous demonstration of carcinogenic potential in the product class that is considered relevant to humans, structure-activity relationship that suggests a carcinogenic risk, evidence of pre neoplastic lesions in repeated dose toxicity studies and long-term tissue retention of parent compound or metabolite(s) resulting in local tissue reactions or other pathophysiologic responses. Initial estimates of cancer risk by human exposures to a new drug are based on results from short-term, genetic toxicology studies.

While it is recognized that nongenotoxic carcinogens may also present a risk, this mechanism of carcinogenesis is thought to have a threshold. Results from chronic carcinogenicity studies are generally not available until the New Drug Application stage of the approval process.

Nucleic Acid binding activity: Most of the antimalarials have affinity for nucleic acids in the cell. These compounds can bind to DNA covalently or non-covalently. Interaction of chloroquine with DNA was studied and confirmed by spectrophotometric analysis by Cohen and Yielding [2]. Nuclear magnetic resonance studies of the interaction of chloroquine with nucleotides were performed by Sternglanz *et al.,* [3] and similar results were reported. Kwakye-Berko and Meshnick [4] reported that the DNA binding properties of 4-aminoquinolines especially chloroquine highly depends upon salt concentration. Binding of 8-aminoquinoline to DNA, RNA and various polydeoxyribo and polyribonucleotides has been confirmed by Morris *et al* [5]. The affinity of aminoquinolines for nucleic acids and polynucleotides decreases in the order chloroquine > pentaquine > plasmocid > primaquine > pamaquine [6].

Both 4-aminoquinolines and 8-aminoquinolines inhibit bacterial DNA and RNA polymerase [7]. Many of these compounds induce aneuploidy and polyploidy. Another antimalarial drug Artemisinin and its derivatives possess the most rapid action of all current drugs against *Plasmodium falciparum*. Its ability to induce cell-cycle arrest, apoptosis [8] and blocking prostate cancer growth and cell cycle progression [9] reveals its nucleic acid binding potential.

Sulfonamides and diaminopyrimidines binds with enzymes and interfere with nucleic acid synthesis. Acridine derivatives are one of the oldest and most successful classes of bioactive agents. Acridines have also found wide use as antimalarial agents from the 1940s primarily in the form of mepacrine and pyronaridine. It has long been known that acridines bind preferentially to DNA and disrupt DNA function in cells [10]. These properties may also contribute to carcinogenic processes.

Mutagenicity and Genotoxicity Testing: Chemical or physical impacts can result in a fixed alteration in the genetic material (mutation) in cells, which lead to lethal or heritable defects. Mutation is defined as a permanent change in the amount or structure of the genetic material in a cell. Mutation applies both to heritable genetic changes that may be manifested at the phenotypic level and to the underlying DNA modifications like specific base pair changes or chromosomal translocations.

The term is used for agents giving rise to an increased occurrence of mutations in populations of cells and/or organisms. In order to identify the chemicals causing mutation, genetic tests and screening procedures have been developed (mutagenicity tests). The scientific field of mutagenicity testing uses various tests to screen newly synthesized chemicals for those that induce mutations.

A) **Test for Gene Mutations in Bacteria:**

Ames Test: This is the most widely used test for assessing the mutagenic properties of chemicals. The *Salmonella typhimurium* histidine reversion system is a microbial assay that uses a set of histidine-requiring strains of bacteria to detect frameshifts and base pair substitution mutations. Treatment with mutagens can induce the

mutations in the histidine operon and shift growth of the strains from a histidine-requiring to a histidine-independent pattern. The change in the growth phenotype represents an indicator of mutagenic response. The role of metabolic activation on the mutagenic effect of chemicals can be addressed by using metabolic activation fraction of rat liver homogenate mimicking *in vivo* situation [11].

B) **Test for Chromosomal Aberrations in Mammalian Cells *In Vitro*:** The chromosome aberration assay in cultured cells has been widely used for many years, and it has proved to be a useful and sensitive test for detection of genotoxic agents. The damage is scored by microscopic examination of chromosomes in mitotic metaphase cells. Tests are carried out with and without extrinsic metabolic activation [12].

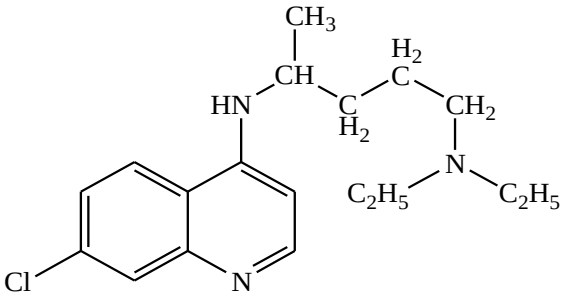
C) ***In vivo* Genetic Assays:** The relevance of this test is that a positive result found in bacteria can additionally studied in a system that has the complex eukaryotic chromosomal structure. This structural complexity also allows the possibility of detection of mutations arising through mechanisms that cannot occur in the simple bacterial genome. Suitable tests include those using mammalian cells designed to detect induction of mutations at specific loci such as those coding for the enzymes hypoxanthine-guanine- phosphoribosyl-transferase or thymidinekinase [13].

D) ***In vitro* and *in vivo* Micronucleus Assay:** The micronucleus test is used for detection of damage to the chromosomes or the mitotic apparatus induced [14]. Micronuclei are small particles consisting of acentric fragments, or entire chromosomes that lag behind at anaphase of cell division. After telophase, these fragments may not be included in the nuclei of daughter cells and form single or multiple micronuclei in the cytoplasm. The assay has been developed into *in vitro* and *in vivo* processes to detect clastogens and aneugens.

E) **Comet Assay:** The single cell gel electrophoresis (Comet assay) can be used to investigate the genotoxicity of industrial chemicals, biocides, agrochemicals and pharmaceuticals. Comet assay detects DNA strand breaks which, when subjected to electrophoresis, will result in migration of DNA fragments out of the nucleus to form the tail of a comet-like structure The extent of migration of DNA fragments is an indication of DNA damage that can be quantified. The comet assay is amenable for both *in vitro* (in any cell type) and *in vivo* or *ex-in vivo* in any species and in any target tissue. As a result, the comet assay can be incorporated in any routine toxicology experiments, which can add value without adding any extra animals. During early drug development, robust genotoxicity screening assays are required that reliably predict the outcome of the time and resource consuming regulatory tests. In this respect, the Comet assay is a promising tool because it is rapid, simple to perform, and requires only a small amount of test substance [15].

Mutagenic and Genotoxic Potential:

Chloroquine: The phosphate salt of chloroquine, that came into use in the mid 1940s, is used in oral dosage form and the hydrochloride salt is administered parentrally. The main site of action involve the lysosome of the parasite-infected erythrocyte. Chloroquine is not effective against exoerythrocytic parasites. Chloroquine is associated with retinopathy, haemolysis and impaired liver function [16]. At high doses chloroquine accumulates in eye and ear tissues [17]. Chloroquine is also accociated with in utero effects on rat lungs near term [18] and on dandritic maturation of hippocampal neurons [19].



STRUCTURE OF CHLOROQUINE

Roy *et al.,* [20] evaluated the genotoxic potential of chloroquine using chromosome aberration, micronucleus, and sperm head abnormality assays *in vivo* in Swiss albino mice. They observed that chloroquine induced chromosome aberration as well as micronucleus in the bone marrow cells.

Further the genotoxicity of chloroquine was evaluated by sperm head abnormality assay. There was a significant increase in the frequency of sperm head abnormality.

Mutagenic potential can be confirmed by mitotic depression of cell cycle. The effects of chloroquine on the mitosis of *Allium cepa* L. was investigated by Wangburuka *et al* [21]. Onion roots were treated with different concentrations of chloroquine. There was a significant difference among the mitotic indices for different concentrations of chloroquine. This suggests that chloroquine induced cell mitotic abnormalities like anaphase bridge, fault polarization of anaphase, chromosome fragmentation, disorderly anaphase, C-metaphase and clumping of chromosomes.

Chloroquine increases the number of chromosomal aberrations at certain doses. Cytogenic effects of chloroquine in human lymphocyte culture was studied by Shalumashvili *et al* [22]. In this finding, addition of chloroquine to a culture of human lymphocytes at the G_1 stage showed that the compound suppresses mitotic activity of the cells in concentrations of 60 and 100 µg/ml.

Ebor *et al.,* [23] used Ames plate reversion and fluctuation tests to evaluate the mutagenic and genotoxic potentials of chloroquine using different strains of *Salmonella typhimurium* and *Escherichia coli*. The results indicated that chloroquine had a potential to cause frame shift mutation.

Potential of chloroquine to cause frameshift mutation in different strains of *Salmonella typhimurium* and *E. coli* with rat liver S9 was studied by Thomas *et al* [24]. The result of the test suggested that chloroquine induces frame shift mutagenesis.

Potential of chloroquine to cause frame shift mutation was also confirmed by Cortinas de Nava *et al.,* [25] using fluctuation assay. The fluctuation assay showed chloroquine diphosphate to be mutagenic in *Salmonella typhimutium strain* TA1537, which detects frameshift mutation. Farombi *et al.,* [26] investigated genotoxicity of chloroquine in rat. They studied the effect of chloroquine using the alkaline comet assay. Chloroquine significantly increased DNA strand breaks of rat liver cells dose-dependently.

Further the concluded that the genotoxicity of chloroquine in rat liver cells might involve reactive oxygen species.

To extend the data of mutagenic effects of chloroquine, Xamena *et al* [27] tested chloroquine for its mutagenicity in *Drosophila melanogaster*. Sex-linked recessive lethals and sex-chromosome loss induction were studied following treatment of adult males using a feeding technique. Result indicated that Chloroquine increased significantly the frequency of sex-linked recessive lethal.

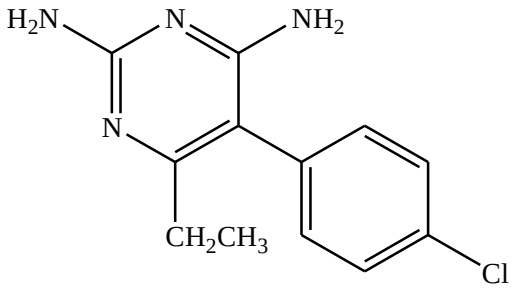
Mutagenic effects of chloroquine were also reported by Schupbach *et al* [28], in different salmonella strains. Similar investigation for mutagenicity of chloroquine in salmonella strains TA 1537, TA 1538, TA 98 and TA 98 by Espinosa-aguirre et al revealed the mutagenic potential of the drug.

In vitro interaction of chloroquine with human polymorphonuclear neutrophils was evaluated by Labro and Chevaye[29]. Phagocytosis was found to decreased in presence of chloroquine. The drug altered neutrophil oxidative metabolism which was assessed by luminol-amplified chemiluminescence.

Chloroquine have been reported to be weakly mutagenic in *Salmonella typhimurium* by Ames and Whitfield[30] and Thomas *et al* [31]. Mutagenic potential of the drug was also evaluated by Kadotani *et al* [32] in *Bacillus subtilis* and similar result was reported.

Genotoxic effects of chloroquine on Escherichia coli was studied by Espinosa-Aguirre *et al* [33]. The result showed that chloroquine was genotoxic in Escherichia *coli* pol A+/pol A−.

Pyrimethamine: Pyrimethamine was developed in 1950s, inhibits the reduction of folic acid and dihydrofolic acid to the active tetrahydrofolate coenzyme form. It is a slow acting erythrocytic schizontocide but does not eliminate the pre erythrocytic phase of *P. falciparum*. Pyrimethamine in combination with sulfadoxine is used for prohylaxis and treatment of chloroquine resistant *P. falciparum.* Megaloblastic anaemia and granulocytopenia occurs at high doses.



STRUCTURE OF PYRIMETHAMINE

The *in vivo* Genotoxic effects of pyrimethamine was studied in mice by Vijayalaxmi and Vishalakshi [34] using the bone marrow micronucleus test and the transplacental micronucleus test. Pyrimethamine was found to induce micronucleus in bone marrow cells.

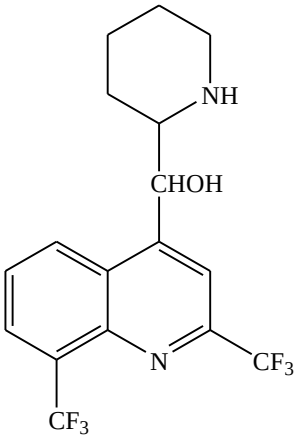
Similar activity was performed in *in vitro* Chinese hamster lung cells and in *in vivo* mouse bone marrow cells by Ono and Yoshimura [35]. Pyrimethamine strongly induced micronuclei in a dose-dependent manner in the *in vitro* micronucleus test using the Chinese hamster lung cell line, when treated at different doses for two days.

Aydemir *et al.,* [36] evaluated the genotoxic effects of pyrimethamine on spermatogenesis in male mice. In this test, the sperm shape abnormality, epididymal sperm counts, and testes weights were evaluated for thirtyfive days. Pyrimethamine increased the frequency of abnormal sperm shape. It is also found to decrease the epididymal sperm counts.

The lethal effects of pyrimethamine in germ cell of mouse was evaluated *in vivo* by Egeli *et al* [37]. They found that pyrimethamine induced dominant lethal mutations in the third, fourth and sixth weeks after pyrimethamine administration and concluded that pyrimethamine is a suspected germ cell mutagen.

Genotoxic effects on long term exposure to pyrimethamine on mice was studied by Tunca *et al* [38]. In this investigation, bone marrow cells of Swiss albino mice were exposed to different doses of pyrimethamine. Chromosome analysis and micro nucleus test were performed for evaluation of genotoxic effect. Structural chromosome aberrations and increased micronuclei revealed the mutagenic and genotoxic effects of pyrimethamine upon long term exposure. Tsuda *et al.,* [39] evaluated the potential of pyrimethamine to induce DNA damage in mouse embryo and maternal organs using alkaline single cell gel electrophoresis assay. Result indicated that the drug induced DNA damage in maternal and fetal placenta and embryos.

Mefloquine: Mefloquine was developed in the 1960s. It is a schizonticide and teratogenic in rats, mice and rabbits. Mefloquine causes skeletal and muscular malformations in rats at 5-20 times the therapeutic dose [40].



STRUCTURE OF MEFLOQUINE

Cytotoxicity and genotoxicity of the mefloquine was tested by Habit *et al* [41]. In this study, male mice were treated with different doses and the possible chromosomal abnormalities including gaps, breaks, and fragments were found to be increased with the higher dose of mefloquine.

Labro and Chevaye [29] also investigated the *in vitro* effect of mefloquine on human polymorphonuclear neutrophil function to correlate its cytotoxic behavior. It was found that mefloquine (100 and 50 micrograms/ ml) significantly altered polymorpho nuclear neutrophil viability. Phagocytosis was also found to decrease.

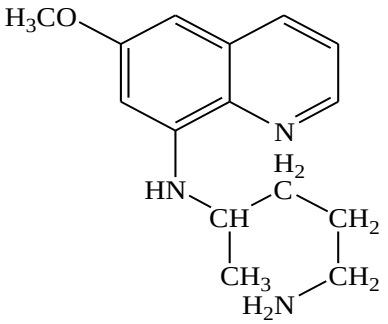
Chatterjee *et al.,* [42] also used antimalarial drug mefloquine to examine the genotoxic and mutatoxic potential. Investigation revealed that the drug is a weak mutagen, but capable of inducing significant sister chromatid exchange and chromosomal aberrations in the bone marrow cells of mice.

Karbwang and White [43] in 1990, detected the induction of lymphocytopenia at certain doses of mefloquine.

Genotoxicity tests by Akerele and Obaseiki-Ebor [44] in 2002, indicated that mefloquine was generally

genotoxic and induces base pair substitution mutagenesis with concentration dependent cytotoxicity. It had the same potential mutagenicity as chloroquine phosphate.

Primaquine: Primaquine has been widely used for the treatment of the hypnozoites (liver reservoirs) responsible for the relapsing forms of *Plasmodium vivax* and *Plasmodium ovale*. However, primaquine was recently reconsidered for malaria chemo-prophylaxis to eliminate *Plasmodium falciparum* at the early stage of infection, when parasite develops in the liver, thus preventing the clinical disease. Despite its good oral absorption, it has a short half-life and needs to be administered daily. Serious toxicity can be a major problem in patients with glucose-6-phosphate dehydrogenase deficiency.



STRUCTURE OF PRIMAQUINE

There are not much investigation on mutagenic or genotoxic effects of primaquine and related antimalarials however Ono *et al.,* [45] evaluatuated the mutagenicity of primaquine, pentaquine and pamaquine using *Salmonella typhimurium* and mammalian microsome assay. Primaquine and pentaquine induced mutations in *Salmonella typhimurium* in the presence and absence of microsomal activation system while Pamaquine was mutagenic to *Salmonella typhimurium* only in the absence of S9 mix.

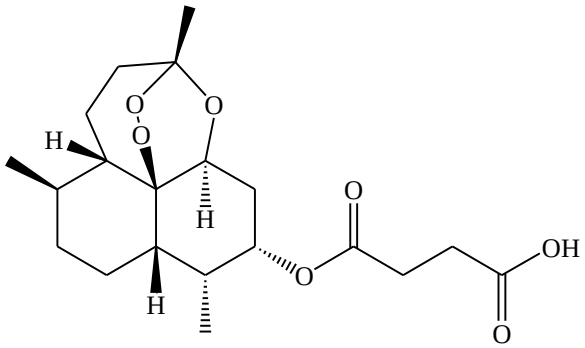
Using plate reversion method Marrs *et al.,* [46] observed mutagenic effect of primaquine in salmonella strain TA 1537 using a liquid preincubation assay.

Ono *et al.,* [47] reported the mutagenic effects in *Salmonella typhimurium* strain TA 97 both with and without S9. Primaquine was also found to be mutagenic in *Salmonella typhimurium* strain TA 100 without S9 by Shubber *et al*.

Noel *et al.,* [48] studied primaquine-induced differential gene expression analysis in mice liver using DNA microarrays. Analysis at RNA transcript level revealed consistent deregulation of 16 probes corresponding to important cellular processes such as protein transportation, transcription regulation, intracellular signaling, protein synthesis, hematopoiesis, cell adhesion and cell proliferation. Large number of affected genes were identified. Results indicated that primaquine affect gene expression in liver.

Mutagenic and genotoxic effects of primaquine were studied by Chatterjee *et al.,* [42] in various strains of *Salmonella typhimurium* using Ames mutagenicity assay, *in vivo* sister chromatid exchange and chromosome aberration assay in bone marrow of mice. Results showed that primaquine had a weak mutagenic effect in *Salmonella* strains. *In vivo* sister chromatid exchange and chromosome aberrataion assay indicated that the drug was genotoxic to bone marrow cells of mice.

Artimisinin: The most widely used artemisinine derivatives artemether and artesunate are semisynthetic derivatives of artemisinine, the active ingredient of the herb sweet wormwood (artemisia annua). They are obtained by reduction of the artemisinine's lacton substructure to a hemiacetal and subsequent methylation to the acetal artemether [49] or acylation to the succinic acid hemiester artesunate. Both compounds are rapidly transformed into dihydroartemisinine which has a rather short elimination half life of 40–60 minutes [50].



STRUCTURE OF ARTESUNATE

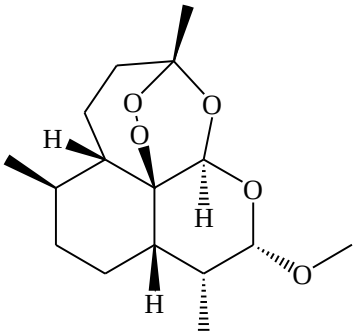


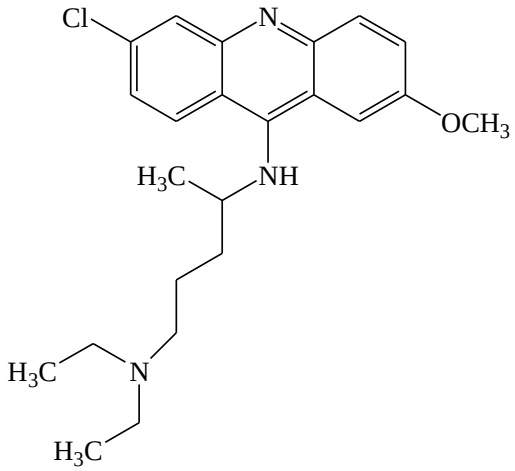
FIGURE 6: STRUCTURE OF ARTEMETHER

Artesunate is a derivate of artemisinin that is both an antimalarial agent and acts cytotoxically on tumor cells. Genotoxicity assessment of the antimalarial compound artesunate in somatic cells of mice was studied by Aquino *et al* [51]. Artesunate induced significant DNA damage in liver cells and high doses of artesunate caused an increase in the mean number of micronucleated polychromatic erythrocytes. The results demonstrate that artesunate exerts a weak genotoxic effects at low doses and clastogenic effects at high doses.

Genotoxicity and cytotoxicity of artisunate were also studied by Mota *et al.,* [52] in human lymphocyte. Comet assay and micronucleus test were used to evaluate the possible genotoxic effects of artesunate. Further, cell death by necrosis and apoptosis were also assessed. Different concentrations of artesunate showed a significant DNA damage and micronuclei formation with increased frequency of apoptotic and necrotic cells. Results showed that artesunate is a genotoxic and cytotoxic compound in cultured human lymphocytes.

Using gastric cell line, Alcantara *et al.,* [53] worked on cytotoxic and genotoxic effects of artemether. In this investigation, MTT (3-(4, 5-methylthiazol-2-yl)-2, 5-diphenyl-tetrazolium bromide) assay, comet assay and ethidium bromide/acridine orange viability staining tests were used to evaluate the cytotoxic and genotoxic effects. Result showed that artemether is genotoxic and cytotoxic to gastric cell line. On comparison with the work on human lymphocyte by Mata *et al*., the human lymphocyte were found to be more sensitive to the cytotoxic effects of the antimalarial drug artemether than the gastric cancer cell line.

Quinarine: Quinacrine is an acridine derivative introduced for malarial therapy in 1930 and was widely used before and after World War II. Quinacrine as an antimalarial has been superseded by other drugs with fewer side effects. At present, uses of quinqcrine include the treatment of giardiasis, systemic lupus erythematosis, and rheumatic diseases. In addition, quinacrine has been used as an anthelminthic and for female sterilization [54]**.**



STRUCTURE OF QUINACRINE

Clarke *et al.,* [55] evaluated the genotoxicity of quinacrine using Ames salmonella assay, mouse lymphoma assay, chromosome aberration test, micronucleus assay and effects on Chinese hamster ovary cells. Quinacrine was found to be mutagenic in Ames tester strain TA 100 and TA1535 with and without S9-activation. The drug was found to be mutagenic in the mouse lymphoma assay in the absence of S9-activation and clastogenic in Chinese hamster ovary cells, with and without S9-activation. The results confirmed that quinacrine was mutagenic and clastogenic *in vitro*.

Another test to confirm the mutagenic and genotoxic effects of quinacrine was done by Gasc and Sicard [56]. In this investigation, quinacrine induced frameshift mutation in pneumococcus. Result suggested that the drug was mutagenic**.** The *in vitro* cytogenetic activity was investigated by Krishnaja and Chauhan [57] in human peripheral blood lymphocytes. Quinacrine dihydro-chloride treated human lymphocytes were grown as whole blood cultures showed a wide range of chromosomal aberrations. Only few analyzable metaphase suggested that quinacrine dihydrochloride was cytotoxic.

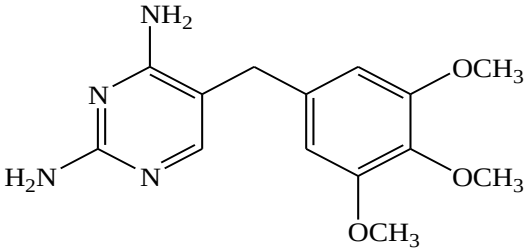
Further, the treatment exhibited chromosome aberrations including dicentrics, ring configurations, translocations, inversions, marker chromosomes, haploid, polyploid, and endoreduplicated cells were also observed.

Mutagenicity of quinacrine was also reported by Xamena *et al.,* [58] in *Drosophila melanogaster*.

Leduc *et al.,* [59] studied the effect of quinacrine on nuclear structure and RNA synthesis in cultured rat hepatocytes. They used ultrastructural cytochemical staining of ribonucleoprotein and DNA method, autoradiography, and measurement of labeled uridine uptake and incorporation method. Three regions of the chromatin were found to altered.

Perinuclear condensed chromatin retracted from the nuclear envelope, remained attached by short DNA-containing bridges, the normally dispersed nucleoplasmic chromatin was condensed into a stainable network which retracted centrifugally and the perinucleolar chromatin became a network of small highly condensed masses interconnected by fibrils which are either decondensed or stretched. These results indicated that quinacrine impaired the nuclear metabolism of rat hepatocytes.

Trimethoprim: Trimethoprim as a single agent used only for the treatment of uncomplicated urinary tract infections. It is closely related to several antimalarials but doesnot have good antimalarial activity by itself [16].



STRUCTURE OF TRIMETHOPRIM

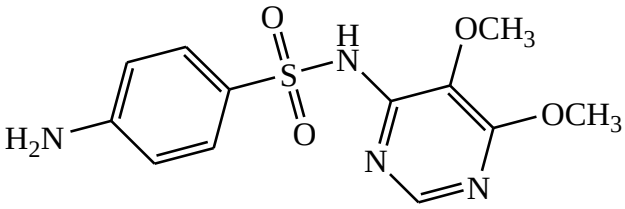
Papis *et al.,* [60] evaluated the *in vitro* cytotoxic and genotoxic effects of trimethoprim on fish and mammalian cell by neutral red retention, comet and micronucleus assays. Concentration- dependent cytotoxic effect of trimethoprim was observed for both the cell lines by all the three tests. The study showed that the cells of different origin exhibited both cytotoxic and genotoxic effects.

Cultured human lymphocytes were used for genotoxicity assessment of trimethoprim by Abou *et al* [61]. Frequency of Sister-chromatid exchange and micronuclei formation increased which revealed the cytotoxic and moderate genotoxic effects.

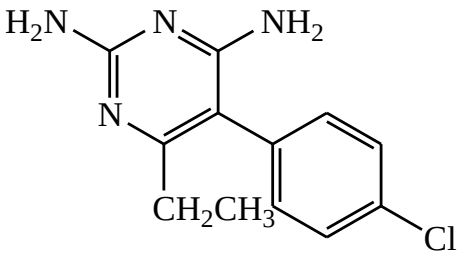
Binelli *et al.,* [62] evaluated cytotoxic and genotoxic effects of trimethoprim on zebra mussel hemocytes. In this study, the potential cellular damage was investigated by in vitro exposure. Genotoxicity was assessed by single cell gel electrophoresis assay and apoptosis frequency evaluation while the cytotoxicity was measured by the lysosomal membranes stability test. The result showed that trimethoprim was accociated with cytotoxic and genotoxic effects.

Another genotoxic evalution of the drug was carried out by Ono *et al* [63]. Bacterial umu test, the bacterial reverse mutation test, the *in vitro* chromosome aberration test, the *in vivo* rodent bone marrow micronucleus test in two different species and the in vivo comet assay in five mouse organs were used. Results of all the tests showed that the drug had a weak genotoxic effects.

Sulfadoxine-pyrimethamine: Pyrimethamine-sulfado xine has been used extensively worldwide for the treatment of chloroquine resistant *Plasmodium falciparum* malaria. Because of the wide usage of pyrimethamine-sulfadoxine in developing countries, the possible genotoxic effects has been evaluated by various scientists.



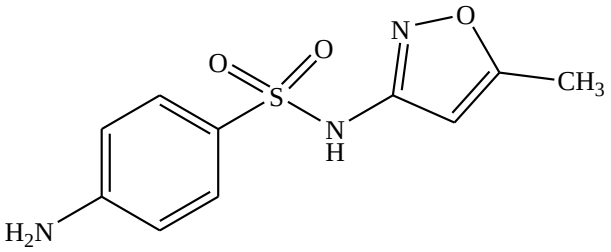
STRUCTURE OF SULFADOXINE



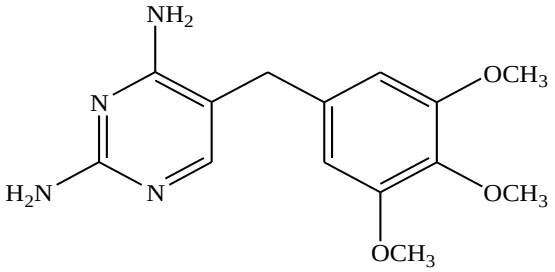
STRUCTURE OF PYRIMETHAMINE

Abou-Eisha and Afifi [64] evaluated the genotoxic effects of fansidar, a pyrimethamine-sulfadoxine combination in cultured human lymphocytes. Cultures were set up by using blood samples from two healthy donors and the treatment was done using different fansidar concentrations. Result indicated that the drug is able to induce moderate genotoxic effects, as there was an increase in sister chromatid exchange and micronuclei formation frequency in cultures.

Sulfamethoxazole-trimethoprim: The combination of trimethoprim and sulfamethoxazole is another widely used drug. Inspite of this there are only few reports on its genotoxicity and mutagenicity.



STRUCTURE OF SULFAMETHOXAZOLE

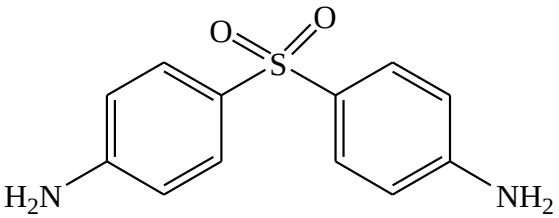


STRUCTURE OF TRIMETHOPRIM

The micronucleus assay is useful for detecting chromosome damage induced by nutritional deficiencies.

Ortiz *et al* [65] evaluated Trimethoprim-sulfamethoxazole combination for genotoxicity in peripheral blood of weanling rats using a flow cytometric analysis technique. The results indicated that drug treatment enhanced the DNA damage in rat peripheral blood reticulocytes.

Dapsone: Dapsone is used in the treatment of lepromatous and tuberculoid type of leprosy. Dapsone is also the drug of choice for dermatitis herpetiformis and is sometimes used with pyrimethamine for treatment of malaria. Its side effects includes hemolytic anaemia, methemoglobinemia and toxic hepatic effects [66].



STRUCTURE OF DAPSONE

Roy and Das [67] evaluated the *in vivo* cytogenic effects of Dapsone in mouse by cytogenetic assays. Adult male mice were treated with different doses of Dapsone and for different periods. Both the metaphase analysis and Micronucleus test in bone-marrow cells revealed significantly higher incidences of clastogenicity and chromosome aberration for all of the dose levels and treatment periods.

CONCLUSION: Long-term clinical trials and careful post marketing surveillance during the next several decades are needed to determine whether some of the antimalarial drugs cause cancer in humans. In the meantime, the results of *in vivo* and *in vitro* experiments in animals and humans suggest that antimalarial drug treatment especially with the quinoline derivatives should be avoided.

REFERENCES:

1. Kar S, Kar S. Control of malaria. Nat Rev Drug Discov 2010; 9:511-512.
2. Cohen SN, Yielding KL. Spectrophotometer studies of the interaction of chloroquine with deoxyribosenucleic acid. J Biol Chem 1965; 240:3123-31.
3. Strenglanz H, Yielding KC, Pruitt KM. Nuclear magnetic resonance studies of the interaction of chloroquine diphosphate with adenosine diphosphate and other nucleotides. Mol Pharmacol 1969; 5:376-81.
4. Kwakye-Berko F, Meshnick SR. Binding of chloroquine to DNA. Mol Bioche Parasitol 1989; 35:51-5.
5. Morris CR, Andrew LV, Whichard LP, Holbrook DJ. The binding of antimalarial aminoquinolines to aucleic acids and polynucleotides. Mol Pharmacol 1970; 6:240-50.
6. Whichard LP, Morris CR, Smith JM, Holbrook DJ. The binding of primaquine, pentaquine, pamaquine, and plasmocid to deoxyribonucleic acid. Mol Pharmacol 1968; 4:630-39.
7. Whichard LP, Washington ME, Holbrook DJ. The inhibition *in vitro* of bacterial DNA polymerases and RNA polymerase by antimalarial 8-aminoquinolines and by chloroquine. Biochim Biophys Acta 1972; 287:52–7.
8. Sen R, Bandyopadhyay S, Dutta A, Mandal G, Ganguly S, Saha P, *et al*. Artemisinin triggers induction of cell-cycle arrest and apoptosis in *Leishmania donovani* promastigotes. J Med Microbiol 2007; 56:1213-18.
9. Willoughby JA, Sundar SN, Cheung M, Tin AS, Modiano J, Firestone GL. Artemisinin blocks prostate cancer growth and cell cycle progression by disrupting Sp1 interactions with the

cyclin-dependent kinase-4 (CDK4) promoter and inhibiting CDK4 gene expression. J Biol Chem 2009; 23:2203-13.
10. Denny WA. Acridine derivatives as chemotherapeutic agents. Curr Med Chem 2002; 9:1655-65.
11. Maron DM, Ames BN. Revised methods for the salmonella mutagenicity test. Mutat Res 1983; 113:173-95.
12. Galloway SM. Cytotoxicity and chromosome aberrations *in vitro*: Experience in industry and the case for an upper limit on toxicity in the aberration assay. Environ Mol Mutagen 2000; 35:191-201.
13. Honma M, Hayashiand M, Shimada H. Mutagenicity testing applied for regulation of developing products. Mutagenesis 1992; 14:5-22.
14. Schmid W. Chemical mutagen testing on *in vivo* somatic mammalian cells. Agents Actions 1973; 3:77-85.
15. Kiskinis E, Suter W, Hartmann A. High throughput comet assay using 96-well plates. Mutagenesis 2002; 17:37-43.
16. Wilson CO, Gisvold O. Antimalarials. In: Troy DB, editor. Textbook of organic medicinal and pharmaceutical chemistry, 11th ed. New York: Lippincott Williams & Wilkins publishing; 2004. p. 287-8.
17. Lindquist NG, Sjostrand SE, Ullberg S. Accumulation of chorioretinotoxic drugs in the foetal eye. Acta Pharmacol Toxicol 1970; 28:64.
18. Okanlawon AO, Ejiwunmi AB, Dada MO, Ashiru OA. Chloroquine-induced retardation of foetal lung maturation in rats. Afr J Med Med Sci 1993; 22:61-7.
19. Pfau G, Westphal S, Dietzmann K, von Bossanyi P, Augustin W. Chloroquine effects on intrauterine and postnatal dendritic maturation of hippocampal neurons and on lipid composition of the developing rat brain. Exp Toxicol Pathol 1997; 49:361-7.
20. Roy LD, Mazumdar M, Giri S. Effects of low dose radiation and vitamin C treatment on chloroquine-induced genotoxicity in mice. Environ Mol Mutagen 2008; 49:488-95.
21. Nwangburuka CC, Oyelana OA. Cytological effects of chloroquine on root mitosis of *Allium cepa* (L.). Actasatech 2011; 4:25-35.
22. Shalumashvili MA, Siqidin YA. Cytogenetic effects of chloroquine in human lymphocyte cultures. Bull Exp Biol Med 1976; 82:1095-7.
23. Obaseiki-Ebor EE, Obasi EE. Aspects of chloroquine mutagenicity. Mutat Res 1986; 175:51-9.
24. Thomas SM, Silburn KA, MacPhee DG. Frameshift mutagenesis by chloroquine in Escherichia coli and Salmonella typhimurium. Mutat Res 1987; 192:233-7.
25. Cornitas de nava C, Espinosa J, Garcia L, Zapata AM, Martinez E. Mutagenicity of antiamoebic and anthelmintic drugs in *Salmonella typhimurium* test system. Mutat Res 1983; 117:79-91.
26. Farombi EO. Genotoxicity of chloroquine in rat liver cells: Protective role of free radical scavengers. Cell Biol Toxicol 2006; 22:159-67.
27. Xamena N, Creus A, Velazquez A, Marcos R. Testing of chloroquine and quinacrine for mutagenicity in *Drosophila melanogaster*. Mutat Res 1986; 158:177-80.
28. Schupbach ME. Mutagenicity evaluation of two antimalarial agents chloroquine and mefloquine using a bacterial fluctuation test. Mutat Res 1979; 68:41-9.
29. Labro MT, Chevaye CB. Effects of amodiaquine, chloroquine, and mefloquine on human polymorphonuclear neutrophil function *in vitro*. Antimicrob Agents Chemother 1988; 32: 1124-30.
30. Ames B, Whitfield H. Frame shift mutagenesis in salmonella. Cold Spring Harb Symp Quant Biol 1966; 31:221-25.
31. Thomas SM, Silburn KA, MacPhee DG. Frame shift mutagenesis by chloroquine in *E. coli* and *S. typhimurium.* Mutat Res 1987; 192:233-7.
32. Kadotani S, Arisawa M, Maruyama HB. Mutagenicity examination of several non steroidal anti-inflammatory drugs in bacterial system. Mutat Res 1984; 138:133-6.
33. Espinosa-Agguirre JJ, Ramirez Santos J, Cornitas de nava C. Influence of the Uvr repair system on the mutagenicity of antiparasitic drugs. Mutat Res 1989; 222:161-6.
34. Vijayalaxmi KK, Vishalakshi M. Evaluation of the genotoxic effects of pyrimethamine, an antimalarial drug, in the in vivo mouse. Teratog Carcinog Mutagen 2000; 20:65-71.
35. Ono T, Yoshimura H. Analysis of micronucleus induction of pyrimethamine in in vitro CHL cells and in *in vivo* mouse bone marrow cells. Mutagenesis 1996; 11:85-8.
36. Aydemir N, Celikler S, Bilaloglu R. In vivo evaluation of the toxic effects of pyrimethamine on spermatogenesis in male mice. J Environ Pathol Toxicol Oncol 2008; 27:287-93.
37. Egeli U, Aydemir N, Akpınar G, Cimen C, Ergul E, Tutar G, *et al. In vivo* dominant lethal effect of pyrimethamine in male mouse germ cells. Mutagenesis 1999; 14:67-9.
38. Tunca B, Egeli U, Aydemir N, Cecener G, Bilaloglu R. Investigation of the genotoxic effect in bone marrow of Swiss albino mice exposed long-term to pyrimethamine. Teratog Carcinog Mutagen 2002; 22:393-402.
39. Tsudaa S, Kosakaa Y, Matsusakaa N, Sasakib YF. Detection of pyrimethamine-induced DNA damage in mouse embryo and maternal organs by the modified alkaline single cell gel electrophoresis assay. Mutat Res 1998; 415:69–77.
40. Palmer KJ, Holliday SM, Brogden RN. Mefloquine. A review of its antimalarial activity, pharmacokinetic properties and therapeutic efficacy. Drugs 1993; 45:430-75.
41. El-Habit OH, Al-Khamash HS. Testing the cytotoxicity and genotoxicity of the antimalarial drug mefloquine. J King Saud Univ-Science. In Press
42. Chatterjee T, Mukhopadhyay A, Khan KA, Giri AK. Comparative mutagenic and genotoxic effects of three antimalarial drugs, chloroquine, primaquine and amodiaquine. Mutagenesis 1998; 13:619-24.
43. Karbwang J, White NJ. Clinical pharmacokinetics of mefloquine. Clin Pharmacokinet 1990; 19:264-79.
44. Okerele JO, Obaseiki-Ebor EE. Studies on the genotoxic and mutagenic potentials of mefloquine. Trop J Pharm Res 2002; 1:91-8.
45. Ono T, Norimatsu M, Yoshimura H. Mutagenic evaluation of primaquine, pentaquine and pamaquine in the Salmonella/ mammalian microsome assay. Mutat Res 1994; 325:7–10.
46. Mars TC, Bright JE, Morris BC. Metheglobinogenic potential of primaquine and its mutagenicity test in Ames test. Toxicol lett 1987; 36:281-7.
47. Ono T, Norimatsu M, Yosimura H. Mutagenic evaluation of primaquine, pentaquine and pamaquine in the salmonella/ mammalian microsome assay. Mutat res 1994; 325:7-10.
48. Noela S, Sharmaa S, Shankerb R, Ratha SK. Primaquine-induced differential gene expression analysis in mice liver using DNA microarrays. Toxicology 2007;239:96–107.
49. Bray PG, Ward SA, O'Neill PM. Quinolines and artemisinin: chemistry, biology and history. Curr Top Microbiol Immunol 2005; 295:3-38.
50. Posner GH, O'Neill PM. Knowledge of the proposed chemical mechanism of action and cytochrome p450 metabolism of antimalarial trioxanes like artemisinin allows rational design of new antimalarial peroxides. Acc Chem Res 2004; 37:397-404.

51. Aquino I, Perazzo FF, Maistro EL. Genotoxicity assessment of the antimalarial compound artesunate in somatic cells of mice. Food Chem Toxicol 2011; 49:1335-9.
52. Mota TC, Cardoso PC, Gomes LM, Vieira PC, Correa RM, Santana PD, *et al*. In vitro evaluation of the genotoxic and cytotoxic effects of artesunate, an antimalarial drug, in human lymphocytes. Environ Mol Mutagen 2011; 52:590-4.
53. Alcantara DD, Ribeiro HF, Cardoso PC, Araujo TM, Burbano RR, Guimaraes AC, *et al. In vitro* evaluation of the cytotoxic and genotoxic effects of artemether, an antimalarial drug, in a gastric cancer cell line (PG100). J Appl Toxicol 2011. In press.
54. Zipper J and Kessel E: Quinacrine sterilization: a retrospective. Int j gynaecol obstet 2003; 83:7-11.
55. Clarke JJ, Sokal DC, Cancel AM, Campen DB, Gudi R, Wagner VO, *et al*. Re-evaluation of the mutagenic potential of quinacrine dihydrochloride dihydrate. Mutat Res 2001; 494:41-53.
56. Gasc AM, Sicard AM. Genetic Studies of acridine-induced mutants in *Streptoccos pneumoniae***.** Genetics 1978; 90:1-18.
57. Krishnaja AP, Chauhan PS. Quinacrine dihydrochloride, the non-surgical female sterilant induces dicentrics, rings, and marker chromosomes in human peripheral blood lymphocytes treated in vitro: a preliminary report. Mutat Res 2000; 466:43–50.
58. Xamena N, Creus A, Velazqez A, Marcos R. Testing of chloroquine and quinacrine for mutagenicity in Drosophilla melanogaster. Mutat Res 1985; 1558:177-180.
59. Leduc EH, Bernhard W, Viron A, Fain J, Puvion E. Effect of quinacrine on nuclear structure and RNA synthesis in cultured rat hepatocytes. Cancer Res 1981; 41:2832-41.
60. Papis E, Davies SJ, Jha AN. Relative sensitivity of fish and mammalian cells to the antibiotic, trimethoprim: cytotoxic and genotoxic responses as determined by neutral red retention, Comet and micronucleus assays. Ecotoxicology 2011; 20:208-17.
61. Abou-Eisha A, Creus A, Marcos R. Genotoxic evaluation of the antimicrobial drug, trimethoprim, in cultured human lymphocytes. Mutat Res.1999; 440:157-62.
62. Binelli A, Cogni D, Parolini M, Riva C, Provini A. Cytotoxic and genotoxic effects of *in vitro* exposure to Triclosan and Trimethoprim on zebra mussel (*Dreissena polymorpha*) hemocytes. Comparative Biochemistry and Physiology Part C: Toxicol Pharmacol 2009; 150:50-6.
63. Ono T, Sekiya T, Takahashi Y, Sasaki YF, Izumiyama F, Nishidate E, *et al*: The genotoxicity of diaveridine and trimethoprim. Environ Toxicol Pharmacol 1997; 3:297-306.
64. Abou-Eisha A, Afifi M: Genotoxic evaluation of the antimalarial drug, fansidar, in cultured human lymphocytes. Cell Biol Toxicol 2004; 20:303-11.
65. Rocio O, Hilda M, Edith C, Elsa C, Leonor R. Trimethoprim-sulfamethoxazole increase micronuclei formation in peripheral blood from weanling well-nourished and malnourished rats. Environ Mol Mutagen 2011; 52:673-80.
66. Wilson CO, Gisvold O. Anti-infective agents. In: Troy DB, editor. Textbook of organic medicinal and pharmaceutical chemistry, 11th ed. New York: Lippincott Williams & Wilkins publishing; 2004. p. 279.
67. Roy B, Das RK. Cytogenetic effect of dapsone, an antileprotic drug, in the mouse in vivo system. Int J Lepr Other Mycobact Dis 1988; 56:574-9.

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