e-ISSN: 2248-9126
Vol 6|Issue 2| 2016 |78-86. Print ISSN: 2248-9118

Indian Journal of Pharmaceutical Science & Research

www.ijpsrjournal.com

# GENOTOXIC AND MUTAGENIC EFFECTS OF THREE COMMONLY USED ANTIAMOEBIC DRUGS: METERONIDAZOLE, TINIDAZOLE AND CHLOROQUINE

**RakeshSahu*[1], Pankaj Kashyap[1], Arpan Kumar Tripathi[1], Dushmanta Kumar Pradhan[1] and Pranita Kashyap[2]**

[1]Department of Pharmacy, Apollo College of Pharmacy, Anjora, Durg, Chhattisgarh, India.
[2]Department of Pharmacy Shri Rawatpura Sarkar Institute of Pharmacy, Kumhari, Chhattisgarh, India.

**ABSTRACT**

Amoebiasis is a major public health problem in developing countries. 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 antiamoebic drugs as a part of pre-clinical trials, there are several literatures confirming the mutagenicity and genotoxicity of marketed antiamoebic 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 antiamoebic drugs are presented.

**Keywords:** Mutagenecity, Genotoxicity, Amoebiasis, Antiamoebic, Metronidazole, Tinidazole, Chloroquine.

## INTRODUCTION

Amoebiasis is a major public health problem in tropical and subtropical countries. Amoebiasis refers to infection caused by protozoan *Entamoeba histolytica* [1]. *Entamoeba histolytica*, has the capacity to invade intestinal mucosa resulting in intestinal amoebiasis and cause extra intestinal amoebiasis [2].Worldwide, 40-50 million symptomatic cases of amoebiasis occur annually and 70,000 to 100,000 deaths due to this infection [3]. There are two distinct, but morphologically identical species of *Entamoeba*: *Entamoeba histolytica*, which is pathogenic and *Entamoeba dispar*, which is non-pathogenic. Other species of *Entamoeba* which infects humans are *Entamoeba hartmanni*, *Entamoeba coli*, *Entamoeba moshkovskii*. This gastrointestinal infection may or may not be symptomatic and can remain latent in an infected person for several years. Symptoms canrange from mild diarrhoea to dysentery with blood and mucus in the stool. Invasion of the intestinal lining by parasite causes amoebic dysentery or amoebic colitis. If the parasite reaches the bloodstream it can spread through the body, most frequently ending up in the liver where it causes amoebic liver abscesses. Liver abscesses can occur without previous development of amoebic dysentery. When no symptoms are present, the infected individual is still a carrier, able to spread the parasite to others through poor hygienic practices. Amoebiasis is usually transmitted by the fecal-oral route, but it can also be transmitted indirectly through contact with dirty hands or objects as well as by anal-oral contact. Infection is spread through ingestion of the cyst form of the parasite. Drugs of choice for invasive

Corresponding Author:-**Rakesh Sahu** Email: rakesh_sahu2010@rediffmail.com

amoebiasis are tissue active agents, like metronidazole, tinidazole and chloroquine or the more toxic emetine derivatives, including dehydroemetine.

**Life cycle of *Entamoeba histolytica***

Humans are infected by the amoeba through ingestion of water or food containing cysts of *E. histolytica*, the infective stage of the parasite. In the host, the cysts become the active living form of the parasite known as trophozoites. These trophozoites live in the mucosal layer of the colon, and occasionally invade other organs. Encystation and excystation are the two major differentiation events essential for completion of *E. histolytica* life cycle in the human intestine [4-6]. Trophozoite lyses the host cell and invades the mucosa. This process may result in dysentery. The parasite then invades the liver resulting in liver abscesses. 'Carriers' passes the cysts in their faeces which infect other individuals [7]. Encystation is a complex process, involving intracellular rearrangements, consumption of glycogen reserves, formation of ribosome aggregates, changes in gene expression and transcription, protein synthesis and the deposition of a chitin cyst wall.

**Antiamoebic drugs**

Antiamoebic drugs are classified into luminal, tissue, and mixed amoebicides. Metronidazole is the major drug of choice in the treatment of amoebasis. Other nitroimidazole derived compounds like tinidazole, secnidazole and ornidazole are equally effective in the treatment. Diloxanidefuroate, diiodohydroxyquin, paromomycin, emetine and chloroquine have also been used as alternate drugs. Diloxanide furoate is the mainstay for treating asymptomatic cyst carriers [8].Chloroquine is used along with metronidazole/emetine in cases of hepatic amoebiasis. However, emetine is rarely used on account of its toxicity. Metronidazole, tinidazole and other 5-nitroimidazole agents which kill the trophozoites by alterations in the protoplasmic organelles of the amoeba are ineffective in treating cyst passers. Whereas, chloroquine acts on the vegetative forms of the parasite and kills it by inhibiting DNA synthesis, emetine kills the trophozoites mainly by inhibiting protein synthesis [9].

**Mechanism of drug resistance in *E. hystolytica***

The mechanisms of drug resistance hypothesized in protozoan parasite are decrease of drug uptake because of loss of a transporter required foruptake, the efflux of drugs from the parasite either by the P-glycoproteins (Pgp) or by ATPases, the alteration of drug target, and loss of drug activation [10].

**GENOTOXIC AND MUTAGENIC PROFILE**

**Metronidazole**

Metronidazole is nitroimidazole derivatives that have proven to be effective against *Entamoeba histolytica*, *Trichomonas vaginalis*, *Giardia lamblia* and *Helicobacter pylori*. It is also a first-line drugs in the prophylaxis of perioperatory infections of the digestive tract or gynecological surgery [11, 12]. The drug is effective orally against both the acute and carrier states of disease. Recently, metronidazole has been found to possess efficacy against obligate anaerobic bacteria, but it is ineffective against facultative anaerobes or obligate aerobes. It is mainly effective against Gram-negative anaerobic *Bacteroide*s and *Fusobacterium* species. It is also effective against Gram-positive anaerobic *Clostridium*, *Peptococcus* and *Peptido streptococcus* species [13].

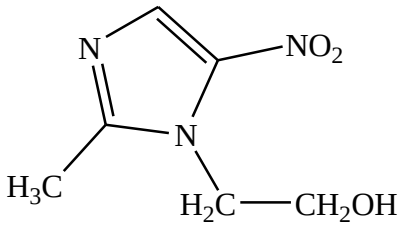



**Fig 1. Metronidazole**

Metronidazole when given intravenously or orally at usual recommended dose, attains concentrations well above the minimum inhibitory concentrations for most susceptible micro-organisms. The drug has an oral bioavailability approaching 100%. Rectal and vaginal administration results in a smaller amount of drug absorption and lower serum concentrations. Metronidazole has limited plasma protein binding but can attain very favorable tissue distribution, including into the central nervous system. The drug is extensively metabolized by the liver to form two primary oxidative metabolites, the hydroxy and acetic acid metabolites. The kidney is responsible for the elimination of only a small amount of the unmetabolized metronidazole [14]. In critically ill patients with liver and renal dysfunction, the half life of metronidazole was found to be beupto 42 hours while renal clearance ranges from 0·281–1·17 ml/min/kg [15].

The reactive intermediate formed in microbial reduction of 5-nitro group of metronidazole covalently binds to DNA of the microorganism [10]. Metronidazole has been reported to bind and damage DNA [16]. Binding of metronidazole to nucleic acids were quantitated and characterized *in vitro* [17].

Using Ames *Salmonella* assay, Dayan *et al* [18] in 1982 concluded that nitroimidazole derivatives are mutagenic in *Salmonella typhimurium*. Further, in 1983, Forty *et al* [19] concluded that Metronidazole causes base-pair mutation in *Salmonella typhimurium* and is thus a potent mutagen.

Potential of metronidazole to cause DNA strand break was studied by Reitz *et al*[58]in lymphocytes of human. Results indicated that the recommended dose of metronidazole induces DNA single strand-breaks.

In another research work, Reitz *et al.*, [20] studied the induction of DNA strand breaks in cultures of human lymphocytes and phytohemagglutinin-stimulated human lymphocytes. Results showed that metronidazole induced DNA single strand-breaks in resting human cells (non-stimulated lymphocytes) and in proliferating human cells dose dependently.

Potential of metronidazole to produce DNA strand break was also evaluated by Ferreiro *et al* [21] in peripheral blood lymphocytes. Findings suggested that primary damage is induced under aerobic conditions and confirmed that metronidazoles is a DNA damaging agent.

The genotoxic effect of metronidazole was studied in primary cultures of both rat and human hepatocytes by Martelli*et al* [22].Results indicated that metronidazole produced DNA fragmentation. The amount of DNA damage was directly related to the dose and the length of exposure of hepatocytes to metronidazole.

In the study of mutagenic effects by Wegman *et al* [23], gene mutations at the HPRT locus were determined in peripheral blood lymphocytes of sheep treated with metronidazole at therapeutic doses for amebiasis. Further, pharmacokinetic studies suggested that the animals with the lowest elimination rates of metronidazole had increased gene mutations.

Mudry *et al* [24] studied the genotoxic activity of metronidazole with the anaphase-telophase test in a CHO cell line, Chromosomal aberration and Micronucleus test in lymphocyte cultures and bone marrow cells. An increase in micronucleated cells and increase in the percentage of abnormal anaphases demonstrated the genotoxic effect of metronidazole.

Another report concerning the evaluation of chromosomal aberrations in cells from patients treated with metronidazole showed an increased frequency of damage after treatment [25].

Using different doses of metronidazole, genotoxic activity was evaluated by Re *et al* [26] in human lymphocytes using Comet assay. Results indicated that metronidazole induced DNA damage in a dose dependent manner but the potential of the drug to cause genotoxicity was decreased in the presence of S9 mix.

Another study was performed by Elizondo *et al* [27] to evaluate the genotoxicity of metronidazole therapy. In this study, chromosome aberration was noticed in peripheral blood lymphocyte cultures suggesting that the drug treatment induced breaking of chromosome and isochromosome.

Mohn *et al* [28] found that the drug induces forward and back mutations in *Escherichia coli*, forward mutation in *Nuerosporacrassa*, mitotic gene conversion in *Saccharomyces cerevisiae* and sex-linked recessive lethal in *Drosophila melanogaster*.

Mutagenic and genotoxic activities of metronidazole were evaluated by Meo *et al* [29] using modified version of Ames test and colorimetric SOS Chromotest. In this experimental work *Salmonella typhimurium* tester strain TA 100 was used with and without the addition of metabolic activation in Ames test. For SOS chromotest, *Escherichia coli* tester Strain PQ 37 was used with and without metabolic activation. Result showed that metronidazole was mutagenic and genotoxic.

DNA damage was determined by Menendez *et al* [30] using single cell gel electrophoresis assay in lymphocytes from healthy subjects treated with therapeutic doses of the drug. Findings suggested that metronidazole induced DNA damage.

Celik*et al* [31] evaluated the mutagenicity and cytotoxicity of metronidazole at therapeutic plasma concentration in cultured human peripheral blood lymphocytes. Result indicated that meronidazole was mutagenic and cytotoxic at plasma concentration.

Buschini*et al* [32] evaluated the cytotoxic and genotoxic potential of metronidazole using a number of assay methods. Metronidazole was found to be mutagenic in *Salmonella* Plate incorporation test. The drug was found to be genotoxic in anoxic condition.

Cavas and Gozukara [33] evaluated the effect of different doses of metronidazole on fish (*Oreochromis niloticus*) by using the piscine micronucleus test. Results revealed that metronidazole had cytotoxic and genotoxic effects.

Genotoxicity of metronidazole in the peripheral blood lymphocytes of *Cebus libidinosus* was evaluated Sister chromatid exchange and mitotic index values were found to be different as compared to that of control suggesting genotoxic nature of the drug.

Herbert *et al* [34] confirmed the mutagenicity of metronidazole. There results indicated that the mammalian enzymes can activate metronidazole to a genetically active intermediate which may have a direct relevance to the carcinogenicity.

Genotoxicity of metronidazole was assessed in vitro by Sekis *et al* [35] in feline peripheral blood mononuclear cells and feline T-cell lymphoma line and *in vivo* in feline peripheral blood mononuclear cells using Comet assay. They detected genotoxicity at all concentration of Metronidazole.

Akyol *et al* [36] used sister-chromatid exchange test to evaluate the genotoxic potential of metronidazole in lymphocytes. Results showed that metronidazole had genotoxic effects in lymphocytes.

The ability of oral metronidazole administration to induce genotoxic damage in somatic cells of mice was evaluated by El-Nahas and El-Ashmawy [37] using mitotic index, micronuclei and chromosomal aberration tests. Reduction in mitotic activity, increase in the frequency of chromosomal aberration and micronucleus showed that metronidazole has the ability to induce genotoxic effects in somatic cells.

Ornelas-Aguirre *et al* [38] worked on the genotoxicity of metronidazole on vaginal cell of rat.

Results indicated that topical administration of high concentration of metronidazole was genotoxic in rat vaginal mucosa cells.

Analysis of induction of chromosomal aberration after incubation of Chinese hamster V79-379A cells with metronidazole in aerobic and hypoxic conditions was done by Korbelik and Horvat [39]. Results showed that metronidazole exhibits a significant clastogenic action.

Metronidazole was tested for mutagenicity on a nitroreductase-positive (TA 100) and a nitroreductase-deficient (TA 100-$FR_1$) strain of *Salmonella typhimurium* by Lindmark and Muller [40]. Result showed that the drug induced mutation in both strains of *Salmonella typhimurium.*

Ong and slade evaluated the mutagenicity of metronidazole in *Neurospora crassa*. Results of their work indicated that the compound was mutagenic in growing vegetative cells of in *Neurospora crassa* [41].

Urine of patients administered with metronidazole also showed mutagenic effect. Paper chromatographic separation revealed that mutagenicity in the urine was associated with unmodified metronidazole and at least four of its known urinary metabolites [42].

The *in vivo* mutagenic potential of metronidazole was evaluated in *Bacteroides fragilis.* The forward and back mutations were identified by nucleotide sequence analysis with GC→CG transversion. Results showed that metronidazole induced base pair substitutions and DNA strand break [43].

Sisson *et al.* [44] reported that metronidazole is mutagenic and causes DNA fragmentation in *Helicobacter pylori* and *Escherichia coli.*

Carcinogenicity of metronidazole was evaluated by Rustia and Shubik [45]. They reported that the drug is able to induce lung tumors and malignant lymphomas in mice.

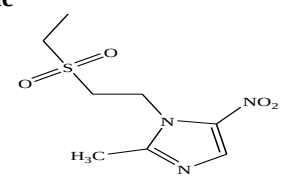
Yeung *et al* [46] evaluated the mutagenicity of metronidazole in *Escherichia coli*. Results suggested that the metabolite of the drug interacted with DNA and produced lesion similar to that caused by ultra violet light.

Wulf concluded that metronidazole is genotoxic as it is has the ability to induce sister chromatid exchange in lymphocytes [47].

The genotoxic effect of metronidazole was studied by Martelli*et al*[48] in primary cultures of both rat and human hepatocytes. The result also suggested that the drug induces genotoxic effect and the amount of DNA damage was directly related to the dose and the length of exposure to the drug.

**Tinidazole**

**Fig 2.Tinidazole**




Tinidazole, a second-generation 5-nitroimidazole compound chemically related to metronidazole, has been widely used throughout Europe and developing countries for the treatment of amoebic and parasitic infections [49].

Metabolism of minidazole is slower and is suited for once daily therapy. Tinidazole exhibits activity against a wide range of pathogenic protozoa (*Tricbomonas vaginalis, Entamoeba histolytica, Giardia duodenalis*), clinically significant anaerobic bacteria (*Bacteroides fragilis, Clostridium difficile*), and the microaerophilic bacterium *Helicobacter pylori*. In susceptible protozoal and bacterial cells, tinidazole is reduced to cytotoxic intermediates that covalently bind to DNA, causing irreversible damage. In human adults, tinidazole has a bioavailability of 100% and a volume of distribution $V_{(d)}$ of 50.7 L. It is almost 12% bound to plasma protein with plasma elimination half life of 12.3 hours. It is eliminated primarily by hepatic metabolism. Clinical cure rates in patients with trichomoniasis, giardiasis, amebiasis, and amebic liver abscess are generally >90%. Tinidazole is more effective in treatment of amebiasis and giardiasis [50].

Tinidazole is a prodrug which is converted to cytotoxic forms *in vivo*. After diffusing into the cells of susceptible organisms (protozoa or anaerobic bacteria), tinidazole is reduced at its nitro group to short-lived, toxic radicals by a ferredoxin-mediated electron transport system. Experimental evidence suggests that the toxic intermediates covalently bind to DNA, resulting in DNA damage in the form of loss of helical structure, impaired template function, and strand breakage, which eventually lead to cell death [51, 52].

Genotoxic and cytotoxic potential on tinidazole at therapeutic concentration was evaluated by Nigro and Carballo [53] in cultured human lymphocytes using mitotic index, replication index, sister chromatid exchange and chromosomal aberrations tests. Results concluded that tinidazole is genotoxic, cytotoxic and is able to modulate cell death through apoptotic mechanisms.

Voogd *et al* [54] evaluated the mutagenicity of tinidazole using fluctuation test in three bacterial strains *Klebsiella pneumonia*, *Escherichia coli* and *Citrobacter freundii.* Result of the test revealed the mutagenic potential of tinidazole.

Possibility of the metabolites of tinidazole to produce mutagenic effect was assessed in bacterial strains by Gupta *et al* [55] They used Ames *Salmonella* plate reversion method in presence of mamalinam liver S9 mix. Results suggested that the metabolite of the drug was mutagenic in *Salmonella typhimurium* strain TA100 and YG 1029.

Similar work was carried out by Lindmark and Muller [56] in *Salmonella typhimurium* strain TA 100 and TA 100-FR(1). Their finding suggested that the drug is a mutagent to both bacterial strains under aerobic and anaerobic conditions.

Coulter and Turner [57] also reported tinidazole to be mutagenic in *Salmonella typhimurium* assay. Potential of tinidazole to produce abnormal sperm was reported by Pylkkanen and Lahdetie [58].

Urine of patients under tinidazole therapy showed greater mutagenic effect as compared to that of nonmetabolized drug in *Salmonella typhimurium*. This suggested that metabolism of the drug produces compounds with greater mutagenic potency [59].

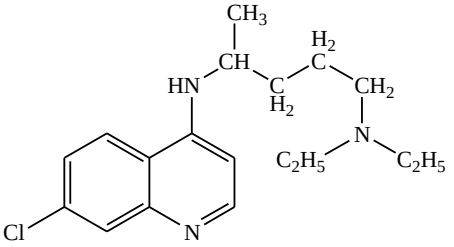
Potential of tinidazole to produce DNA strand break was evaluated by Ferreiro *et al* [60] in peripheral blood lymphocytes. Findings suggested that primary damage is induced under aerobic conditions and confirmed that tinidazole is a DNA damaging agent.

Mutagenic activity of tinidazole was screened by Melo and Ferreira [61] using Simultest in *Salmonella typhimurium* starins TA 97, TA 98, TA 100 and TA 102. The drug gave positive result indicating its mutagenic potency.

Nigro *et al* [62] evaluated the potential of tinidazole to cause DNA damage in human lymphocytes.The results revealed the genotoxic and cytotoxic effect of tinidazole in human peripheral blood cultures *in vitro*.

**Chloroquine**

**Fig 3.Chloroquine**




Chloroquine, an antiamebic and anti-inflammatory drug, is used in the treatment of malaria, giardiasis, extraintestinal amebiasis, lupus erythematosus, and rheumatoid arthritis. The phosphate salt of chloroquine came into use in the mid 1940s, is used as an oral dosage form and the hydrochloride salt is administered parentrally. The main site of action involves the lysosome of the parasite. Chloroquine is associated with retinopathy, haemolysis and impaired liver function [63]. At high doses chloroquine accumulates in eye and ear tissues [64]. Chloroquine is also associated with in utero effects on rat lungs near term [65] and on dandritic maturation of hippocampal neurons [66].

Roy *et al* [67] 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* was investigated by Wangburuka *et al* [68]. Onion roots were treated with different concentrations of chloroquine. There was a significant difference among the mitotic indices for different concentrations of chloroquine. This suggested 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* [69] 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* [70] used Ames plate reversion and fluctuation tests to evaluate the mutagenic and genotoxic potential 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 frame shift mutation in different strains of *Salmonella typhimurium* and *Escherichia coli* with rat liver S9 was studied by Thomas *et al.*[71] The result of the test suggested that the drug induced frame shift mutation.

Potential of chloroquine to cause frame shift mutation was also confirmed by Cortinas de Nava *et al* [72] using fluctuation assay. The fluctuation assay showed chloroquine diphosphate to be mutagenic in *Salmonella typhimutium strain* TA1537, which detects frame shift mutation.

Farombi*et al* [73] 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* [74] 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 significantly increased the frequency of sex-linked recessive lethal.

Mutagenic effects of chloroquine were also reported by Schupbach *et al* [75], 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 [76]. Phagocytosis was found to decrease 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 [77] and Thomas *et al* [78] Mutagenic potential of the drug was also evaluated by Kadotani *et al.*, [79] in *Bacillus subtilis* and similar results were reported.

Genotoxic effect of chloroquine on *Escherichia coli* was studied by Espinosa-Aguirre *et al* [80] Result showed that chloroquine was genotoxic in Escherichia coli pol A+/pol A−.

## CONCLUSION

Genotoxic effects of antiamoebic drugs should be taken into account considering its wide human consumption. Long-term clinical trials and careful post marketing surveillance during the next several decades are needed to determine whether some of the antiamoebic drugs cause cancer in humans.

## ACKNOWLEDGEMENT

The authors are thankful to the faculties, Apollo college of Pharmacy, Durg, for providing support for the completion of this work.

## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

## REFERENCES

1. World Health Organization. Amoebiasis. Report of a WHO Expert Committee.*WHO Technical Report Series*, 421, 1969, 1-52.
2. Diamond LS, Clark CG. A redescription of *Entamoeba histolytica* Schaudinn 1903 (emended walker, 1911) separating it from *Entamoe badispar* Brumpt.*J Eukaryot Microbiol*, 40,1993, 340-344.
3. Walsh JA. Problems in recognition and diagnosis of amoebiasis: estimation of the global magnitude of mortality and morbidity.*Rev Infect Dis*,8, 1986, 228-238.
4. Chavez-Munguia B, Omana-Molina M, Gonzalez-Lazaro M *et al*. Ultrastructure of cyst differentiation in parasitic protozoa. *ParasitolReserch*, 100, 2007, 1169-1175.
5. Eichinger D. A role for a galactoselectin and its ligands during encystment of *Entamoeba*. *Eukaryot Microbiol*, 48, 200, 17–21.
6. Mirelman D, Avron B. Cyst formation in *Entamoeba*. In: Ravdin JI, editor. Amebiasis: Human infection by *Entamoebahistolytica*. New York, Wiley Medical Publication, 1988, 768-781.
7. Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology. 5th ed. London, Churchill livingstone, 2003.
8. Knight R. The chemotherapy of amoebiasis. *J Antimicrob Chemother*, 6, 1980, 577-593.
9. Ravdin JI, Petri WA. *Entamoeba histolytica*. Mandell, Douglas and Bennett's principles and practice of infectious disease. New York: Churchill Livingstone, 1995, 2395-2408.
10. Borst P, Ouellette M. New mechanisms of drug resistance in parasitic protozoa. *Annu Rev Microbiol*, 49, 1995, 427-460.
11. Brismar B, Edlund C, Malmborg A, Nord C. Ecological impact of antimicrobial prophylaxis on intestinal microflora in patients undergoing colorectal surgery. *Scand. J Infect Dis*, 70, 1990, 25-30.
12. Richle Von R, Scholer HJ, Angehrn P *et al*. Basic chemotherapy of thrichomoniasis and amoebiasis with ornidazole. *Arzneim. Forsch*, 28, 1978, 612-625.
13. Beale JM. Anti-infective agents, In Troy DB, editor. Textbook of organic medicinal and pharmaceutical chemistry, New York, Lippincott Williams & Wilkins, 2004, 260.
14. Lau AH, Lam NP, Piscitelli SC, Wilkes L, Danziger LH. Clinical pharmacokinetics of metronidazole and other nitroimidazole anti-infectives. *Clin Pharmacokinet*, 23, 1992, 328-364.
15. Plaisance KI, Quintiliani R, Nightingale CH. The pharmacokinetics of metronidazole and its metabolites in critically ill patients. *J. Antimicrob. Chemother*, 21, 1988, 195-200.
16. Edwards DI. Reduction of nitroimidazoles and DNA damage. *Biochem Pharmacol*, 35, 1986, 53-58
17. Lopez Nigro MM, Carballo MA. Genotoxicity and cell death induced by tinidazole (TNZ).*ToxicolLett*, 180, 2008, 46-52.
18. Dayan J, Crajer MC, Deguingand S. Mutagenic activity of four active-principle forms of pharmaceutical drugs: comparative study in the *Salmonella typhimurium* microsome test, and the HGPRT and Na+/K+ATPase systems in cultured mammalian cells. *Mutat Res*, 102, 1982, 1-12.
19. Cantelli-Forti G, Aicardi G, Guerra MC, Barbaro AM, Biagi GL. Mutagenicity of a series of 25-nitroimidazolesand two nitrothiazoles in *Salmonella thypimurium*. *Teratogen Carcinogen Mutagen*, 3, 1983, 51-63.
20. Reitz M, Rumpf M, Knitza R. Metronidazole induces DNA strand-breaks in cultures of human lymphocytes and phytohemagglutinin-stimulated human lymphocytes. *Drug Res*, 41,1991, 65-69.

21. Ferreiro GR, Badias LC, Nigro ML *et al*. DNA single strand breaks in peripheral blood lymphocytes induced by three nitroimidazole derivatives. *Toxicology Letters*, 132, 2002, 109-111.
22. Martelli A, Allavena A, Robbiano L, Mattioli F, Brambilla G. Comparision of the sensitivity of human and rat hepatocytes to the genotoxic effects of metronidazole. *PharmacolToxicol,* 66, 1990, 329-334.
23. Ostrosky-Wegman P, Lares I, Santiago P, Elizondo G, Montero R. Metronidazole hprt mutation induction in sheep and the relationship whit its elimination rate. *Mutat Res*, 307, 1994, 253-259.
24. Mudry MD, Carballo M, Labal de Vinuesa M, Cid MG, Larripa I. Mutagenic bioassay of certain pharmacological drugs. III. Metronidazole (MTZ). *Mutat Res*, 305, 1995, 127-132.
25. Mitelman F, Hartley-Asp B, Ursing B. Chromosome aberrations and metronidazole. *Lancet*, 2, 1976, 802.
26. Donald GL and Miklos M. Antitrichomonad Action, Mutagenicity, and Reduction of Metronidazole and Other Nitroimidazoles. *Antimicrob Agents Chemother*, 10, 1976, 476–482.
27. Elizondo G, Gonsebatt ME, Salazar AM *et al*. Genotoxic effects of metronidazole. *Mutat Res*, 370, 1996, 75-80.
28. Mohn GR, Ong T, Callen DF, Kramers PG, Aaron CS. Comparasion of the genetic activity of 5-nitroimida-zole derivates in *Escherichia coli*, *Neurosporacrassa, Saccharomyces cerevisae* and *Drosophila melanogaster*. *J Environ Pathol Toxicol*, 2, 1970, 657-670.
29. Meo MD, Vanelle P, Bernadini E *et al*. Evaluation of the mutagenic and genotoxic activities of 48-nitroimidazoles and related imidazole derivatives by the Ames test and the SOS chromotest. *Environ Mol Mutagen*, 19, 1992, 167-181
30. Menendez D, Rojas E, Herrera LA *et al*. DNA breakage due to metronidazole treatment. *Mutation Research*, 478, 2001, 153-158.
31. Celik A, Ates NA. The Frequency of Sister Chromatid Exchanges in Cultured Human Peripheral Blood Lymphocyte Treated with Metronidazole in Vitro Frequency of Sister Chromatid Exchanges. *Drug and Chemical Toxicology*, 29, 2006, 85-94.
32. Buschini A, Ferrarini A, Franzoni S *et al*., Genotoxicity Revaluation of Three Commercial Nitroheterocyclic Drugs: Nifurtimox, Benznidazole and Metronidazole. *J Parasitol Res***,** 2009, 2009, 463-66.
33. Cavas T, Gozukara SE. Genotoxicity evaluation of metronidazole using the piscine micronucleus test by acridine orange fluorescent staining. *Environ Toxicol Pharmacol*, 19, 2005, 107-111.
34. Rosenkranz HR, Speck WT. Mutagenicity of metronidazole: Activation by mammalian liver microsomes. *Biochemical and Biophysical Research Communications*, 66, 1975, 520-525.
35. Sekis I, Ramstead K, Rishniw M *et al.* Single-dose pharmacokinetics and genotoxicity of metronidazole in cats. *J Feline Med Surg*, 11, 2009, 60-68.
36. Akyol D, Mungan T, Baltaci V. A comparative study of genotoxic effects in the treatment of *Trichomonasvaginalis* infection: metronidazole or nalidixic acid. *Arch GynecolObstet*, 264, 2000, 20-23.
37. El-Nahas AF, El-Ashmawy IM. Reproductive and Cytogenetic Toxicity of Metronidazole in Male Mice. *Pharmacology & Toxicology*, 94, 2004, 226–231.
38. Ornelas JM, Gomez BC, Zamora AL, Ramos ML, Batista-Gonzalez CM, Zuniga-Gonzalez GM. Micronucleus induction by metronidazole in rat vaginal mucosa. *Environ Mol Mutagen*, 47, 2006, 352-256.
39. Korbelik M, Horvat D. The mutagenicity of nitroaromatic drugs: effect of metronidazole after incubation in hypoxia in vitro. *Mutat Res*, 78, 1980, 201-207.
40. Donald G and Miklos M. Antitrichomonad Action, Mutagenicity, and Reduction of Metronidazole and Other Nitroimidazoles. *Antimicrob Agents Chemother*, 10, 1976, 476–482.
41. Ong T, Slade B. Mutagenicity and mutagenic specificity of metronidazole and niridazole in Neurosporacrassa. *J Toxicol Environ Health*, 4, 1978, 815-824.
42. Speck WT, Stein AB, Rosenkranz HS. Mutagenicity of metronidazole: presence of several active metabolites in human urine. *J Natl Cancer Inst*, 56, 1976, 283-284.
43. Trinh S, Reysset G. Mutagenic action of 5-nitroimidazoles: in vivo induction of GC→CG transversion in two *Bacteroidesfragilis* reporter genes. *Mutat Res*, 398, 1998, 55-65.
44. Sisson G, Jeong JY, Goodwin A *et al*. Metronidazole Activation Is Mutagenic and Causes DNA Fragmentation in *Helicobacter pylori* and in *Escherichia coli* Containing a Cloned *H. pylori rdxA*$^{+}$ (Nitroreductase) Gene. *J Bacterio*, 182,2000, 5091-5096.
45. Rustia M, Shubik P. Induction of lung tumors and malignant lymphomas in mice by metronidazole. *J Natl Cancer Inst*, 48, 1972, 721-729.
46. Yeung TC, Beaulieu BB, McLafferty MA, Goldman P. Interaction of metronidazole with DNA repair mutants of *Escherichia coli*. *Antimicrob Agents Chemother*, 25, 1984, 65-70.
47. Wulf HC. Sister chromatid exchanges in lymphocytes treated with metronidazole. Preliminary communication. *Dan Med Bull*, 27, 1980, 38-40.

48. Menendez D, Rojas E, Herrera LA *et al*. DNA breakage due to metronidazole treatment. *Mutation Research*, 478, 2001, 153-158.
49. Melo ME, Ferreira LS. Screening the mutagenic activities of commonly used antiparasite drugs by simultest, a simplified *Salmonella*/microsome plate incorporation assay. *Rev. Inst. Med. Trop. Sao Paulo*, 34 (2), 1990, 269-274.
50. Fung HB, Doan TL. Tinidazole: a nitroimidazole antiprotozoal agent. *Clin Ther*, 27, 2005, 1859-1884.
51. RaetherW, Hanel H. Nitroheterocyclic drugs with broad spectrum activity. *Parasitol Res*, 90, 2003, 19-39.
52. Tsaca T, Borges FP, Bonan CD *et al*. Effects of metronidazole and tinidazole on NTPDasel and ecto-5'- nucleotidase from intact cells of *Trichomonasvagmalis. FEMS Microbiol Lett*, 226, 2003, 379-384.
53. Lopez Nigro MM, Carballo MA. Genotoxicity and cell death induced by tinidazole (TNZ).*ToxicolLett*, 180, 2008, 46-52.
54. Voogd CE, Van der Stel JJ, Jacobs JJ. The mutagenic action of nitroimidazoles. II. Tinidazole, ipronidazole, panidazole and ornidazole. *Mutat Res*, 48, 1977, 155-161.
55. Gupta RL, Vats V, Juneja TR. Activation of tinidazole, an antiprotozoal drug to a mutagen by mammalian liver S9. *Mutat Res*, 370, 1996, 195-201.
56. Donald G and Miklos M.Antitrichomonad Action, Mutagenicity, and Reduction of Metronidazole and Other Nitroimidazoles. *Antimicrob Agents Chemother*, 10,1976, 476–482.
57. Coulter JR, Turner JV. Tinidazole (TNZ) ethyl[2-(2-Methyl -5-Nitro-1-imidazolyl) ethyl]sulphone) is mutagenic in a *Salmonella Typhimurium* assay. *Mutat Res*, 57,1978, 97-101.
58. Pylkkanen L, Lahdetie J. Sperm abnormality assay of metronidazole and tinidazole. *Mutat Res*, 140, 1984, 137-140.
59. Espinosa-Aguirre JJ, Torre RA, Lares-Asseff I, Rubio J, Dorado V, Wong M, Hernandez JM. Bacterial mutagens in the urine of patients under tinidazole treatment. *Mutat Res*, 359, 1996, 133-140.
60. Celik A, Ates NA. The Frequency of Sister Chromatid Exchanges in Cultured Human Peripheral Blood Lymphocyte Treated with Metronidazole in Vitro Frequency of Sister Chromatid Exchanges. *Drug and Chemical Toxicology*, 29, 2006, 85-94.
61. Melo ME, Ferreira LS. Screening the mutagenic activities of commonly used antiparasite drugs by simultest, a simplified *Salmonella*/microsome plate incorporation assay. *Rev. Inst. Med. Trop. Sao Paulo*, 34 (2), 1990, 269-274.
62. Lopez Nigro MM, Gadano AM, Carballo MA, Evaluation of genetic damage induced by a nitroimidazole derivative in human lymphocytes: Tinidazole (TNZ). *Toxicology in Vitro*, 15, 2001, 209-213.
63. Wilson CO, Gisvold O. Antimalarials. In: Troy DB, editor. Textbook of organic medicinal and pharmaceutical chemistry, New York: Lippincott Williams & Wilkins publishing, 11th edition, 2004, 287-288.
64. Lindquist NG, Sjostrand SE, Ullberg S. Accumulation of chorioretinotoxic drugs in the foetal eye. *Acta Pharmacol Toxicol*, 28, 1970, 64.
65. Okanlawon AO, Ejiwunmi AB, Dada MO, Ashiru OA. Chloroquine-induced retardation of foetal lung maturation in rats. *Afr J Med MedSci*, 22, 1993, 61-67.
66. 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*, 49, 1997, 361-367.
67. Roy LD, Mazumdar M, Giri S. Effects of low dose radiation and vitamin C treatment on chloroquine-induced genotoxicity in mice. *Environ Mol Mutagen*, 49, 2008, 488-495.
68. Obaseiki-Ebor EE, Obasi EE. Aspects of chloroquine mutagenicity. *Mutat Res*, 175, 1986, 51-59.
69. Shalumashvili MA, Siqidin YA. Cytogenetic effects of chloroquine in human lymphocyte cultures. *Bull ExpBiol Med*, 82,1976, 1095-1097.
70. Obaseiki-Ebor EE, Obasi EE. Aspects of chloroquine mutagenicity. *Mutat Res*, 175, 1986, 51-59.
71. Trinh S, Reysset G. Mutagenic action of 5-nitroimidazoles: in vivo induction of GC→CG transversion in two *Bacteroidesfragilis* reporter genes. *Mutat Res*, 398, 1998, 55-65.
72. 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*, 117, 1983, 79-91.
73. Farombi EO. Genotoxicity of chloroquine in rat liver cells: Protective role of free radical scavengers. *Cell BiolToxicol*, 22, 2006, 159-167.
74. XamenaN, Creus A, Velazquez A, Marcos R. Testing of chloroquine and quinacrine for mutagenicity in Drosophila melanogaster. *Mutat Res*, 158, 1986, 177-180.
75. Schupbach ME. Mutagenicity evaluation of two antimalarial agents chloroquine and mefloquine using a bacterial fluctuation test. *Mutat Res*, 68, 1979, 41-49.
76. Labro MT, Chevaye CB. Effects of amodiaquine, chloroquine, and mefloquine on human polymorphonuclear neutrophil function in vitro. *Antimicrob Agents Chemother*, 32, 1988, 1124-1130.
77. Ames B, Whitfield H. Frame shift mutagenesis in salmonella. *Cold Spring Harb Symp Quant Biol*, 31, 1966, 221-225.

78. Thomas SM, Silburn KA, MacPhee DG. Frame shift mutagenesis by chloroquine in *E. coli* and *S. typhimurium. Mutat Res*, 192, 1987, 233-237.
79. Kadotani S, Arisawa M, Maruyama HB. Mutagenicity examination of several non steroidal anti-inflammatory drugs in bacterial system. *Mutat Res*, 138, 1984, 133-136.
80. Espinosa-Agguirre JJ, Ramirez Santos J, Cornitas de nava C. Influence of the Uvr repair system on the mutagenicity of antiparasitic drugs. *Mutat Res*, 222, 1989, 161-166.