Chapter One: Introduction

1. Introduction

The conception of herbal safety has undergone a shift in recent years. Previously, two concepts depicting the fundamental safety of herbs have prevailed. One was religious: Allah had put the herbs on this earth to heal the illnesses of mankind; they were designed to be helpful and not harmful. Man, turning away from Allah teachings, dabbled in things that were outside man's more limited role, and could then cause harm. Therefore, returning to Allah natural pharmacy would assuredly provide help. The other concept was based on the scientific view of nature: plants and animals had evolved together over hundreds of millions of years, and there was a natural balance in the constituents of plants and in their interaction with animals (and, therefore, with humans). Thus, while isolated chemicals from plants and their synthetic derivatives used, as drugs would be harmful because they lacked nature's balance, whole herbs would be healing because each of the myriad ingredients balanced the others and produced a harmonious interaction with the numerous substances in the body of the consumer. Both the religious and scientific interpretations converged on the concept that herbs and natural foods are inherently safe, while those substances modified or created by man often do harm (Subhuti, 2000).

Pelargonium odoratissimum (L.) soland is grown mainly for aromatic and ornamental reasons, edible. *P. odoratissimum* used as astringent , antiseptic, gastro-enteritis and hemorrhage (Uphof, 1959; Westwood, 1993).

Metronidazole (Flagyl) is an important drug that provides significant efficacy in treating anaerobic and parasitic infections (Darbon *et al.*,1962). Metronidazole reaches good levels in nearly all body tissues, including the cerebral spinal fluid, saliva, bone, and abscesses. Bioavailability studies show equal efficacy with oral and intravenous dosing (Gilbert, 2001).

Metronidazole and its principal metabolites are mutagenic in bacteria. The tumorgenicity was observed in animal studies after oral administration of metronidazole e.g. pulmonary tumorigenesis , malignant hepatic tumors and malignant lymphomas confirmed its tumorgenicity (Internet, 2005).

Many medicinal herbs and drugs are therapeutic at low doses and toxic at high doses (Ernest and Pittler, 2002).

The interactions between herbs and drugs may increase or decrease the pharmacological or toxicological effects of either component (Adriene, 2000)

The aim of this work was to reduce the side effects of existing drugs. Researchers could develop matched drug-antidote pairs at the beginning of the drug development process to enable the control of drug activity in patients. This approach could be reached by testing genome stability through the following:-

1. Detecting the active compounds of *P. odoratissimum* (L.) soland .

2. Studying the cytogenetic effect of metronidazole and *P. odoratissimum* aquatic extract by using (mitotic index, chromosomal aberration assays) in mouse bone marrow cells (*in vivo*) and in human blood lymphocytes cultures (*in vitro*).

3. Studying the effect of metronidazole and plant aquatic extract on the blood leukocyte both total and differential count in mice.

4. Investigating the ability of aquatic extract of *P. odoratissimum* in reducing the genotoxic effects produced by metronidazole.

Chapter Two: Literature Review

2.1: Metronidazole

Metronidazole (MTZ) was discovered in 1955 and was found to be highly effective against several protozoan infections. In 1962 MTZ had been used by clinicians as the mainstay of therapy of giardia (Levi *et al*., 1977; Tracy and Webster**,** 1996). MTZ initially approved by Food and Drug Administration (FDA) in 1963 (Dobell**,** 1990).

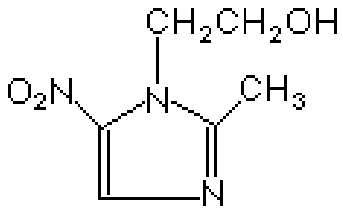
Metronidazole is a synthetic antibacterial and antiprotozoal agent that belongs to the nitroimidazole class (Figure 2.1). It is an effective therapy against protozoa such as *Trichomonas vaginalis*, amebiasis, and giardiasis. In addition, it is one of the most effective drugs available against anaerobic bacterial infections (Darbon *et al***.,** 1962; Dubreuil *et al*., 1996).

Therapeutic concentrations of the drug are attained throughout most body compartments after either oral or intravenous administration (Stranz and Bradleyi, 1981).

Metronidazole has shown minimal to no activity against clinically relevant facultative anaerobes or obligate aerobes (Ralph and Kirby, 1975).

2.1.1: Uses of Metronidazole

Metronidazole is indicated in the treatment of extraintestinal amebiasis, as well as acute intestinal amebiasis caused by *Entamoeba histolytica* (Internet , 2005).



$C_6H_9N_3O_3$

Figure (2-1) Structure of Metronidazole (Ralph and Kirby, 1975).

Metronidazole is also indicated in the treatment of bone, joint and brain abscess infections caused by *Bacteroides* species, including the *B. fragilis* group (*B. fragilis*, *B. distasonis*, *B. ovatus*, *B. thetaiotaomicron*, *B. vulgatus*) (Dobell, 1990).

Another important infections treated by MTZ is that of the central nervous system (CNS) infections including meningitis and in the treatment of endocarditis caused by *Bacteroides* species, including the *B. fragilis* group (Kusumi *et al.*,1980).

Also, this drug is usually indicated in the treatment of intra-abdominal infections, including peritonitis, intra-abdominal abscess, and liver abscess, also used in the treatment of female pelvic infections, and prophylaxis of preoperative infections during colorectal surgery and in treatment of lower respiratory tract infections caused by *Bacteroides* species, including the *B. fragilis* group, *Clostridium* species, *Eubacterium* species, *Peptococcus* species, and *Peptostreptococcus species* **(** McEvoy, 1988 ; Rauws and Tytgat ,1990).

Oral MTZ is used in the treatment of giardiasis and bacterial vaginosis caused by *Gardnerella vaginalis, Mobiluncus* spp*, mycoplasma hominis* and anaerobes (*Peptostreptococcus* spp. and *Bacteroides* spp.) caused by *Giardia lamblia*, MTZ was indicated in the treatment of symptomatic and asymptomatic trichomoniasis, in males and females, caused by *Trichomonas vaginalis,* also MTZ was used in the treatment of inflammatory bowel disease (Durel *et al*., 1990; Mandell *et al*., 1990; Weil, 1990).

However, MTZ resistance may occur, especially in patients who have been previously exposed to MTZ (Borsch *et al*., 1989; Rauws and Tytgat, 1990; Glupczynski *et al.*, 1990; Ormand and Talley, 1990; Becx, 1990 ; Weil , 1990). Some studies indicated that resistance of anaerobic bacteria to MTZ is almost nonexistent **(**McEvoy, 1988).

2.1.2: Mechanisms of Metronidazole Activity against Microbial Agents

Metronidazole is amebicidal, bactericidal, and trichomonicidal. Unionized MTZ is readily taken up by anaerobic organisms and cells. Its selectivity for anaerobic bacteria is a result of the ability of these organisms to reduce MTZ to its active form intracellularly. The electron transport proteins necessary for this reaction are found only in anaerobic bacteria. Reduced MTZ then disrupts DNA's helical structure, thereby inhibiting bacterial nucleic acid synthesis. This eventually results in bacterial cell death (Townson *et al*., 1994; Gillis and Wiseman, 1996; Upcroft and Upcroft, 1998; Samuelson, 1999).

Metronidazole is also equally effective against dividing and non-dividing cells**.** Because of its mechanism of action, low molecular weight,

and limited binding to serum proteins, MTZ is a highly lethal antimicrobial agent (McEvoy, 1988).

2.1.3: Distribution of Metronidazole

Metronidazole is distributed to saliva, bile, seminal fluid, breast milk, bone, liver and liver abscesses, lungs, and vaginal secretions; crosses the placenta and blood-brain barrier (Lau *et al*., 1992; Tracy and Webster, 1996)**.**

2.1.4: Pharmacokinetics of Metronidazole

Metronidazole is administered orally, intravenously, intravaginally, and topically.It was found that both intravenously and oral MTZ are widely distributed into most body tissues and fluids, the drug crosses the placenta and enters breast milk. Protein binding of MTZ is roughly 10%. . Bioavailability of oral absorption is at least 90%, food decreases the rate, but not the extent of absorption. Another rout, which is intravaginally administered MTZ, is absorbed systemically; but peak serum concentrations after intravaginal administration are < 2% of the levels achieved with 500 mg oral doses (Kucers *et al*., 1997).

A significant amount of MTZ (30-60%) is metabolized in the liver by hydroxylation, oxidation, and glucuronide conjugation. The major metabolite is 2-hydroxymethyl MTZ, which has some antibacterial and antiprotozoal activity (Scully, 1988).

2.1.5: Elimination of Metronidazole

Within 24 hours, approximately 20% of MTZ is excreted in urine and 3% in feces. After 5 days, the amount excreted in the urine increases to 77% and that excreted in feces to 14%. Both unchanged drug and metabolites are excreted in the urine and the feces. Geriatric patients may have a decreased urinary excretion of MTZ (Roe**,** 1985).

This medication is removed by hemodialysis, but not by peritoneal dialysis, and it can color the urine reddish-brown due to water-soluble pigments formed during metabolism. The mean elimination half-life of MTZ is roughly 8 hours (Tracy and Webster**,** 1996).

2.1.6: Interaction of Metronidazole with other Medicinal Products

Metronidazole may interact and make the following clinical problems: -

Potentiation of the anticoagulant effect and increased hemorrhagic risk caused by decreased hepatic catabolism. Lithium retention observed by increased plasma lithium levels, accompanied by evidence of possible renal damage has been reported in patients treated simultaneously with lithium and MTZ. Patients should be advised not to take alcohol, (or drugs containing alcohol) during MTZ therapy and for at least 48 hours afterwards because of a disulfiram-like (antabuse effect) reaction (flushing, vomiting, tachycardia) (Townson *et al*., 1994**).**

2.1.7: Mutagenicity and Carcinogenicity of Metronidazole

Metronidazole has been shown to be carcinogenic in a number of studies in mice. Pulmonary tumorigenesis has been reported in six studies , malignant hepatic tumors have also been noticed in male mice given very high doses (approximately 500 mg/kg/day). Malignant lymphomas have been reported in one lifetime feeding study in mice (Beard *et al*.,1988; Internet , 2005).

It was found that Malignant liver tumors were increased in male mice treated at approximately 1500 mg/ml. This dose is approximately 3 times the recommended dose. While malignant lymphomas and pulmonary neoplasms are also increased with lifetime feeding of the drug to mice.

Mammary and hepatic tumors were increased among female rats administered oral MTZ compared to concurrent controls (Heinonen *et al*., 1977).

Many studies showed that MTZ is mutagenic in bacteria and carcinogenic in mice and rats at high doses over long periods. Metronidazole has been shown to be carcinogenic in rats whereas other studies found that MTZ causes a statistically significant increase in the incidence of various neoplasms, especially mammary and hepatic tumors, in female rats (Lindmarkand Muller, 1976; Bost, 1977; Voogd, 1981; Tracy and Webster, 1996).

In vitro assay systems was done including the Ames test to show MTZ mutagenic activity while studies in mammals *in vivo* had failed to demonstrate a potential for genetic damage (Beard *et al.*, 1979 ;Goldman, 1980; Roe, 1985;Falagas *et al.*, 1998).

The single major drawback to the use of MTZ is uncertainty about its carcinogenic potential in humans. MTZ was carcinogenic in animals and mutagenic *in vitro*, but had not increased the incidence of cancer in humans followed for relatively short periods (Stranz and Bradley, 1981).

Other tumorigenicity studies in hamsters had been performed and reported to be negative (Beard *et al*., 1979).

2.1.8: Metronidazole and fetotoxicity

Fertility studies have been performed in mice at doses up to six times the maximum recommended human dose based on mg/ml and have revealed no evidence of impaired fertility (Internet , 2005).

Metronidazole crosses the placenta and enters the fetal circulation rapidly. Adequate and well-controlled studies in humans have not been

done. Meanwhile studies in rats, given doses of up to 5 times the human dose, have not shown that MTZ causes impaired fertility or birth defects in the fetus (Goldman, 1980**).**

Heinonen *et al*., 1977 found that intraperitoneally administered MTZ to pregnant mice at approximately the human dose, had been shown to cause fetotoxicity, however a study of 50,000 mother-child pairs, demonstrated that first-trimester exposure to MTZ in 31 women showed a possible association with malformations.

While Berget and Weber**,** 1972 found no evidence of congenital abnormality; MTZ has not been shown to be carcinogenic or tumorigenic in women who took MTZ in pregnancy, with using the drug in the first trimester

2.2: *Pelargonium odoratissimum* (L.) soland

Kingdom	*Plantae* – Plants
Subkingdom	*Tracheobionta* – Vascular plants
Superdivision	*Spermatophyta* – Seed plants
Division	*Magnoliophyta* – Flowering plants
Class	*Magnoliopsida* – Dicotyledons
Subclass	*Rosidae*
Order	*Geraniales*
Family	*Geraniaceae* – Geranium family
Genus	*Pelargonium* – geranium
Species	*Pelargonium odoratissimum* (L.) soland - Rose geranium

(Watson and Dallwitz, 1992)

There are around 300 species of pelargoniums coming from different parts of the world (Becker and Brawner, 1996).

Some of the species are cultivated for the distillation of the volatile oil, in France, Spain, Algiers, and the Island of Reunion. Several species of *Pelargonium* yield a volatile oil by distillation.The oil is obtained from the leaves. Oil of Geranium is derived from this and allied species. The constituents include esters of geraniol, citronellol and linalool (Budavari, 1996; Balchin and Roth, 2000). The composition of the oil can vary according to the source (Algerian, French, and Bourbon) *(*Martin, 1987*).*

P. odoratissimum also called Arabic odor to distinguish it from the Italian odor (Saad and Abdul-Kady, 1988).

2.2.1: Plant Distribution

The majority of the plants species are native to the southern part of the African continent, for example the Republic of South Africa and Namibia. However, the distribution of the genus extends further North.Throughout the East African countries: Zimbabwe, Malawi, Tanzania, Kenya, Ethiopia and extends to the Arabian subcontinent Yemen, in East Turkey and Middle East Iraq, Iran (Becker and Brawner, 1996).

2.2.2: Plant Description

Rose Geraniums have palmate separated leaves, rough and covered with tiny hair. Leaves and stems have special odor, the stem is succulent, and the medical parts are leaves and stem (Saad and Abdul-Kady, 1988).

2.2.3: Cultural Notes

An easily grown plant, succeeding in a well-drained ordinary good soil (Bown, 1995). It requires a light well-drained neutral to alkaline soil in a sunny position (Brickell, 1990). Plants are tolerant to shade (Bown, 1995).

The plants need to be kept fairly dry in winter. When grown in pots, the plants require regular repotting in order to stay vigorous (Phillips and Rix, 1998).

Very tolerant to pruning, they can be cut right down to the base in the autumn when bringing them back indoors, or in the spring to encourage lots of fresh growth. The plant is cultivated for its essential oil. Oil of geranium is obtained by distillation from the leaves of *P. odoratissimum* (Bown, 1995).

2.2.4: Propagation Notes

Cuttings succeed at almost any time in the growing season but early summer is the best time in order for the new plant to become established before winter (Saad and Abdul-Kady, 1988).

2.2.5: Edible Uses

The leaves are crushed and used to flavour salads, soups, fruit dishes, jellies, sorbets, ice-cream, cakes and tea (Bown, 1995).

2.2.6: Medicinal Uses

The whole plant is an aromatic herb with astringent produces contraction in living tissue, reducing the flow of secretions and discharges of blood, mucus, it brings steady improvement and antiseptic effects preventing sepsis, decay or putrefaction, it destroys or arrests the growth

of micro-organisms.It is used internally in the treatment of debility, gastro-enteritis and hemorrhage. Externally, it is used to treat skin complaints, injuries, neuralgia and throat infections. The essential oil is used in aromatherapy. It is used in the treatment of burns, sores and shingles (Westwood, 1993).

2.2.7: Other Uses

An essential oil is obtained from the plant that is used in perfumery, paint solvents and insect repellents. The growing plant will repel flies, especially if the leaves are touched occasionally to release their scent (Bown, 1995).

2.3: Herbal – Drug Interaction

Herbal medicines include dietary supplements that contain herbs, either singly or in mixtures. Also called botanicals, herbs are plants or plant parts used for their therapeutic properties.

Since herbal medicines are classified as dietary supplements, there are no Food and Drug Administration (FDA) regulations regarding accuracy of active ingredients content or efficacy and safety of active ingredients. Concurrent use of herbs may mimic, magnify, or oppose the effect of drugs (Ernst and Pittler, 2002).

Herb-drug interactions can impact our health and the effectiveness of our treatments. For example, some herbal therapies might:

- Increase the side effects of drugs, possibly leading to toxicity
- Decrease the therapeutic effect of drugs, possibly leading to treatment failure.

- Modify the action of drugs, possibly leading to unexpected complications
- Enhance the therapeutic effect of drugs, possibly leading to over medication

Likewise, prescription and non-prescription drugs can alter the way human body reacts to herbal therapies (Adriene, 2000).

2.3.1: The Mechanisms of Herb-Drug Interaction

Pharmacokinetic interactions involve changes in the way herbs and drugs move through the body and can alter the amount, or level, of drug(s) in the body. If the interaction increases the level of a drug, this might lead to experience side effects and/or toxicity. If the interaction decreases the level of a drug, it might not work as well, possibly leading to treatment failure and/or drug resistance (Donavon and Zhiyang, 2004).

Herbs can affect the way in which drugs are absorbed, leading to changes in the amount of drug that enters the bloodstream. For example, some herbs can change the physical environment of the stomach, such as the pH level, while others might chemically bind to drugs, causing them to remain in the stomach instead of entering the bloodstream (Horowitz, 2000).

In vivo studies with pre-clinical species are not predictive of the human clinical situation. Although *in vitro* kinetic data also have limitations when extrapolating *in vivo*, *in vitro* testing has become more common due to reduced cost and higher throughput (Donavon and Zhiyang, 2004).

2.4: Mechanisms of Antimutagenicity

Naturally occurring substances in foods have been shown to serve as dietary antimutagens. Therefore, many studies have been expanded on natural compounds which had the antimutagenic effect, which work in different mechanism to reduce the action of these mutagenic compounds, in contrast these mutagenic compounds have a mechanism to convert into electrophilic radicals capable to reach into target site protein, RNA and DNA and made damage (Newmark, 1987).

The mechanisms of antimutagenesis have been classified into two major processes by Morita *et al.* 1978 one desmutagens and other bioantimutagens.

Antimutagens in the diet can be broadly classified into two groups, the bioantimutagens and the desmutagens, the former acting on DNA and the latter not affecting genetic material directly (Clarke and Shankel, 1975; Kuroda, 1999; Samejima *et al*., 1995).

Desmutagens encompass all agents that affect mutagenicity through mechanisms other than DNA repair or replication. Dietary desmutagens may function as chemical inactivaters, enzymatic inducers, mutagen scavenging or antioxidants; they create this effect without directly affecting the genetic material (Ferguson, 1994). Garlic extract, onion and flavonoids exhibit activity against a wide range of chemical mutagens by metabolic inactivation and increase glutathione (Dorant *et al*.,1993). Chlorophyll inhibits the mutagenicity of aflatoxin B_1, *in vitro.* Ascorbic acid (vitamin C) and E are antioxidants, they reduce free radicals produced by mutagenic compound. Vitamin C has the ability to form complex with the mutagene ethylmetylsulfonate (EMS) reducing its effect (Kuroda and Hara, 1990).

The bioantimutagens are naturally occurring substances that reduce mutant yield by acting on the DNA repair or replicative processes. These compounds act after a DNA adduct has formed but before the DNA lesion is fixed into a mutation (Clarke and Shankel, 1975). Bioantimutagens may *a*) inhibit the induction of

strand-on-strand DNA repair, reducing replication of mutated strands; *b*) in cells containing mutations, make the "proofreading" in repair more like that seen in normal cells; or *c*) accelerate the recombination strand-on-strand repair rate, thus reducing the number of mutated strands (Kuroda, 1990). An example of a bioantimutagen is vanillin, present in vanilla beans, which appears to enhance post replication recombination repair under certain conditions (Ohta *et al*., 1988).

2.5: Cytogenetic Analysis

Cytogenetic assays have been used since the early 1960s. Cytogenetic analysis is a widely employed indication system for the evaluation of physically, chemically and biologically induced mutations. It allows for the objective evaluation of the genetic material damages and is a method that permits direct image analysis for the chromosome damage. Cytogenetic analyses have been proved to be good and reliable for the mutagen–carcinogen exposure and chromosomal aberration (Nakanishi and Schneider, 1979; Gebhart, 1981).

2.5.1: Blastogenic Index

It is term used to describe the morphological changes that resulted when small lymphocyte were transformed into lymphoblast on exposure to non specific mutagen or mitogen (Soren , 1973).

The theory that inherited characteristics are classified into three types, one: transmittion from parent to offspring through germ plasm, two: reproduction of an organism by budding, and three: the transformation of small lymphocytes into larger cells that is capable of undergoing mitosis (Evans *et al*., 1967).

Phytohemagglutinin (PHA) is a mitogen used along with a minimal essential medium or RPMI-1640 medium for initiating the lymphocyte culture in *in-vitro*. The PHA is known to stimulate the lymphocytes to

undergo mitosis. PHA stimulate human lymphocytes are widely used to detect chromosome-damaging agents, possible human exposure to mutagenic carcinogens and the immune response of blood, the results are affected by the number of cell divisions(Savage, 1979).

After stimulation of blood lymphocytes with PHA the cultures soon contain cells that have divided different numbers of times (Morimoto and Wolff, 1980). This heterogeneity has been explained variously as a difference in cell-cycle times (Crossen and Morgan, 1979), or in the times when the cells start blasto-genesis by responding to PHA (Younkin, 1975).

2.5.2: Mitotic Index (MI)

Proliferating cells go though a regular cycle of events, the mitotic cell cycle, in which the genetic material is duplicated and divided equally between two daughters. This is brought by the duplication of each chromosome from two closely adjacent sister chromatids, which separate from each other to become two daughter chromosomes. Test along with other chromosome of each set, are then packaged in to two genetically identical daughter nuclei. The molecular mechanisms underlying the cell cycle are highly conserved in all organisms with in nucleus eukaryotes (Eva , 2001).

Research has indicated that this assay is affected by the mutant and carcinogenic material in which all of these materials can cause decrease in MI either *in vivo* or *in vitro*.

Mitotic index (MI) is determined as a ratio of mitotic cells to inter-phase nuclei in 1000 cells (Shubber and Al-Allak, 1986).

2.5.3: Chromosomal Aberrations (CAs)

Chromosomes are the structures that hold our genes. Genes are the individual instructions that tell our bodies how to develop and keep our bodies running healthy (Becher *et al*., 1983).

Living organisms may expose to different kinds of effects, which may cause chromosomal abnormalities (or aberrations). CAs are considered as a genetic damage of chromosomal level observed as an alteration either in chromosome number or in chromosome structure (Catherine *et al.*,1998).

The most important thing that the chromosomal structure abnormalities can be distinguished when the cell is in the metaphase of the mitosis (Evans, 1976)

Many agents induce visible chromosome damage after the cells undergo a round of DNA replication. However, damaged cells may not survive for more than one or two cell cycles after aberrations have been induced (Evans and Lioyd, 1978; Catherine *et al.*, 1998).

The increasing variety of chemicals, radiation and other physical agents we are expose to nowadays have stimulated the development of many reliable assays for the detection of the mutagenicity or carcinogenicity of such agents. One of these methods is the chromosomal aberration assay (Lambert *et al*., 1978, Ardito *et al.*, 1980).

The chromosomal studies are rapid, sensitive, and reproducible, and yield quantitative measurement of breaks and gaps in mitotic chromosomes resulting from mutagenesis in G_2 cells. The principle used consists of scoring microscopically identifiable breaks and gaps in mitotic chromosomes (Ishii and Watatani, 1983; Becher *et al*., 1983).

Numerical and structural aberrations are important both in congenital abnormalities and tumors (Preston, 1987).

Therefore every one should have 46 chromosomes in somatic cell of their body. If a chromosome or piece of a chromosome is missing or duplicated, there are missing or extra genes respectively. When a person has missing or extra information (genes) problems can develop for that individual's health and development (Ishii and Watatani, 1983)

Structural aberrations of chromosomes are common in nature and have apparently played a significant role in evolution. They occur spontaneously, but the frequency is increased by ionizing radiation and chemical mutagens (Evans and Oriordan, 1977; IAEA, 2001).

Most chemical mutagens and non-ionizing mutagenic radiations are unable to cause double-strand breaks in DNA and act mainly in the single-strand DNA synthesis phase of the cell cycle. Such agents produce mainly chromatid-type aberrations (OECD, 1997).

The types of chromosomal aberrations which have been observed are:

1-Chromatid-type aberration is structural chromosome damage expressed as breakage of single chromatids or breakage and reunion between chromatids.

2-Chromosome-type aberration is structural chromosome damage expressed as breakage, or breakage and reunion, of both chromatids at an identical site.

3-Gap is a chromatic lesion smaller than the width of one chromatid, and with minimum misalignment of the chromatids.

4-deletions - loss of segment, a chromosomes deletion is when a part of a chromosome(s) has been deleted. A deletion can occur on any chromosome, at any band, and can be any size (large or small). What a deletion causes depends on how big a piece is missing and what genes are missing in the section.

5- Ring chromosome could happen in two ways. First the end of the p and q arm breaks off and then stick to each other resulting in loss of information. Second, the ends of the p and q arm stick together (fusion), usually without loss of material (Tamarin, 1996). However the ring can cause problems when the cell divides and can cause problems for the individual. It is also possible to have a ring and be apparently healthy with no delays in development. As with all chromosome abnormalities it depends on what is actually found, the size of the ring, how much material was lost, which chromosomes are involved (Internet , 2004).

6- Acentric fragment when centromere is lost, an acrocentric chromosome is created which is usually lost during meiosis (death of cell or loss of fertility) (Obe and Natarajan, 2004).

2.5.4: Sister Chromatid Exchanges (SCE)

Sister chromatid exchange (SCE) is the process where in the two sister chromatids break and rejoin with one another, physically switching positions on the chromosome. Because the exchanges occur with tremendous precision with respect to the DNA sequence, and the sister chromatids are genetically identical, no information is altered during the exchange. Such exchanges are natural events during cell replication with each cell typically undergoing three to four SCEs during each replication cycle (Hasgafvel, 1987;Shubber *et al.*, 1991).

Sister chromatid exchange is useful for genetic toxicology studies as chemicals material that cause mutations, the result often increased the frequency of SCEs (Shubber *et al.,* 1991).

Attention has been devoted to the frequency of SCE, it was reported to be significantly higher in lymphocytes of patients with Bloom syndromes (Chaganti *et al.*, 1974), acute lymphoblastic leukemia (Otter *et al.*, 1979), chronic myeloid leukemia (Shirishi and Sadberg, 1980) and in patient with schistosomiasis (Shubber, 1987; Shubber *et al.*, 1991; Juma *et al.*,1999).

During the S-phase of the cell cycle, DNA is replicated and each chromosome is present in a duplicated state with the two genetically identical chromatids joined together at the centromere. These two sister chromatids are readily apparent in late prophase or early metaphase of mitosis (Keshava and Ong, 1999).

SCE frequency was a more sensitive marker of mutagenesis than the chromosomal abnormalities, although they occur after exposure to many genotoxic agents or various diseases and are believed to indicate DNA damage, it could be visualized by growing cells in a medium, which contains the DNA base analog bromodeoxyuridine (BUdR). BUdR closely resembles thymidine, and gets incorporated into the growing DNA strand during replication. During replication of DNA the BUdR was taken up into the newly synthesized strand, with the template strands remaining free of BUdR. During a second round of growth in BUdR medium, the two sister chromatids differ in the amount of BUdR present (Latt, 1974).

The sister chromatid which has the original template strand of DNA has one strand of normal DNA and one which contains BUdR. With chemical and ultraviolet light treatment, the two sister chromatids can be

distinguished from each other. SCEs can then be identified by differences in staining patterns. Many people are occupationally exposed to various biohazardous agents, organic and inorganic chemicals. These hazardous agents typically are used as raw materials or an intermediate manufacturing processes (Keshava and Ong , 1999).

Damage to genetic material can be cytologically observed as chromosome aberration, micronuclei or SCE (Zhang *et al*., 1991)

2.6: The Cell cycle

The phases through which a cell passes as it divides, the cell cycle consists of four well characterized stages (phases).During the first stage known as the G1 phase , the cell increase in size and prepares to duplication its DNA which will occur in the second stage known as the S phase. During the S phase, the cellular DNA was duplicated and a complete copy of the chromosome complement was made. The cell then enters the third stage termed the G2 phase during which the cells prepared itself for division, the cell divides when it enters the fourth and final stage termed the M phase, during M phase cell divided to produce two daughter cells each with complete set of chromosomes, each daughter cell then usually enters the first stage G1 and the cycle repeated therefore (Alan and Robert, 1997;Prosperi,1997), as shown in figure (2.2).

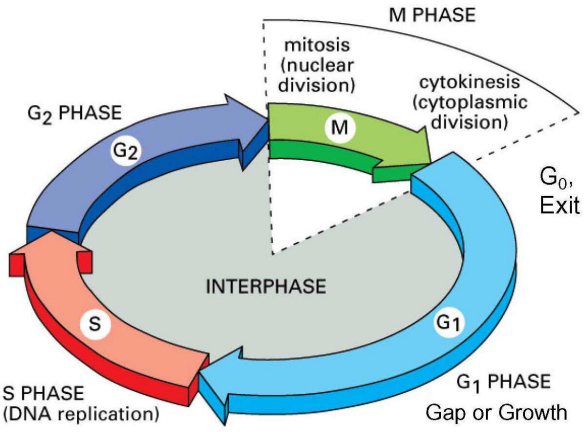


Figure (2.2): Cell cycle (Alan and Robert, 1997).

2.6.1: Cell Cycle Progression (CCP)

Cell cycle progression is the ability of cells to replicate for three cell division M1, M2 and M3.Cell cycle progression is used as an assay to know the ability of chemical and physical material in reduction cell replication kinetics and it is measured by decreasing the percentage of M3 and increasing the percentage of M1 (Tice *et al.*,1976; Lamberti *et al.*,1983).The CCP consists of three types of division:-

1- First cell division

These groups of cells have no incorporated BUdR or in single DNA strand during S phase. The chromosomes of this phase all appear bright under the light microscope (Latt, 1973).

2- Second cell division

This group have the ability to corporate BUdR during two S phases and display a typical differential staining of sister chromatids one dull and one bright.

3-Third cell division

These cells corporated BUdR during three S phases so they contained BUdR-substituted DNA in both sister chromatids, half number of the chromosomes will express differential stain while the other half will loss the brightness because of BUdR (Lamberti *et al.*,1983; Shubber and AL-Allak, 1986).

2.6.2: Replicative Index (RI)

Cell cycle progression (CCP) may be defined using a parameter that considers, at the same time, the number of M1, M2 and M3 metaphase cells.

Many indicators were considered, but the most suitable was the RI. This index is formulated using the following equation:

RI= (1xM1%) + (2xM2%) + (3xM3%)/100

(Lamberti *et al.*, 1983).

2.7: Hormesis

The word "hormesis" is derived from the Greek word "hormaein" which means "to excite". It has long been known that many popular substances such as alcohol and caffeine have mild stimulating effects in low doses but are detrimental or even lethal in high doses. In the early 1940s it was found that despite the fact that high concentrations of Oak bark extract inhibited fungi growth, low doses of this agent stimulated fungi growth. They modified starling's word "hormone" to "hormesis" to describe stimulation induced by low doses of agents which are harmful or even lethal at high doses (Calabrese and Baldwin,1999).

Generally, hormesis are any stimulatory or beneficial effect, induced by low doses of an agent that can not be predicted by the extrapolation of detrimental or lethal effects induced by high doses of the same agent (Edward and Linda , 1998).

Whether small doses of toxins are beneficial or harmful seems to depend on what the substances are, how small the doses are, what diseases are being studied and who's exposed to them (Feinendengen *et al*., 1987).

The idea behind hormesis is that parts of human cells change when challenged with small doses of toxins. These changes often make the cell stronger at first, but larger doses can still damage it. It is similar to the immunity-building effects produced by small and weakened doses of viruses used in vaccines (Lucky,1982).

The concept of hormesis is now being used to enhance cognitive function in patients with neurodegradative diseases such as Alzheimer's disease , boost immune function to prevent diseases in people and in commercial fisheries and avoid harmful tumor-promoting effects of anti-cancer drugs (Edward and Linda , 1998).

The following theories may explain the mechanism of hormetic phenomena:-

1-DNA Repair (Molecular Level)

According to this theory, low doses of chemical substances induce the production of special proteins that are involved in DNA repair processes (Ikushima *et al*., 1996).

2-Free Radical Detoxification (Molecular Level)

In 1987 Feinendengen and his co-workers indicated that low doses of ionizing radiation or chemical substances cause a temporary inhibition in DNA synthesis. This temporary inhibition of DNA synthesis would provide a longer

time for infected cells to recover. This inhibition also may induce the production of free radical scavengers, so infected cells would be more resistant to any further exposures.

3-Stimulation of Immune System (Cellular Level)

Despite the fact that high doses of chemical substances or ionizing radiation are immunosuppressive, many studies have indicated that low doses chemical may stimulate the function of the immune system. In 1990, Russ first showed that mice treated with low-level radiation were more resistant against bacterial disease.

The example of hormesis was the survivors of the bomb blast in Japan had lived a little longer and healthier they were, this was at low levels (Luckey, 1982).

Chapter Three: Materials and Methods

3.1. Materials:

3.1.1. Equipments and Apparatus:

The following equipments and apparatus were used in this study:

No.	Apparatus	Company
1.	Autoclave	Gallenkamp(England)
2.	Centrifuge	Gallenkamp(England)
3.	Water path	Gallenkamp(England)
4.	Cooling incubator	Gallenkamp(England)
5.	Electric balance	Metler (Switzerland)
6.	Electric oven	Gallenkamp(England)
7.	Micropipette	Gelson(France)
8.	Microscope	Olympus(Japan)
9.	pH-Meter	Orien Research (USA)
10.	U.V. light lamp	CAMAGLL,1987
11.	Laminar air flow	Metalab (France)
12.	Reflex	Buchi (Switzerland)
13.	Electric shaker	Merk(Germany)
14.	Rotary evaporator	Buchi (Switzerland)

3.1.2. Chemical Materials:

The following chemical materials were used in this study and their origin:

No.	Material	Company
1.	phytohaemoaglutinine	Radiobiology center of The Ministry of Science and Technology(Iraq)
2.	Anhydrous sodium	BDH(England)
3.	Methanol	Fluka(Switzerland)
4.	Glacial acetic acid	Fluka(Switzerland)
5.	Hydrochloric acid (HCl)	BDH(England)
6.	Potassium chloride(KCl)	Fluka(Switzerland)
7.	Heparin	Denemarca
8.	RPMI(1640)	Flow laboratories –V.K.
9.	Hepes	Sigma chemical company(USA)
10.	Sodium bicarborate($CaCl_2$)	Sigma chemical company(USA)
11.	Antibiotics(penicilline, Streptomycin)	Samara drug factory(Iraq)
12.	Giemsa stain	Fisher(German)
13.	Colchicin	Houde(France),Ibn-Hayan (Syria)
14.	5-Bromodeoxyuridine	BDH(England)
15.	Fetal calf serum	Sigma chemical company(USA)
16.	NaCl	Sigma chemical company(USA)
17.	Na_2HPO_4	Sigma chemical company(USA)
18.	KH_2PO_4	Sigma chemical company(USA)
19.	Hoechst	BDH(England)
20.	Metronidazole	Samara drug factory(Iraq)

3.2. Solutions Preparation:

1. Colchicine: two colchicine products were used in this study.

a. Colchicine (Ibn Hayan / Syrian)

This colchicine was prepared by dissolving one tablet 0.5 mg of colchicine in 0.5 ml of PBS to be used for mouse injection. Each animal was injected intraperitoneally (IP) with 0.25 ml of this solution.

b. Colchicine (Houde/France)

This colchicine was prepared by dissolving one tablet 0.5 mg of colchicine in 10 ml of D.W. (sterile) to make a stock solution .This solution was stored at -20 ˚C until used for human blood culture (Al-Kayat, 1999).

2. Phosphate Buffer Saline (PBS)

The solution was prepared by dissolving of the following chemical in 1000 ml of D.W. and the pH was adjusted to 7.2 ,these chemicals are:-

Sodium chloride (NaCl)	8gm
Potassium chloride (KCl)	0.2 gm
Sodium phoshate hydrate (Na_2HPO_4)	1.15gm
Potassium phosphate dehydrate (KH_2PO_4)	0.2gm

This solution was sterilized by autoclaving and stored at 4˚C (Allen *et al*., 1976).

3. Potassium Chloride (KCl) (Hypotonic Solution):

The hypotonic solution was prepared by dissolving 5.75 gm of KCl in one liter D.W. to get 0.075 M concentration of KCl. The solution was sterilized by autoclaving and stored at 4˚C (Allen *et al*., 1976).

4. Fixative Solution:

This solution was freshly prepared by mixing 3 parts of absolute methanol with 1 part of glacial acetic acid and then kept at 4˚C until use (Allen *et al*., 1976).

5. Giemsa Stain:

Giemsa stock solution was prepared by dissolving 1gm of giemsa powder in 33 ml of glycerol in water bath at 60˚C for 2 hours with continuous shaking, then left for 30 min. at room temperature, then add 66 ml of absolute methanol with continuous shaking; the stain solution was then filtered though out filter paper and stored at room temperature in dark bottles.

The stain was diluted from the stock in ratio of 1 ml of giemsa stock solution in 1.25 absolute methanol and 0.5 ml of sodium bicarbonate and 40 ml of D.W. (Shubber *et al.,* 1991).

6. Sodium Bicarbonate Solution:

This solution was prepared by dissolving 4.4 gm of ($NaHCO_3$) in 100ml of sterile D.W. this solution kept at 4˚C until used (Allen *et al*., 1976).

7. RPMI 1640 Medium:

This medium contained the following components :-

RPMI 1640 medium base	10 gm
Fetal calf serum (heat inactivated)	15%
Hepes	1%
Sodium bicarbonate	1%
Penicillin	100 Iu/ml
Streptomycin	100 µg/ml
BUdR	15µg/ml

The volume was completed with sterile D.W. to 1000 ml, and the pH was fixed to 7.2 and sterilized by filtration using 0.22µm size filter under aseptic conditions, after that 2 ml of the medium was transferred into sterile test tubes and kept at 4˚C until used (Shubber *et al.,* 1991).

8. Bromodeoxyuridine (BUdR):

This solution was prepared by dissolving 50 mg of 5-bromo-deoxyuridine powder in 37.5 ml of D.W.The solution was sterilized by filtration, distributed in sterile tubes with final concentration 1.33 mg/ml and stored at -20˚C until used (Shubber *et al.,* 1991).

9. Hoechst (33258) Stain:

This fluorescent stain was prepared by dissolving 0.025 gm in 100 ml phosphate buffer saline (stock), and 2 ml (stock) then was taken and added to 100 ml of D.W. (Shubber *et al.,* 1991).

10. Antibiotic Preparation:

Streptomycin was prepared by dissolving 1 g of streptomycin in 100ml D.W. Ampicillin was prepare by dissolving 1000000 unite /100 ml D.W., both antibiotic were sterilize by filtration under aseptic conditions (Allen *et al*., 1976).

11. Normal Saline:

It was prepared by dissolving 8.5 gm of NaCl in 1 liter of D.W.

12. Leukocyte Diluent's Solution (Catalano, 2002):

Two ml of glacial acetic acid was added to 98 ml distilled water and few drops of methylen blue was added as a color detector, kept at 4˚C until used.

13. Sourensens Buffer:

Prepared by dissolving 9.47 gm of Na_2HPO_4 and 9.08 gm KH_2PO_4 in 100 ml of D.W. pH was fixed to 6.8, kept at 4˚C until used (Allen *et al*., 1976).

3.3. Laboratory Animals:

Seventy Albino Swiss mice belong to the strain of *Mus musculus* were received from Biotechnology Research Center (Al-Nahrain University) with weight 25-30 grams. Their age ranged between (8-12) weeks. They were divided into (12) groups, each group includes 4 mice that were put in separated plastic cage

and those cages were kept in normal condition 23-25˚C room temperature. All those animals were fed with suitable quantity and quality of complete diet and water, which include the locally prepared feeding from the following materials:

Product	Percentage %
Crushed barley	24.50
Crushed wheat	30.00
Crushed yellow corn	22.50
Soya bean	15.20
Sodium chloride NaCl	0.45
Calce stone	0.20
Animal protein	7.15

3.4:Preparation of Metronidazole:

Metronidazole was obtained from (Samara Drug Industry company) at concentration of 500mg/tablet, one tablet of metronidazole was dissolved in 5 ml of sterile PBS to make a stock solution (100mg/ml), and from this solution we made the doses of 1, 100, 200 or 400mg/kg (Legator *et al*., 1975) to be used in mouse studies as shown in the figure (3.1).

While in human blood culture studies, one tablet of metronidazole was dissolved in 100ml of sterile D.W. to make a stock solution (5mg/ml). Different concentrations (2.5, 5, 10, 20, 40 or 80µg/ml) were prepared from this stock solution, and then sterilized by filtration and kept at 4˚C until being used as shown in figure (3.2).

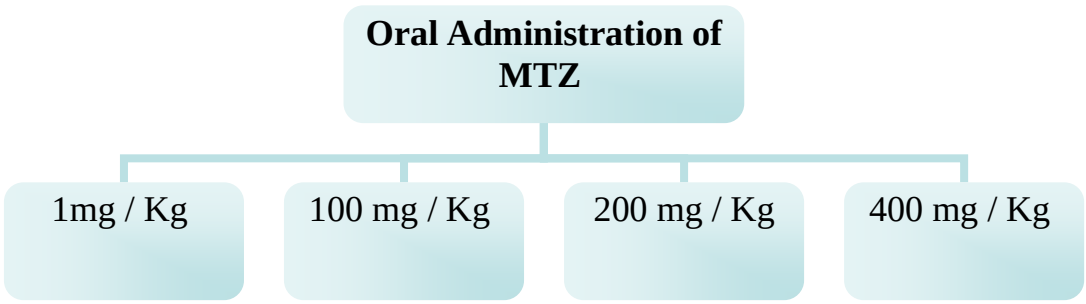


Fig (3.1): Oral administration of Metronidazole in mice.

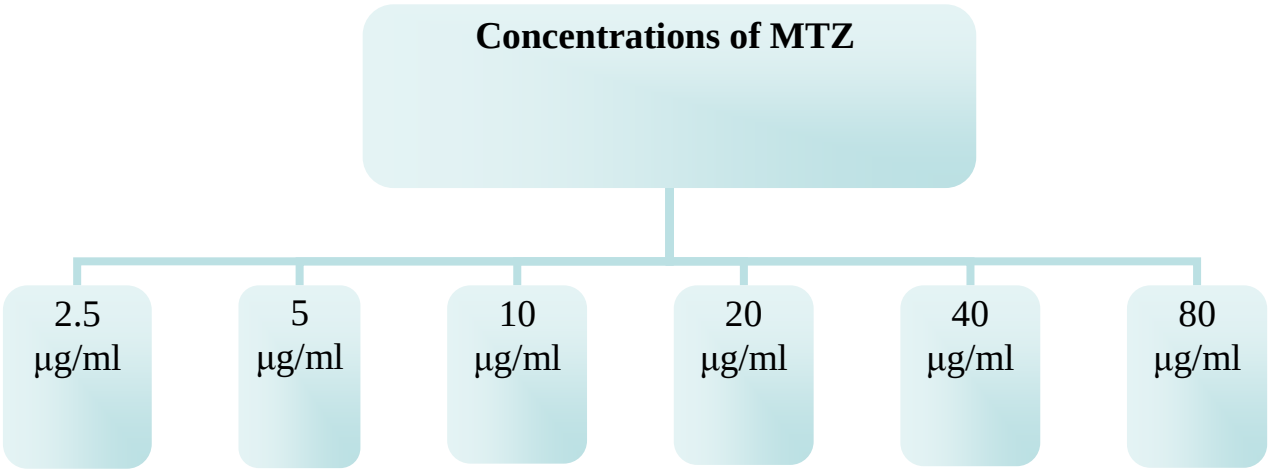


Fig (3.2): Concentrations of MTZ in human blood lymphocyte culture (*in vitro*).

3.5: Collection of *P. odoratissimum* (L.)soland:

The plant was collected from a local garden in AL- Kadisya in November, and identified by Dr. Ali Al- Mousawy Herbarium of the College of Science, Department of Biology in Baghdad University .The leaves were air dried at room temperature and grieved into powder form (Figure 3.3).

Figure (3.3): *Pelargonium odoratissimum* (L.) soland (Taken by the author).

3.6. Plant Extraction:

Water extraction:

The powdered leaves were macerated with D.W.in ratio 1:5(w/v) (50 g of the power was mixed with 250 ml distilled water). Then extracted in the reflex for 3 hours, filtrated and placed in the rotary evaporator until it became dry.

3.7. Preparation of *P. odoratissimum*:

Two grams of dried plant extract were taken and dissolved in 10 ml of sterile PBS to make a stock solution (200mg/ml), and from this solution different doses of 100, 200, 400 or 800mg/kg were prepared to be used in mouse studies as shown in figure (3.4).

While in human blood culture studies, 0.5 gm of plant extract was dissolved in 10 ml to make a stock solution (50mg/ml). Different concentrations 5 , 10 , 20 , 40 ,80 or 100µg/ml were prepared from this stock solution, and then sterilized by filtration and kept at 4˚C until being used as shown in figure (3.5).

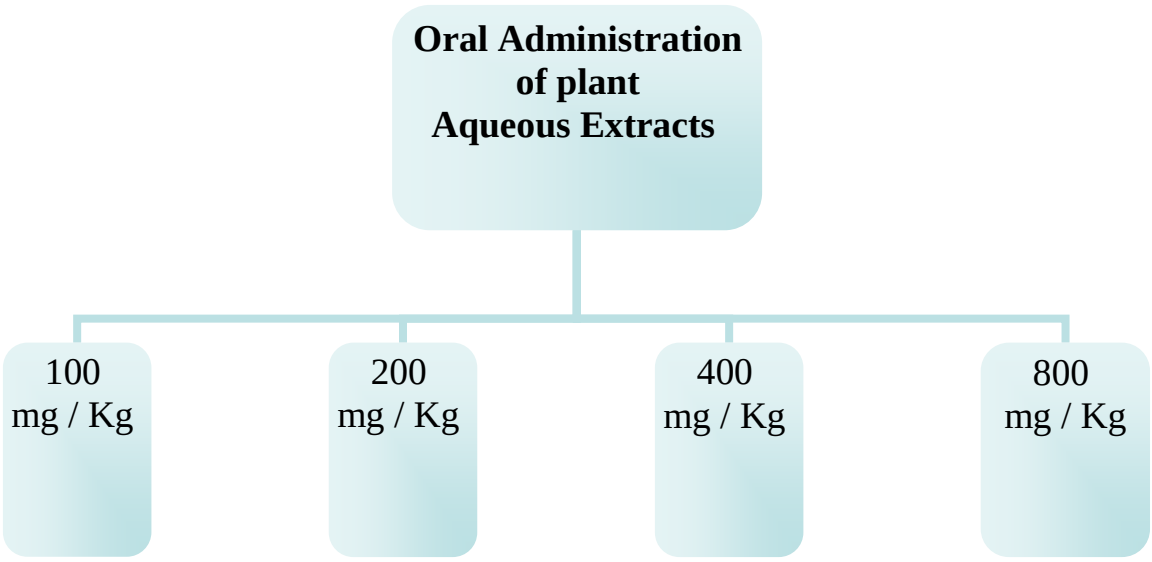


Fig (3.4): Oral administration of plant aqueous extract.

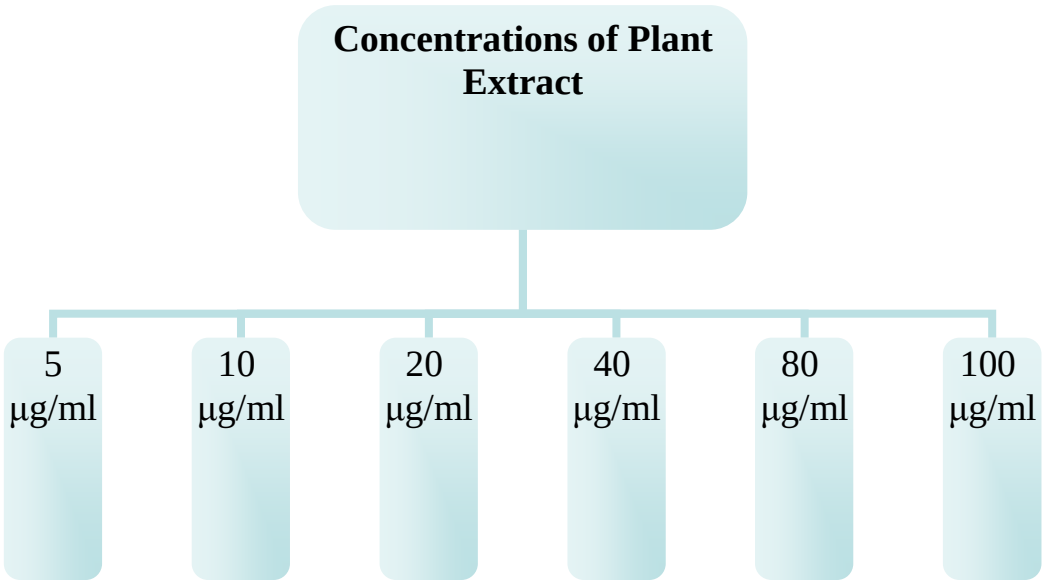


Fig (3.5): Concentrations of plant aqueous extracts in human blood lymphocyte culture (*in vitro*).

3.8. Chemical study of Plant Active Compounds:-

3.8.1 Preparation of Reagents:-

1. Mayer's Reagent:

This reagent was prepared from the following solutions:

Solution (A) prepared by dissolving 1.58 g of mercuric chloride ($HgCl_2$) in 60 ml of D.W. while solution (B) was prepared by dissolving 5g of KI in 10 ml of water , solutions A and B were mixed and the volume was completed to 1000 ml by D.W. (Smolensk *et al*.,1972).

2. Fehling Reagent:

This reagent was prepared from the following solutions:

Solution (A) was prepared by dissolving 35 g of cupric sulfate (CuSO4) in 100 ml of D.W. and then diluted with 500 ml D.W., while solution (B) was prepared by dissolving 7 g of sodium hydroxide (NaOH) and 175g (Rochelle ´s salt) in 100 ml of D.W. and the volume completed to 500 ml with D.W., equal volumes of A and B were mixed **(**Shihata, 1951).

3. Ferric Chloride ($FeCl_2$)Solution (Shihata, 1951)

This solution was prepared by dissolving 1 g of FeCl2 in 100 ml of D.W.

3.8.2 Detection of Some Active Plant Components:

This study included detection of some active component: alkaloids, flavinoids, tannins, saponins and glycoside.

1. Detection of Tannins:

This was done according to the method described by Shihata (1951). Five grams of the plant extract were mixed with 25ml of distilled water in a magnetic stirrer; the mixture was boiled in a water bath for a few minutes, and then filtered, and the filtrate was treated with few drops of 1% of lead acetate solution. The development of greenish-blue precipitate was an indication of the presence of tannins.

2. Detection of Saponins:

This was done according to the method described by Stahl (1969).

Saponins were detected by two methods:

a - Aqueous extract of the *P. odoratissimum* was shaken vigorously with distilled water in a test tube. The formation of foam standing for a time indicated the presence of saponins.

b-Five milliliters of aqueous extracts of the *P. odoratissimum* were added to 3ml of 3% ferric chloride solution, a white precipitate developed which represented an indication of the presence of saponins.

c- Haemolytic test was done according to Cheeke (1971), two test tubes in each one 5ml of 10% solution of blood in normal saline, and 5ml of normal saline

solution were added and to the other 5ml of aqueous extract of plant was added to one test tube, both tubes were shaked gently

3. Detection of Glycosides:

a.Non Hydrolysed Extracts:

Equal amounts of the aqueaous extract of the plant was mixed with fehling reagent in a test tube, then boiled in a water bath for 10 minutes. Red precipitate was formed, this was an indication of the prescience of glycosides (Shihata, 1951).

b. Hydrolysed Extract:

Few drops of diluted HCl was added to 5 ml of the aqueous extract of the plant , then left in a boiling water bath for 20 minutes, the acidity was neutralize by NaOH solution, equal volume of Fehling reagent was added .The development of red precipitate was an indication for the ą glycon part of the glycoside(Shihata, 1951).

4. Detection of Flavanoids:

Ethanolic extract of the plant was partitioned with petroleum ether ; the aqueous layer was mixed with the ammonia solution. The appearance of dark color was an evidence for the presence of flavonoids (Harborne, 1973).

5. Detection of Alkaloids:

Ten grams of plant powder was boiled in 50 ml of D.W. then cooled and filtrated.The supernatant was placed into clock glass and mixed with mayer´s reagent. The appearance of white precipitate indicated the presence of alkaloids (fahmy, 1951).

3.9. Administration of Laboratory Animals:-

3.9.1. Metronidazole

The animals in this experiment were treated with cumulative doses of metronidazole in a short time. The main aim of this experiment was to evaluate the acute treatment effect of MTZ by applying cytogenetic analysis and examining the inhibition in mitotic activity and the induction of chromosomal aberrations in normal bone marrow cells. Also to examine the total and differential count of leukocytes in normal mouse blood. It also aimed to select the most suitable administration duration of metronidazole treatment which is harmful to be used in the next experiments.

Metronidazole doses were chosen according to (Legator, *et al.,* 1975).

Five groups of mice were used in this experiment and treated as follows:

Group I: Negative control (4 mice), treated with (0.1 ml) PBS.

Group II: MTZ treatment (4 mice), treated with (0.1 ml) of MTZ (1 mg/kg)

Group III: MTZ treatment (4 mice) , treated with (0.1 ml) of MTZ (100 mg/kg)

Group IV: MTZ treatment (4 mice) , treated with (0.1 ml) of MTZ (200 mg/kg)

Group V: MTZ treatment (4mice), treated with (0.1 ml) of MTZ (400 mg/kg)

The MTZ was given orally for 4 successive days as in figure (3.1), then the mice were sacrificed after one day. Bone marrow and blood samples were taken and cytogenetic analyses and blood count were carried out as described later see (3.11.1) and (3.13).

3.9.2 *P. odoratissium* :Aqueous Extract

Evaluation of the effects of *P. odoratissimum* aqueous extracts was carried on by applying cytogenetic analysis , examining the mitotic activity and the chromosomal aberrations in normal bone marrow cells examination of the total and differential count of leukocytes in normal mouse blood cells. The most suitable

concentration of *P. odoratissimum* aqueous extract was selected which is useful to be used in the treatment of animals.

Five groups of mice were used for this experiment and treated as follows:

Group I: Negative control (4 mice) were treated with (0.1 ml) PBS.

Group II: Treated (4 mice) with *P. odoratissimum* aqueous extract, (0.1 ml) of plant extract (100mg/kg)

Group III: Treated (4 mice) with aqueous extract, (0.1) ml plant extract (200 mg/kg)

Group IV: Treated (4 mice) with aqueous extract, (0.1 ml) plant aqueous extracted (400 mg/kg)

Group V Treated (4 mice) with aqueous extract, (0.1 ml) of plant aqueous extract (800 mg/kg).

P. odoratissimum aqueous extract was given orally for 4 successive days as shown in figure (3.4), and then the mice were sacrificed after one day. Bone marrow and blood samples were taken and cytogenetic analyses and blood count were carried out as described later see (3.11.1) and (3.13).

3.9.3 The Interaction Study Between Metronidazole and Plant Aqueous Extract in Mice (*In vivo*).

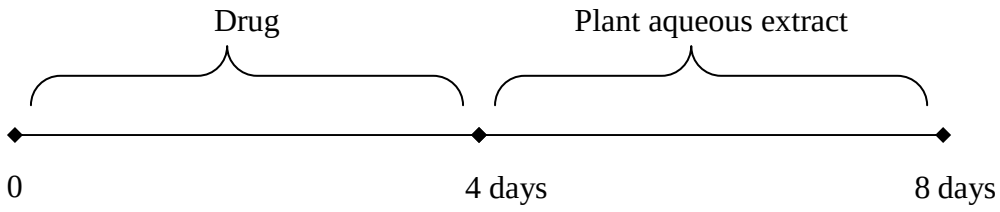
3.9.3.1: Pre-Drug Treatment with Plant Aqueous Extract:

This group of (10) mice was divided into four subgroups as in figure (3.6), as follow:

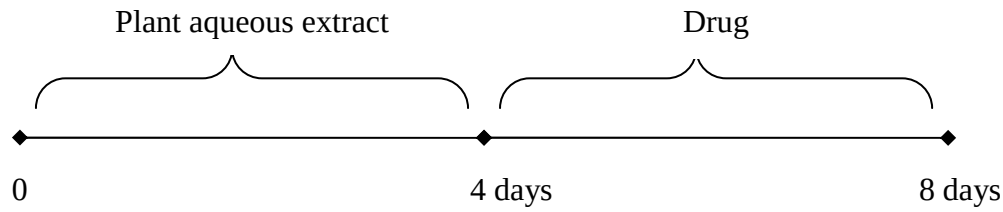
Group I: negative control (2 mice), treated with (0.1 ml) PBS for eight days.

Group II: positive control (2 mice), treated with (0.1 ml) of MTZ (400mg/kg) for four successive days.

Group III: Treated with (0.1 ml) of *P. odoratissimum* aqueous extract (100mg/kg) for four successive days (2 mice).



(A)



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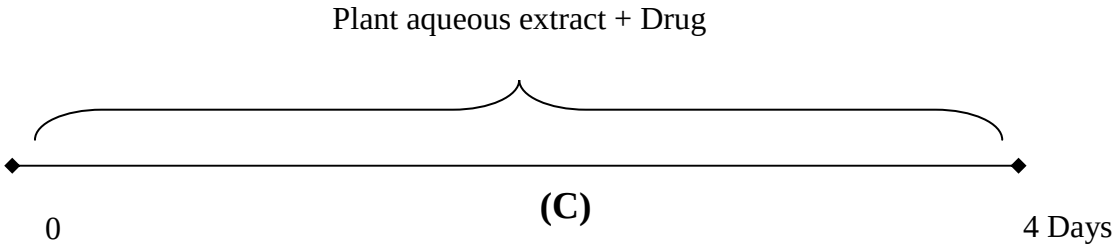


(C)

Figure (3.6) :The Interactions between MTZ and *P. odoratissimum* aqueous extract in mice (*In vivo*),(A) Post-treatment,(B)Pre-treatment and (C) Simultaneous treatment.

Group IV: Pre-treatment group (4 mice) Treated with (0.1 ml) of plant extract (100mg/kg) for four successive days, then treated with (0.1 ml) of MTZ (400mg/kg) after four successive days as in figure (3.6).

3.9.3.2 Post-Drug Treatment with Plant Aqueous Extract:

This group of (10) mice was divided into four subgroups, as follow:

Group I: negative control (2 mice), treated with (0.1 ml) PBS.

Group II: positive control (2 mice), treated with (0.1 ml) of MTZ 400mg/kg for four successive days.

Group III: Treated with (0.1 ml) of plant aqueous extract (100 mg/kg) for four successive days (2 mice).

Group IV: Post-treatment group (4 mice), treated with (0.1 ml) of MTZ 400mg/kg for four successive days with (0.1 ml), then treated with (0.1 ml) of plant extract (100mg/kg) for four successive days as in the figure (3.6).

3.9.3.3: Simultaneous Treatment with Plant Aqueous Extract and Drug:

This group of (10) mice was divided into four subgroups, as follow:

Group I: negative control (2mice), treated with (0.1 ml) PBS.

Group II: positive control (2 mice), treated with (0.1 ml) of MTZ (400mg/kg) for four successive days.

Group III: Treated with (0.1 ml) of plant extract (100 mg/kg) for four successive days (2 mice).

Group IV: Simultaneous treatment group (4 mice), treated with (0.1 ml) of metronidazole (400mg/kg) and of plant extract of (100 mg/kg). The drug and plant aqueous extract were mixed together and incubated at (37˚C) for three hours before giving orally to the mice, the tests were carried out for four successive days as in the figure (3.6).

3.10: Human Blood Lymphocyte Culture (*In vitro*):

The blood samples were collected randomly from the students of AL-Nahrain University. There age ranged between 20-28 years, 5 ml were collected in hyperinized syringe. Ten different samples of human blood lymphocyte were collected for MTZ to examine the six different concentrations as shown in figure (3.2), to select the most harmful concentration ,and the negative control 0.2 ml of PBS was added .

For plant extract five different samples of human blood lymphocyte we collected to examine the six different concentrations as shown in figure (3.5), to select the lowest side effects to be used, and the negative control 0.2 ml of PBS was added .

3.10.1 The Interaction between MTZ and *P. odoratissiumn* Aqueous Extract in Human Blood Lymphocyte Culture (*In vitro*).

In this study, five different blood samples were used.

Group 1: negative control (normal media), culture of human blood lymphocyte was initiated *in vitro* under optimum conditions; 0.2ml of PBS was added.

Group 2: positive control (MTZ) at concentration of 80µg/ml 0.2 ml was added to the human blood culture for 72 hr.

Group 3: plant extract at concentration of 10µg/ml and 0.2 ml was added to the human blood culture for 72 hr.

Group 4: Post –drug treatment: after normal culturing of human blood cells, MTZ (80µg /ml) was added to the culture after 48 hr. The tubes taken and centrifuged for 10 min. at 1500 rpm. The supernatant was discarded and normal media and plant extract (10 µg /ml) were added for other 24 hours, i.e. the culture period would be 72 hr. as shown in figure (3.7).

Group 5: Pre –drug treatment: after culturing of normal human blood, plant aqueous extract (10 µg/ml) was added to the culture for 48 hr. The tubes were centrifuged for 10 minutes at 1500 rpm and the supernatant was discarded and

normal media and MTZ (80µg/ml) were added for the last 24 hours of culture periods as shown in figure (3.7).

Group 6: Simultaneous treatment: after normal culturing of human blood lymphocytes, a mixture of plant extract and MTZ were added to the culture for 72 hr. as shown in figure (3.7)

Group 7: After normal culturing of human blood, plant extract 0.2 ml at a concentration (10µg/ml) added to the culture after 48 hr. The solutions were centrifuged then the supernatant was discarded and only normal media was added as shown in fig. (3.8).

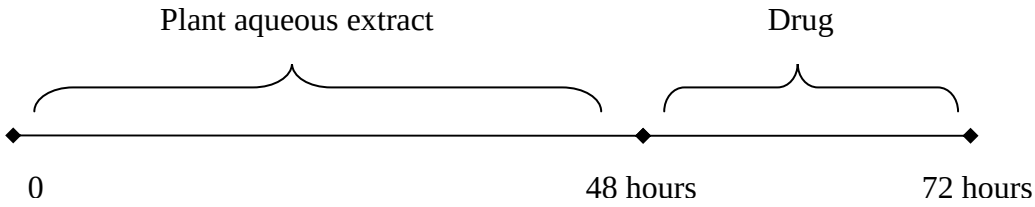
Group 8: After normal culturing of human blood, MTZ 0.2 ml at concentration (80µg/ml)was added then after 48 hr.The tube were centrifuged the supernatant was discarded and only normal media was added as in fig.(3.8).

3.11: Cytogenetic Experiments:

3.11.1 Chromosomal Preparation from Somatic Cells of the Mouse Bone Marrow (i*n vivo*):

The experiment was done according to Allen *et al*. (1976) as follows:

1. The animal was injected with 0.25 ml of colchicine with concentration of 0.25mg/ml intraperitoneally (IP) 2hr. before sacrificing the animal.
2. The animal was sacrificed by cervical dislocation.
3. Then it was fixed on its dorsal side on the anatomy plate and the abdominal side of the animal and its thigh region were swabbed with 70% ethanol.
4. The femur bone was taken and cleaned from the other tissues and mussels, then gapped from the middle with a forceps in a vertical position over the edge of a test tube, by a sterile syringe, (5ml) of PBS were injected so as to wash and drop the bone marrow in the test tube.



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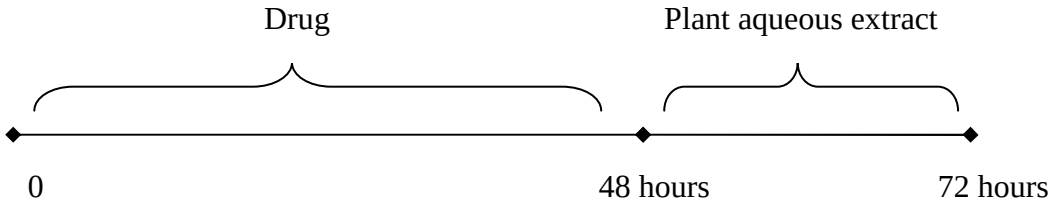

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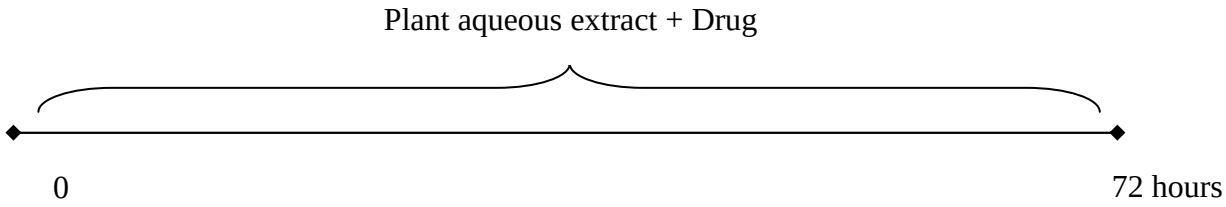
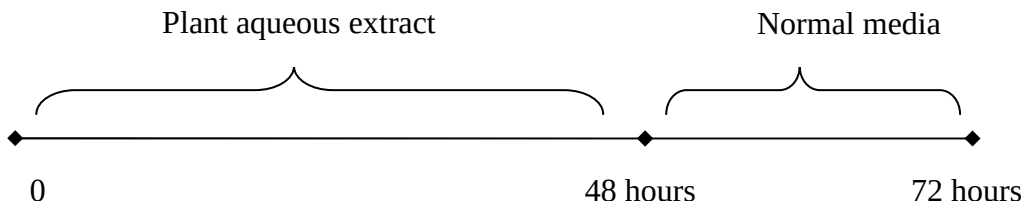
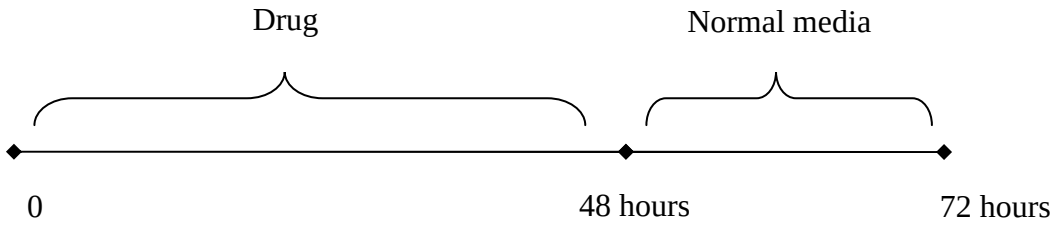


(C)

Figure (3.7): Study the interaction between MTZ and *P. odoratissimum* aqueous extract in human blood lymphocyte culture (*in vitro*), (A) Pre-treatment, (B) Post-treatment and (C) Simultaneous treatment.



(A)



(B)

Figure(3.8): Study the normal culturing of MTZ and *P. odoratissimum* aqueous extract in human blood lymphocyte culture (*in vitro*)for 48 hours. (A) normal culturing of drug and (B) normal Culturing of plant aqueous extract.

5. The test tubes were taken and put in the centrifuge at speed of 2000 rpm for 10 min.

6. The supernatant was removed and (5ml) of potassium chloride (KCl) was added as a hypotonic solution at (0.075M), then the test tubes were left for 30 min in a water bath at 37˚C, and shaked from time to time.

7. The tubes were centrifuged at 2000 rpm for 10 min.

8. After that, the supernatant was removed and the fixative solution was added (as drops) on the inside wall of the test tube with continuous shaking, the volume was fixed to (5ml) and the contents were shaked well.

9. The tubes were kept at 4˚C for 30 min to fix the cells.

10. The tubes were centrifuged at 2000 rpm for 10 min.

The process was repeated for 3 times and the cells were suspended in (2ml) of the fixative solution.

11- By a pasture pipette, few drops from the tube were dropped vertically on the chilled slide from a height of 3 feet at a rate of (4-5) drops to give the chance for the chromosomes to spread well. Later, the slides were kept to dry at room temperature..

12- The slides were stained with Giemsa stain and left for (15 min),and then washed with D.W.

13-Two slides for each animal were prepared for cytogenetic assays.

3.11.2: Cytogenetic Analysis of Human Blood Lymphocytes (Shubber, 1987)

1. Human blood was collected into heparin coated syringe.

2. Peripheral bloods(0.25 ml)was added into test tube containing 2 ml of culture medium (RPMI-1640).

3. PHA (0.25 ml)was added, the components were mixed very well.

4. A portion of 0.2 mg/ml of colchicine was added to each tube, 2hr. before cell harvesting.

5. The test tube ontents were centrifuged at speed 2000 rpm for 10 min.

6. The supernatant was removed, and 5 ml of potassium chloride (KCl) added as hypotonic solution at (0.075 M), then the test tubes were left for 30 min in the incubator at 37°C, and shaked from time to time.

7. The tubes were centrifuged at 2000rpm for 10 min.

8. After that, the supernatant was removed and the fixative solution was added as drops on the interior wall of the test tube with continuous shaking and then, the volume was fixed to 5 ml and the contents were shaked well.

9. The tubes were kept at 4°c for 30 min to fix the cells.

10. The tubes were transferred in to the centrifuge at 2000 rpm for 10 min. The process was repeated for 3 times, the cells were then suspended in (2 ml) of the fixative solution.

11. By a pasture pipette, few drops from the tube were dropped vertically on the chilled slide from a height of 3 feet at a rate of (4-5) drops to give the chance for the chromosomes to spread well .The slides were kept to dry at room temperature..

12. The slides were stained with giemsa stain and left for (15 min), then washed with D.W.

13. Two slides for each tube were prepared for cytogenetic assays.

Another slides for each concentration were stained with Hoechst stain (33258) for the analysis of cell cycle progression and sister chromatid exchange.

3.11.3: Hoechst (33258) Staining

(Ian-Freshney, 2000).

1. The slides were immersed in a coplin jar Hoechst 33258 at a concentration of 20µg/ml for 10 min.
2. The slides were transferred to a slide rack, and 500 µl of 2x SSC were dropped.
3. The slides were covered with 22-mm x 50- mm cover slips, and the edges were sealed with a temporary seal, such as cow gum, to prevent evaporation.

4. The covered slides in the slide rack (cover slip facing downwards) were placed on a short-wave UV box. Maintain a distance of approximately 4 cm between the slides and the UV source. The longer the pale chromatid will become, expose the slides for about 24-60 min.
5. The covered slips were removed, and the slides were washed three times in ultra purified water (UPW), 5min. per wash. The holder was covered with aluminum foil.
6. The slides were air dried in the dark.
7. The slides were stained in a coplin jar containing 3.5% Giemsa solution in sourensens buffer (pH 6.8) for 3-5 min.
8. The slides were carefully rinsed in tap water, and drained using a paper tissue.
9. The slides were air dried on the bench for 1 hour, and dipped into xylene, 4 drops of DPX mountant were dropped onto the slide and a 22-mmX50- mm cover slip was lowered, expressing any air bubbles with tissue.
10. The slides were air dried in a fume hood overnight

3.12. Total and Differential Count of Mouse Blood Leukocytes

All mice groups which were used for the cytogenetic experiment (3.11.1) and shown in figures (3.1) and (3.3), were heart punctured before being injected with colchicine , to collect the blood for total and differential count of leukocytes.

3.12.1: Total Count of Leukocytes (Catalano, 2002):

1. The blood was taken by heart puncher and put into heparinized tube.
2. A diluting solution (190 µl) was pipetted into test tube.
3. The heparinzed blood (10µl) was pipetted and mixed well with diluting fluid for at least 2 minutes.
4. The heomcytometer was set up with its cover glass in position, by a pasteur pipette, both sides of the hemocytometer were filled with the diluted blood.

5. The cells were allowed 2 minutes to be settled.

6. The cells were counted in the four large squares on both sides of chamber, by using the 40 X objective and subdued light.

7. The WBCs were calculated on the basis of cells counted, counted area, and the dilution.

No. of cells (cells/mm^3blood) =no. of cells in four square × volume correct × dilution correct /4

3.12.2: Differential Count of Leukocytes (Catalano, 2002**):**

1. A small drop of heprinazed blood was put on the end of clean, dry slides. A pusher slide was place at an angle of 30° to 45°to the slide and then moved it back to make contact with the drop. The forward movement of the pusher spreads the blood on the slide.
2. The blood film was allowed to dry in the air.
3. The slides were completely covered with Giemsa stain, after 3 minutes the slides were washed gently and then examined under light microscope.

No. of cells (cells/mm3blood) = (total no. of leukocyte × cells %)

3.13. Cytogenetic Parameters Analysis:

1- Mitotic Index (MI) Assay:

The slides were examined under high power(40 X) of compound light microscope and (1000) of divided and non divided cells were counted and the percentage rate was calculated for only the divided ones according to the following equation :

Mitotic index =no. of the divided cells/ total no. of the cells (1000) ×100

2-Blasto Index (BI) Assay:

The slides were examined under high dry power (40X) of compound light microscope and (1000) cells were counted to calculate the percentage rate of the blast cells according to the following equation:

Blasto index (BI) =no. of the blast cells/total no. of cells (1000) ×100

3-Chromosomal Aberrations (CAs) Assay:

The prepared slides were examined under the oil immersion lens for 100 divided cells per each animal or blood lymphocytes culture, and the cells should be at the first metaphase stage of the mitotic division where the chromosomal aberrations are clear and the percentage of these aberrations was estimated.

4-Sister Chromatid Exchange (SCE) Assay:

Sister chromatid exchange was counted in 50 well spread second metaphases.

5-Replicative index (RI) assay

The replicative index (RI) was determined by counting the number of cells at the first, second and the third metaphase in (100) cells at metaphase, the RI was calculated according to the following equation:

RI= (1xM1%) + (2xM2%) + (3xM3%)/100 (Lamberti *et al*.,1983)

3.14: The Protective Value of *P. odoratissimum* Aqueous Extract:

The protective value of *Pelargonium odoratissimum* aqueous extract was calculated according to the following equation:-

Protective value = A-C/A-B×100

A = (+) ve control (treatment with MTZ only)

B = (-) ve control (treatment with PBS only)

C = interaction group (treated with MTZ and plant aqueous extract).

(Rawat *et al*., 1997)

3.15: Statistical Analysis:

One or two way analysis of variance was performed to test whether group variance was significant or not, the comparison between groups were made using SAS package (SAS, 2001).

Chapter Four: Results and Discussion

4.1: Detection of Some Active Compounds in *P. odoratissimum*:

Using different chemical reagents and solutions for detection of various active compounds found in the *P. odoratissimum*, results are represented in table (4-1). The results indicated that *P. odoratissimum* contains flavonoids and saponins, while glycosides, alkaloids and tannins are absent in this plant.

Table (4-1): Active Compounds in *P. odoratissimum*

Chemical Components	Reagents Used	Indication	Results of Detection
Saponins	1.Saking water extract	(Foaming form)	+ve
	2.Ferric chloride 1%	(White ppt.)	+ve
	3.Blood	(Hemolysis)	+ve
Tannins	Lead acetate 1%	(Greenish blue)	-ve
Glycosides (before hydrolysis)	Fehling reagent	(Red ppt.)	-ve
Glycosides (after hydrolysis)	Fehling reagent	(Red ppt.)	-ve
Flavonoids	Ammonia	(Dark color)	+ve
Alkaloids	Mayer´s reagent	(White ppt.)	-ve

+ve : presence of active compound.

-ve : absence of active compound.

4.2: Cytogenetic Effect of Metronidazole:

4.2.1 Cytogenetic Effect of Metronidazole on Mouse Bone Marrow Cells *in vivo*:

The mitotic activity and the chromosomal aberrations in mouse bone marrow cells were examined in mice treated with four doses of MTZ administrated orally.

4.2.1.1: Metronidazole Effect on Mitotic Index:

Depending on our results, white mice have a mitotic index (6.70%) in their bone marrow cells, this considered as a negative control.

Table (4-2) revealed that the mitotic index of mouse bone marrow cells treated with MTZ was decreased with the increase of drug doses, a significant reduction ($p<0.05$) in mitotic index to (5.4, 4, 3.4 and 3.30%) on their bone marrow cells was seen at doses of MTZ (1, 100, 200 and 400mg/kg) respectively.

This reduction in MI may be related to several factors: first of all the proteins required for mitosis were not produced at the same quantities, or the code did not reach the cells to induce it for proliferation, or the drug may cause the death of bone marrow cells (Turner *et al*., 1988), or the mitotic activity of the cells affected by MTZ was not repaired, or it may be due to a defect occurred in the mitotic spindle composition during cell division (Shiraishi, 1978).

There are other chemotherapentic drugs that also cause mitotic index inhibition, like cyclophosphamide (CP) (Al-Robiaay, 2000), methotrexate (MTX) (Al –Amiry, 1999) , mitomycin-C (MMC) (Littlefield *et al*., 1979; Shubber,1981; Shubber *et al.*,1985) and Tamoxifen (TMA)(Al-Sudany, 2005).

Table (4.2): Cytogenetic Effect of Metronidazole on Mouse Bone Marrow Cells (*In vivo*).

Doses of MTZ mg/kg	Mitotic Index %mean±S.E.	Chromosomal Aberrations % mean± S. E.							
		Ring	Gap	Acentric	Dicenteric	Chromosome breaks	Chromatid breaks	Deletion	Total
Negative Control	6.70±0.04 A	0.021±0.004 A	0.451±0.011 A	0.042±0.001 A	0.026±0.011 A	0.02±0.001 A	0.02±0.001 A	0.015±0.004 A	0.595±0.04 A
1mg /kg	5.40±0.02 B	0.012±0.001 A	0.690±0.02 A	0.0451±0.002 A	0.027±0.001 A	0.010±0.007 A	0.143±0.09 B	0.036±0.001 B	0.9631±0.020 A
100mg /kg	4.00±0.20 C	0.040±0.001 B	0.495±0.10 A	0.075±0.003 A	0.681±0.04 B	0.015±0.008 A	0.184±0.08 B	0.058±0.004 C	1.548±0.016 B
200mg /kg	3.40±0.05 D	0.122±0.002 C	0.465±0.05 A	0.131±0.005 B	0.901±0.06 C	0.026±0.010 A	0.263±0.016 C	0.087±0.006 D	1.795±0.018 B
400mg /kg	3.30±0.05 D	0.191±0.09 D	0.392±0.03 A	0.165±0.007 C	1.262±0.12 D	0.025±0.018 A	0.312±0.07 D	0.49±0.09 E	3.837±0.013 C

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

4.2.1.2: Metronidazole Effect on the Chromosomal Aberrations (CAs):

The types of CAs which have been observed are ring, gap, acentric, dicenteric, chromosome breaks, chromatid breaks and deletion (Figures (4.1) (4.2) (4.3) and (4.4)). These CAs were noticed in negative control and in mice treated with MTZ as shown in table (4.2).

The spontaneous levels of CAs observed in untreated mouse bone marrow cells was (0.595%). This percentage increased in comparison with the animals treated with MTZ for the four different doses.

Rings (Figure 4.1) were non significantly reduced ($p<0.05$) to (0.012%) at the dose (1 mg/kg) when compared with the negative control (0.021%) whereas significantly increased ($p<0.05$) at the doses (100, 200 and 400 mg/kg) to reach a percentage of (0.191%) at the dose of (400 mg/kg) which was the highest doses used.

Gaps (Figures 4.2 and 4.4) frequency in the negative control was (0.451%), in the treated animals this percentage was significantly different ($p<0.05$) to (0.69, 0.495, 0.465, and 0.392%) at the doses (1, 100, 200 and 400 mg/kg) respectively.

Dicentric and acentric chromosomes were found to be increased non significantly ($p<0.05$) at the dose (1mg/kg). Dicentric increased significantly ($p<0.05$) at the doses (100, 200 and 400 mg/kg) in comparison with negative control (0.026%).

While the acentric (Figure 4.2) percentage was (0.131 and 0.165%) at the doses (200 and 400 mg/kg) respectively in comparison with negative control, which was (0.0421%), table (4.2).

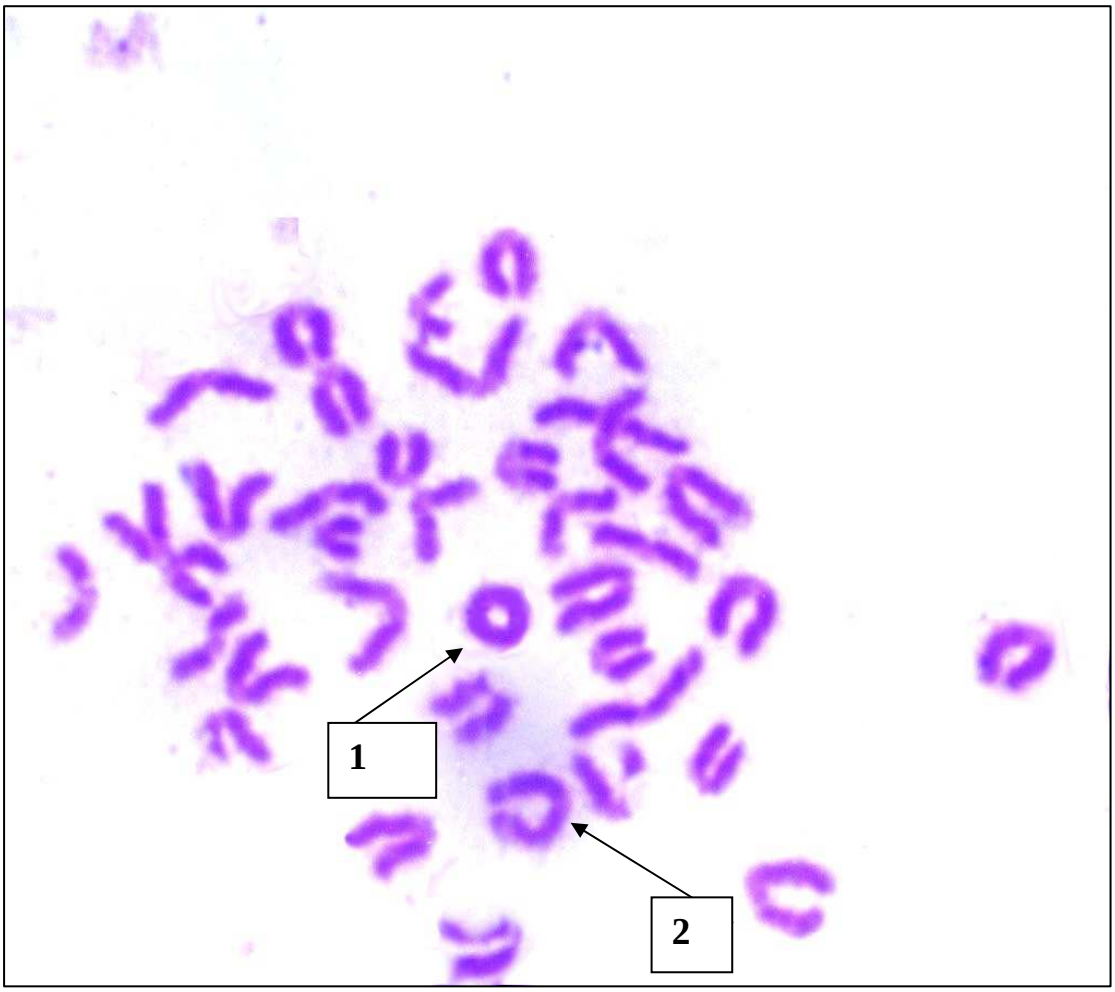


Figure (4.1)Metaphase of bone marrow cells from animals treated with MTZ showing (1) ring and (2) chromosome break(1000x) .

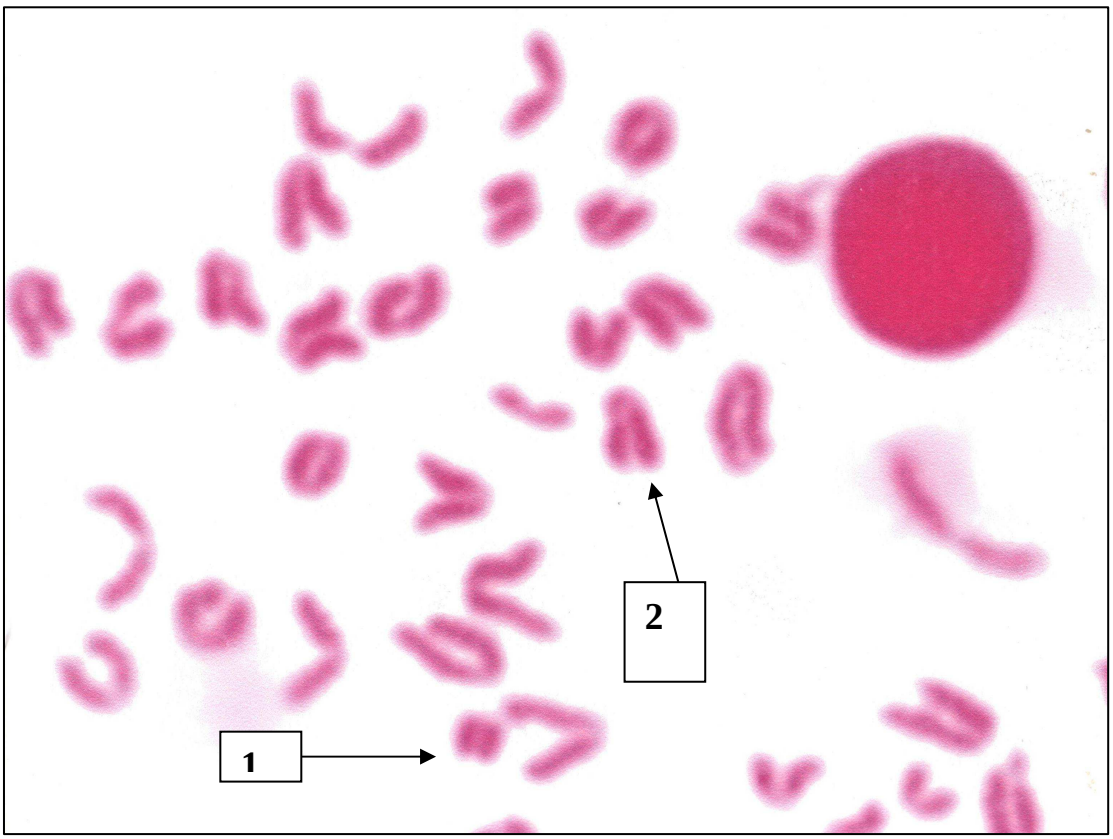


Figure (4.2): Metaphase of bone marrow cells from animals treated with MTZ showing (1) a centric and (2) gap (1000x) .

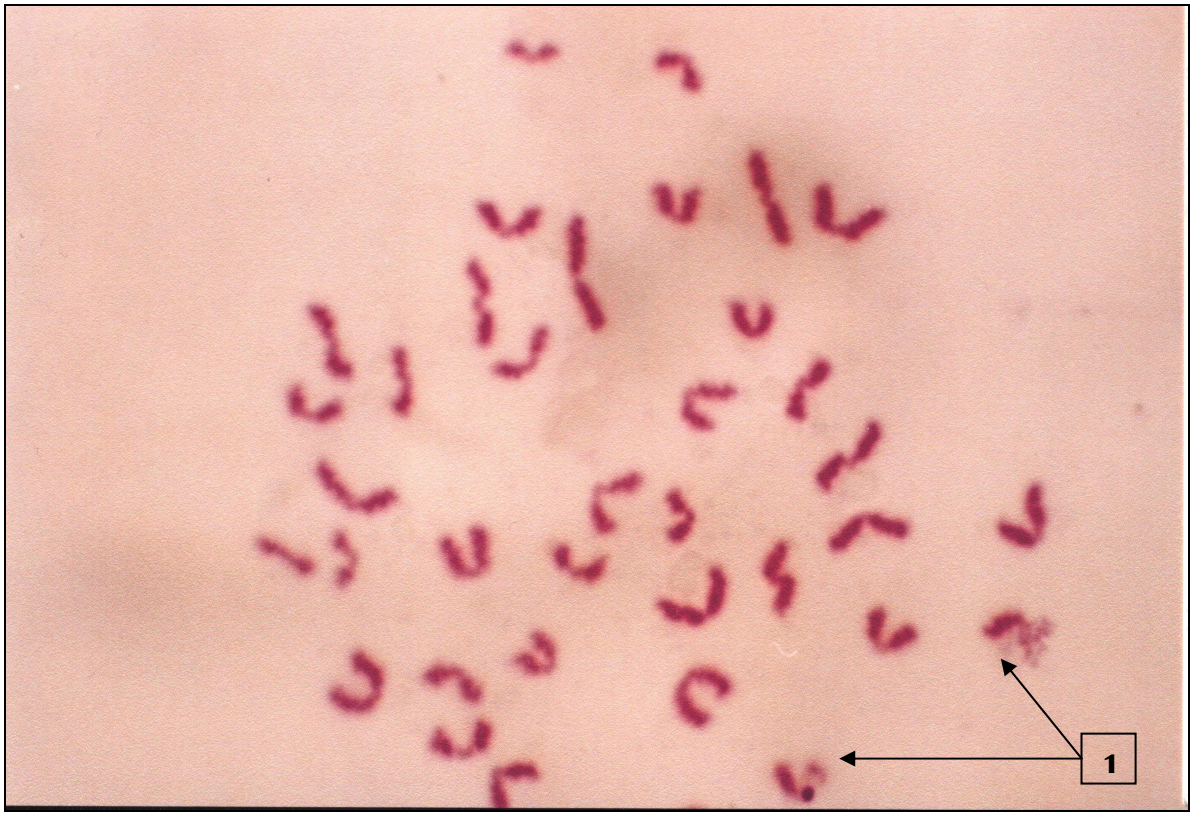


Figure (4.3): Metaphase of bone marrow cells from animals treated with MTZ showing (1) deletion(1000x).

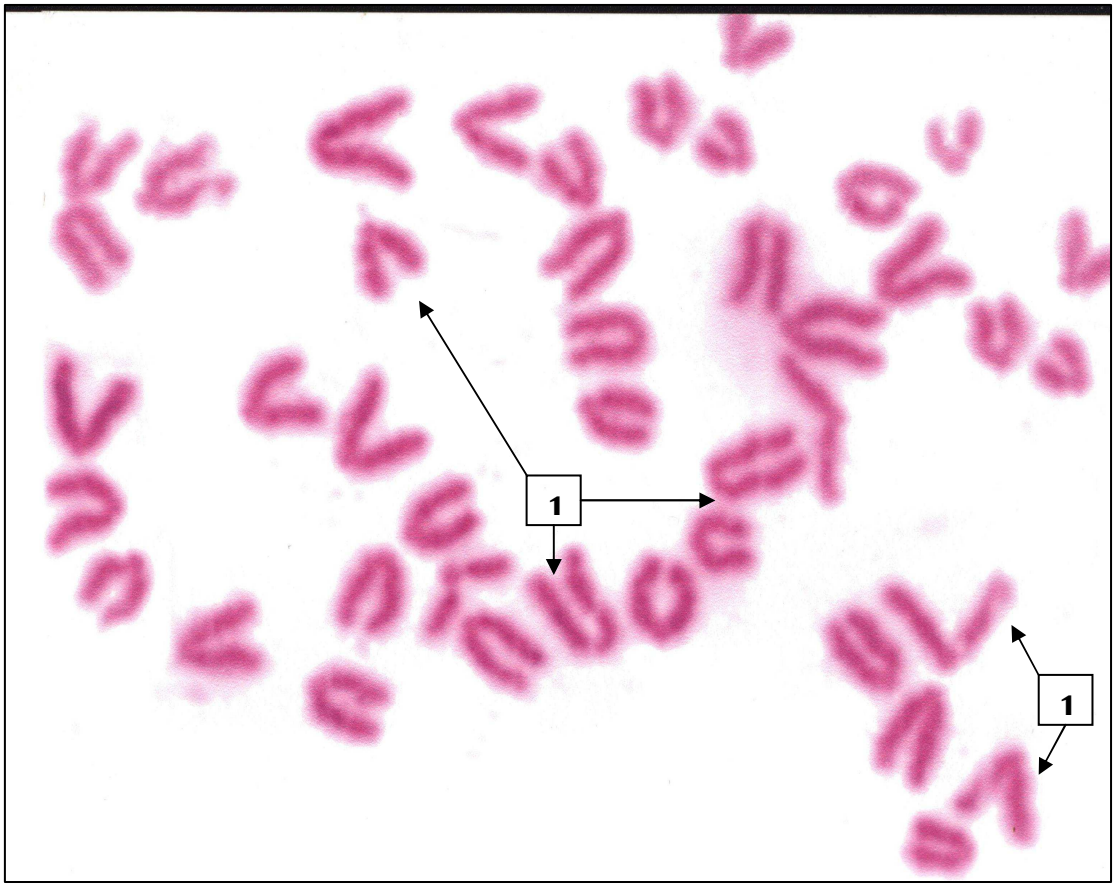


Figure (4.4): Metaphase of bone marrow cells from animals treated with MTZ showing the (1) gap(1000x).

The percentages of chromosome breaks (Figure 4.1) was found not effected by MTZ at the doses (1,100,200 or 400mg/kg). This result appeared clearly when compared with the negative control percentage which was (0.02%).

Chromatid breaks were induced in the presence of the drug and increased significantly ($p<0.05$) with the increase of drug doses. The dose (400 mg/kg) chromatid breaks percentage reached (0.312%) in comparison with the negative control (0.02%).

The deletion (Figure 4.3) percentage increased from (0.015%) in the negative control to (0.036, 0.058, 0.087 and 0.49%) at the doses (1, 100, 200 and 400 mg/kg) respectively, as shown in table (4.2). These results are significantly different ($p<0.05$) from the negative control and from each other.

It was noticed that the percentage of chromatid breaks was higher than the percentage of chromosome breaks after treatment with MTZ. This may be related to the differences in the repair systems of each change. The double-stranded DNA breaks will induce the production of ADP- ribose enzyme more than the single -stranded DNA break. ADP- ribose will activate the repair of DNA break (Gill, 1980).

Treatment with MTZ induced and significantly increase ($p<0.05$) in the levels of CAs in mouse bone marrow cells to (0.9631, 1.548, 1.795 and 3.837%) at the doses (1, 100, 200 and 400 mg/kg) respectively.

These results were in agreement with those of Mitelman *et al.*, 1988. They noticed that CAs increased in prolonging treatment with high doses of MTZ and disagree with those of Roe, 1985 who was unable to demonstrate a potential genetic damage *in vivo*.

4.2.2: Cytogenetic Effect of Metronidazole on Human Blood Lymphocytes *in vitro:*

4.2.2.1: The Effect of Metronidazole on Mitotic Index and Blasto Index:

The mitotic index of normal untreated blood lymphocytes (negative control) was (4.10%) and blasto index was (40.66%), as shown in table (4-3).

A gradual inhibition in MI and BI at the concentrations (10,20,40 and 80µg/ml) was noticed after using different concentrations of MTZ in human blood culture, this inhibition was concentration dependent.

The concentrations of MTZ (2.5 and 5µg/ml) caused significantly increase ($p<0.05$) in the blasto index percentage and non significantly increase ($p<0.05$) in MI. These represent the hormesis effect of MTZ in which very small amounts of chemicals either stimulated the body's repair mechanism or free radical detoxification or by stimulation of immune system (Ikushima *et al.*, 1996).

The highest reduction in MI and BI have been shown at the concentrations of (10, 20, 40 and 80 µg/ml) , of MTZ in comparison with the negative control. This indicates that MTZ inhibits mitosis which was either associated with formation of an incomplete metaphase of chromosomes or an altered arrangement of spindle microtubules that strongly resembled the abnormal organization that occurs in antimitotic compounds (Jordan and Thrower, 1991).

Also MTZ might inhibit the growth and induced apoptosis of cycling normal human peripheral blood lymphocytes that were induced by mitogen (PHA), or that lymphoblast treated with DNA-damaging agents of several chemotherapeutic agents, which intercalates into DNA and inhibits DNA synthesis (Ashizawa *etal*., 1996).

Table (4.3): Cytogenetic Effect of Metronidazole in Human Blood Lymphocytes Culture (*in vitro*).

Concentrations of MTZ (µg/ml)	Blasto Index % mean± S. E.	Mitotic Index % mean± S. E.	Cell Cycle Progression % (mean)			Replicative Index %mean± S. E.	Sister Chromatid Exchange %mean± S. E.
			M1	M2	M3		
Negative Control	40.66±0.01 E	4.10±0.080 E	33	32	35	2.02±0.03 E	6.33±0.02 A
2.5 µg/ml	50.08±0.03 F	4.45±0.035 E	31	32	37	2.06±0.06 E	6.03±0.001 A
5 µg/ml	49.35±0.03 F	4.35±0.049 E	29	38	33	2.04±0.04 E	8.90±0.01 B
10µg/ml	24.28±0.04 D	3.14±045 D	49	28	23	1.74±0.01 D	12.04±0.06 C
20µg/ml	17.39±0.021 c	2.02±0.06 C	63	22	15	1.52±0.05 C	18.50±0.007 D
40µg/ml	12.72±0.029 B	1.10±0.02 B	71	18	11	1.40±0.01 B	24.10±0.003 E
80µg/ml	8.29±0.006 A	0.60±0.01 A	78	21	1	1.23±0.03 A	32.40±0.002 F

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

The RI percentage of normal control was (2.02%), the effect of MTZ on RI in human blood cultures treated with concentrations (2.5 and 5µg/ml) gave us (2.06 and 2.04%)respectively, in which there was non significant decrease ($p<0.05$) in comparison with untreated cultures, except when the concentrations (10, 20, 40 and 80µg/ml) were used, which caused a significant decrease ($p<0.05$) in the RI in comparison with the negative control giving (1.74,1.52,1.4 and 1.23%) respectively.

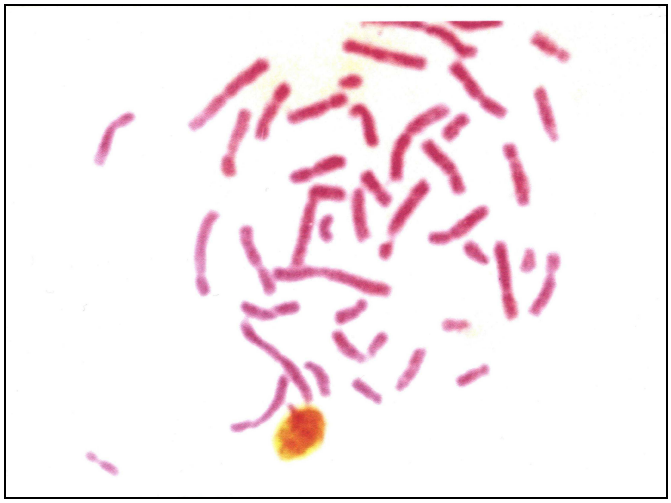
The numbers of cells in M3 reduced gradually with increasing concentrations figure (4.5). These results indicate that MTZ affects cell cycle in comparison with negative control. The cells that have the ability to reach M3 decrease and it depends on concentration except at the 2.5µg/ml which increased to 37 in comparison with negative control which was 35. At concentrations (5, 10, 20, 40 and 80µg/ml) the reduction were (33, 23, 15, 11 and 1) respectively as shown in table (4.3).

These results indicate that MTZ caused a reduction in growth rate of normal blood lymphocytes stimulated by mitogen (PHA) in dose dependent manner. MTZ and its metabolite might inhibit the proliferation of lymphocytes (Ashizawa *etal*.,1996).

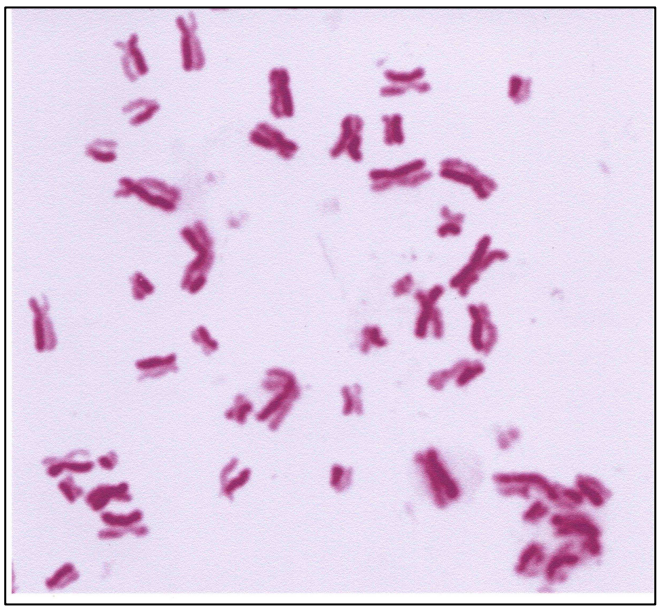
The SCE percentage of negative control was (6.33%), the SCE appear to be affected by MTZ (Figure 4.6). The reduction occurred at the concentrations (5, 10, 20, 40 and 80µg/ml) reaching (8.9, 12.04, 18.5, 24.10 and 32.40%) respectively, in which the number of lymphatic cells with SCE increased significantly ($p<0.05$) as shown in table (4.3).

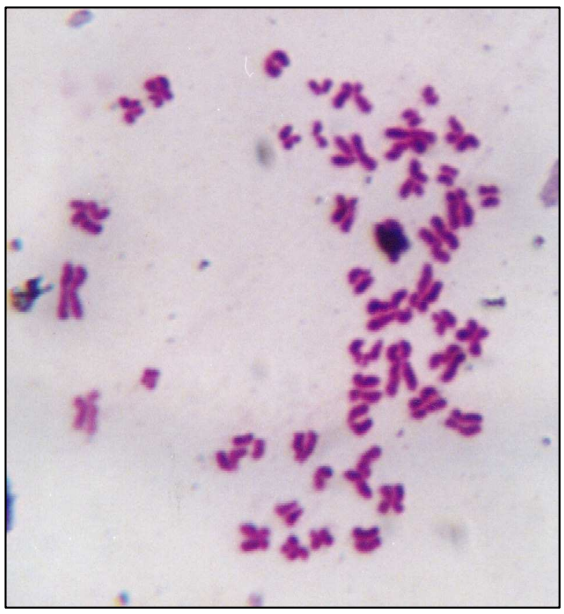
4.2.2.2: The Effect of Metronidazole on the CAs:

Other cytogenetic effects of MTZ in human blood cells *in vitro* were the increase in spontaneous frequencies of CAs as shown in table (4-4).



M1





M2 **M3**

Figure (4.5): Metaphase of human blood lymphocytes stained with Hoechest stain, showing: M1 cells, M2 cells with sister chromatid exchanges and M3 (1000x).

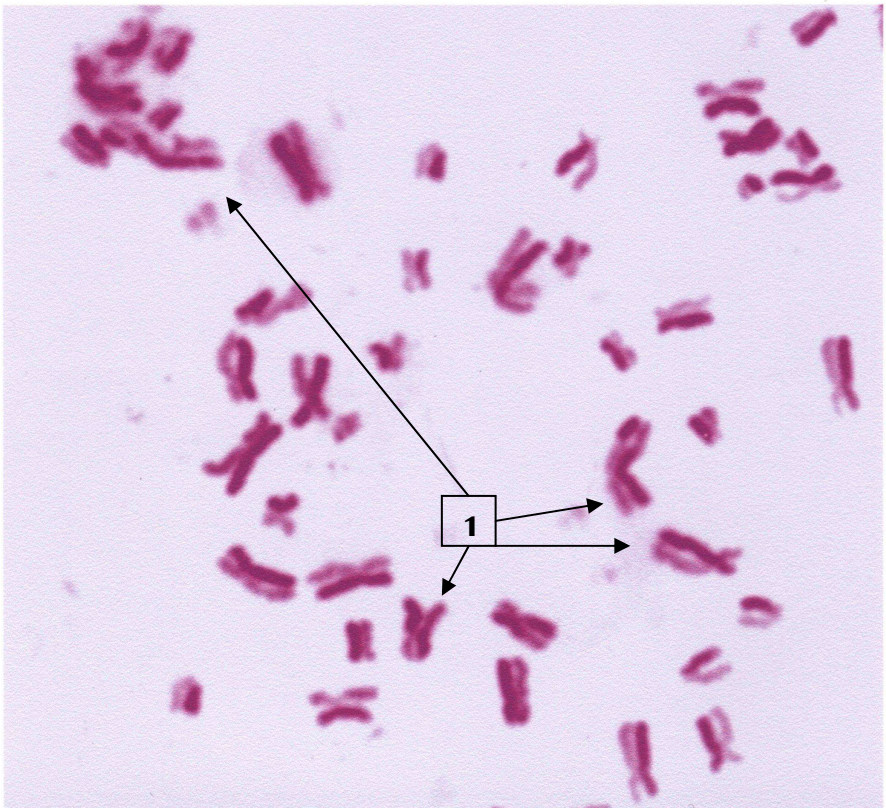


Figure (4.6):Metaphase of human blood lymphocyte culture in M2 showing (1) sister chromatid exchange(1000x).

Table (4.4): Chromosome Aberrations Induction by MTZ in Human Blood Lymphocyte Culture (*in vitro*)

Concentrations of MTZ (µg/ml)	Chromosomal Aberration % mean± S. E.						
	A centric	Dicenteric	Chromosome breaks	Chromatid breaks	deletion	gap	Total
Negative Control	0.00 A	0.00 A	0.00 A	0.00 A	0.00 A	0.230±0.03 A	0.230±0.03 A
2.5µg/ml	0.00 A	0.00 A	0.15±0.00 B	0.00 A	0.00 A	0.27±0.04 A	0.42±0.09 B
5µg/ml	0.00 A	0.00 A	0.10±0.01 B	0.15±0.002 B	0.00 A	0.30±0.07 B	0.55±0.010 C
10 µg/ml	0.150±0.001 B	0.100±0.01 B	0.210±0.05 C	0.267±0.06 C	0.153±0.002 B	0.420±0.05 B	1.30±0.020 D
20 µg/ml	0.280±0.08 C	0.190±0.01 C	0.297±0.03 D	0.316±0.06 D	0.237±0.07 C	0.487±0.10 B	1.807±0.025 E
40 µg/ml	0.350±0.03 D	0.260±0.03 D	0.344±0.01 E	0.411±0.009 E	0.310±0.003 D	0.500±0.15 C	2.175±0.018 F
80 µg/ml	0.420±0.04 E	0.350±0.02 E	0.412±0.009 F	0.463±0.01 E	0.400±0.013 E	0.573±0.012 C	2.618±0.026 F

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

Frequencies of CAs in the negative control (Figure 4.7) was (0.23%) which represented the gap aberration only. Other parameters of the CAs were not observed. CAs increased with increasing the concentration of MTZ to a percentage of (2.618%) at the concentration of (80 µg/ml).

At the concentrations (2.5 and 5 µg/ml) the acentric, dicentric chromosomes and deletion were not observed. At concentrations (10, 20, 40 and 80µg/ml) the acentric (Figure 4.9) results were (0.150, 0.280 , 0.35 and 0.42%) respectively, this indicated that there was a slight increase at the concentrations (20µg/ml and 40µg/ml) but a sharp increasing occurred at 80µg/ml as shown in table (4.4).

These results might differ in dicentric (Figure 4.8) in which a significant increase ($p<0.05$) occurred at concentrations (10, 20, 40 and 80µg/ml) reaching a percentages of (0.10, 0.19, 0.26 and 0.35%) respectively.

For the deletion, the percentage increased to (0.153, 0.237, 0.310 and 0.40%) at concentrations (10, 20, 40 and 80µg/ml) respectively. These results are significantly different ($p<0.05$) from the negative control and from each other.

There was no chromatid breaks in the negative control and at the concentration (2.5µg/ml), but it induced first at the concentration 5µg/ml reaching(0.15%), then increased gradually until reached (0.463%) at concentration (80µg/ml).

These results were slightly differ for chromosomal breaks which increased to reach (0.15, 0.10, 0.21, 0.297, 0.344 and 0.412%) at the concentrations (2.5, 5, 10, 20, 40 and 80µg/ml) respectively.

Gap (Figure 4.9) was found in both the control and in MTZ treated cultures, it increased gradually with increasing concentrations, and at concentration (80µg/ml) the

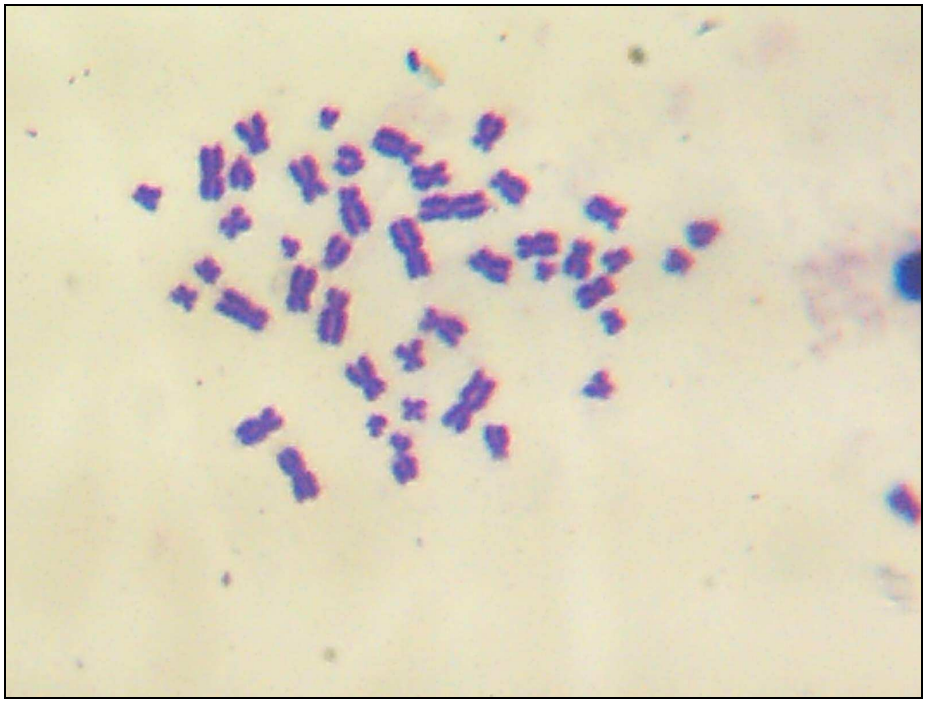


Figure (4.7): Metaphase of normal human blood lymphocytes(1000x).

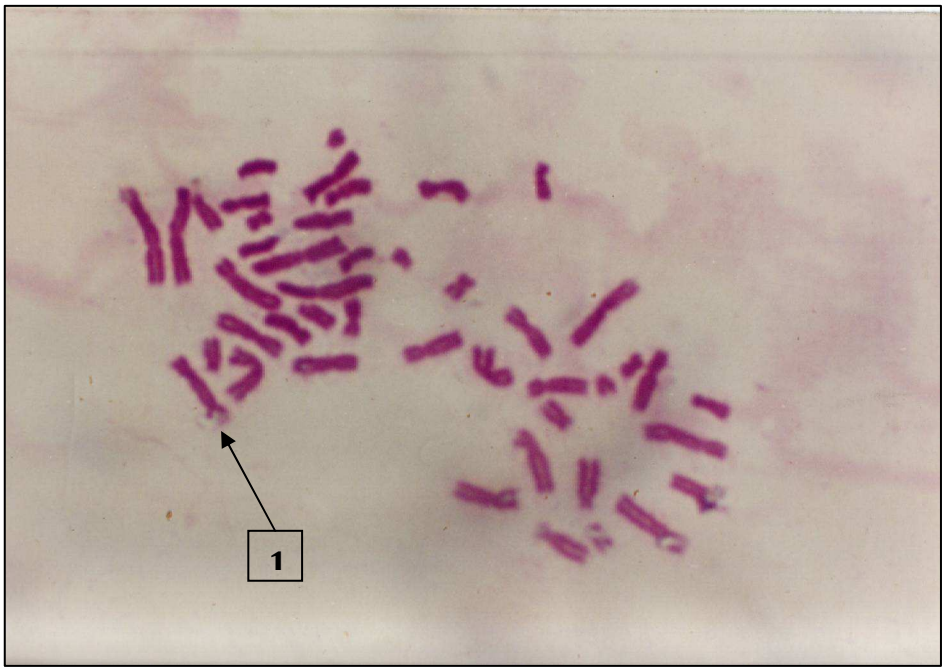


Figure (4.8):Metaphase of human blood lymphocytes treated with MTZ showing (1) dicentric(1000x) .

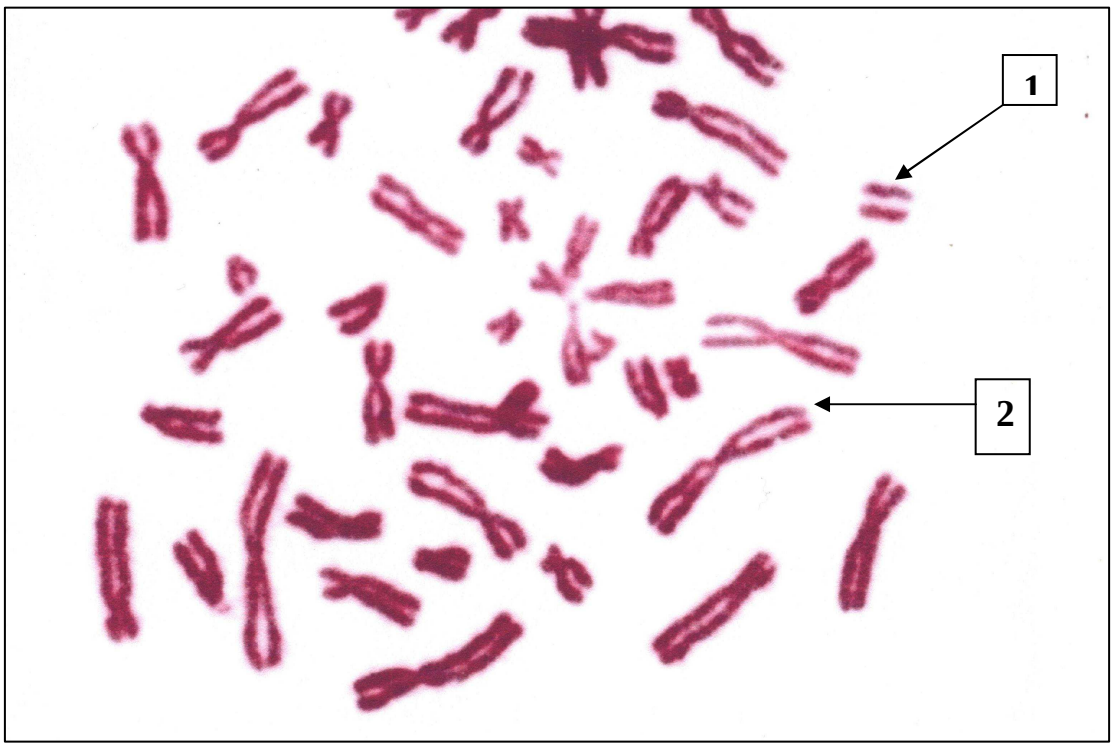


Figure (4.9): Metaphase of human blood lymphocytes treated with MTZ showing (1) acentric and (2)gap(2000x).

percentage reached (0.573%) in comparison with the negative control (0.23%) as shown in table (4.4).

The increase in the percentage of CAs after the addition of MTZ indicated that MTZ have a genotoxic effects on human blood cells *in vitro.*

4.3: Cytogenetic Effect of *P. odoratissimum* Aqueous Extract:

4.3.1: Cytogenetic Effect of *P. odoratissimum* Aqueous Extract on Mouse Bone Marrow Cells:

4.3.1.1: The Effect *P. odoratissimum* Aqueous Extract on Mitotic Index:

Under normal experimental conditions, white mice have a mitotic index of (6.60%) and this considered as negative control.

Table (4-5) shows that the mitotic index of the mouse bone marrow cells treated with *P. odoratissimum* aqueous extract with four different doses significantly decreased at the doses (400 and 800 mg/kg) reaching (5.30 and 5.05%)respectively.

4.3.1.2: Effect of *P. odoratissimum* Aqueous Extract on CAs:

The CAs of normal white mice was (0.595%) this considered as negative control. The results in table (4.5) showed that the plant aqueous extract cause an increase in CAs with the increase of doses. This indicated that plant aqueous extract is genotoxic at high doses on mouse bone marrow cells.

The Ring, gap and acentric in normal and animal treated with plant aqueous extract was slightly increased while chromosome breaks and chromatid breaks induced with all doses of the plant aqueous extracts, chromosome breaks in the negative control was (0.02%), in the treated animal the percentage was significantly increased to (0.025, 0.055, 0.051 and 0.054%) at the doses (100, 200, 400 and 800 mg/ml) respectively as shown in table (4.5).

Table (4.5): Cytogenetic Effect of *P. odoratissimum* (L.) Soland Aqueous Extract in Mouse Bone Marrow Cells(*in vivo*).

Doses of plant extract(mg/kg)	Mitotic Index %mean±S.E.	Chromosomal Aberrations % mean ± S. E.							
		Ring	Gap	Acentric	Dicenteric	Chromosome breaks	Chromatid breaks	Deletion	Total
Negative Control	6.60±0.01 A	0.021±0.004 A	0.451±0.011 A	0.042±0.011 A	0.026±0.011 A	0.02±0.001 A	0.02±0.001 A	0.015±0.004 A	0.595±0.04 A
100 mg/kg	6.25±0.05 A	0.025±0.001 A	0.40±0.003 A	0.037±0.003 A	0.00±0.000 A	0.025±0.008 B	0.03±0.009 A	0.016±0.004 A	0.533±0.014 A
200 mg/kg	6.15±0.05 A	0.018±0.009 A	0.48±0.001 A	0.038±0.016 A	0.032±0.001 B	0.055±0.003 C	0.041±0.010 B	0.12±0.009 B	0.784±0.012 B
400 mg/kg	5.30±0.10 B	0.025±0.003 A	0.51±0.004 A	0.035±0.010 A	0.057±0.002 C	0.051±0.0010 C	0.0516±0.009 B	0.130±0.009 C	0.8596±0.017 B
800 mg/kg	5.05±0.15 B	0.028±0.001 A	0.50±0.005 A	0.037±0.002 A	0.055±0.003 C	0.054±0.080 C	0.0513±0.006 B	0.1275±0.005 C	0.8528±0.012 B

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

The chromatid breaks in mouse bone marrow of negative control was (0.02%). This percentage was significantly increased in animals treated with plant aqueous extract.

While the percentage of dicentric chromosomes in the negative control was (0.026%), at the dose (100 mg/ml). But at doses (200, 400 and 800mg/ml), they were increased significantly (0.032, 0.057 and 0.055%) respectively.

Deletion appeared in the negative control (0.015%) and this percentage increased non significantly at the dose 100 mg/ml, but at the doses (200, 400 and 800 mg/kg) the results increased significantly to (0.12, 0.13 and 0.1275%) respectively.

Treatment with plant aqueous extract caused a significant increase in the levels of CAs in mouse bone marrow cells reaching (0.8528%) at the dose 800 mg/ml.

4.3.2: Cytogenetic Effect of *P. odoratissimum* Aqueous Extract on Human Blood Lymphocyte Culture:

4.3.2.1: Effect of *P. odoratissimum* Aqueous Extract on MI and BI:

The mitotic index of human blood cells from healthy human was (3.94%) and the blasto index was (39.01%) (Table 4.6).

There was a significant decrease in MI in comparison with the negative control, except in concentrations of (5 and 10µg/ml). However, a significant decrease was observed in MI at the concentrations (20, 40, 80 and 100 µg/ml) compared with controls.

Blasto index increased gradually in correlation with concentrations of plant aqueous extract when compared with that of normal human blood lymphocyte cultures (39.01%), the percentage was (58.80%) at the concentration (100µg/ml).

Table (4.6): Cytogenetic Effect of *P. odoratissimum* Aqueous Extract in Human Blood Lymphocyte Culture (*in vitro*)

Concentrations of Plant extract (µg/ml)	Blasto Index %mean ±S.E.	Mitotic Index %mean ± S.E.	Cell Cycle Progression % (mean)			Replicative Index %mean± S.E.	Sister Chromatid Exchange %mean± S.E.
			M1	M2	M3		
Negative Control	39.01±0.01 AB	3.94±0.050 B	36	31	33	1.97±0.01 AB	6.52±0.01 A
5 µg/ml	38.8±0.02 A	3.97±0.071 B	46	21	33	1.87±0.04 A	6.39±0.01 A
10 µg/ml	40.02±0.013 A	4.10±0.011 B	33	31	36	2.03±0.07 A	6.32±0.03 A
20 µg/ml	45.07±0.017 A	2.70±0.035 A	25	26	49	2.24±0.02 A	6.77±0.009 B
40 µg/ml	51.13±0.023 B	2.75±0.030 A	20	18	62	2.42±0.08 B	6.80±0.03 C
80 µg/ml	55.43±0.085 B	2.65±0.050 A	8	25	67	2.59±0.01 B	6.85±0.03 C
100 µg/ml	58.80±0.012 C	2.75±0.02 A	6	20	74	2.68±0.09 B	6.63±0.010 C

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

Results of blasto index indicated that crude plant extract contains vitamins, minerals, and active constituents which may stimulate cell mediated immunity by stimulating blastogenesis or blast transformation of lymphocytes (Mowery,1986) .

The values of cells in M3 vary with different concentrations, in the negative control. The number of cells in M3 was 33 which was similar to the concentration 5µg/ml but at 10µg/ml the cells in M3 increased to 36. This value increased to 49 at concentration 20µg/ml, 62 at 40µg/ml, 67 cells at 80µg/ml and at the concentration 100µg/ml the cells reached 74 as shown in table (4.6).

The RI increased significantly with the increased concentrations of plant aqueous extract in comparison with the negative control.

The SCE in the negative control was (6.52%) and this percentage was increased significantly ($p<0.05$) with the increase in concentrations of plant aqueous extract.

4.3.2.2: Effect of *Pelargonium odoratissimum* Aqueous Extract on CAs:

The experiment declared that the plant aqueous extract at high dose induced CAs in human blood lymphocyte (*in vitro*) as shown in table (4.7).

The CAs that were noticed include: gap, deletion, chromosomal breaks and chromatid breaks which appeared after treatment with different concentrations of plant aqueous extracts.

The deletions were found at the concentration (40 µg/ml), and their percentages increased non significantly when the plant aqueous extract concentrations increased, in which at the concentrations (40, 80 and 100µg / ml) the deletion percentage was (0.11, 0.11 and 0.13%) respectively.

The Chromosome breaks appeared at the concentration 20µg/ml and induced significantly with the increasing of the concentrations in comparison with the negative control, which had no chromosome breaks.

Table (4.7) : Chromosomal Aberrations induction by *P. odoratissimum* Aqueous Extract in Human Blood Lymphocyte Culture(*in vitro*).

Concentrations of plant extract (µg/ml)	Chromosomal Aberration % mean ±S.E.				
	Chromosome breaks	Chromatid breaks	Deletion	Gap	Total
Negative Control	0.00 A	0.00 A	0.00 A	0.25±0.01 A	0.250±0.01 A
5 µg/ml	0.00 A	0.013±0.03 B	0.00 A	0.23±0.02 A	0.243±0.05 A
10 µg/ml	0.00 A	0.015±0.02 B	0.00 A	0.24±0.01 A	0.260±0.03 A
20 µg/ml	0.031±0.007 B	0.021±0.008 B	0.00 A	0.31±0.06 A	0.364±0.02 B
40 µg/ml	0.035±0.003 B	0.028±0.009 C	0.11±0.01 B	0.32±0.04 A	0.493±0.016 C
80 µg/ml	0.037±0.003 B	0.031±0.009 C	0.11±0.005 B	0.35±0.04 B	0.5285±0.016 C
100 µg/ml	0.047±0.003 B	0.027±0.009 C	0.13±0.01 B	0.37±0.04 B	0.58±0.016 C

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

The chromatid breaks were found to induce at all concentrations of plant aqueous extract.

Gap appeared in the negative control (0.25%) at the concentrations (5, 10, 20, 40, 80 and 100 µg/ml) the gaps noticed are (0.23, 0.245, 0.312, 0.32, 0.35 and 0.375%) respectively.

Plant aqueous extract contains classes of chemical compounds which may act to increase the activity of the detoxification enzymes, such as superoxide dismutase (SOD) and glutathione-S-transferase (GST) that scavenging free radicals from the cell, or may act to decrease the activity of arylhydrocarbon hydroxylase (AHH) enzyme which has the ability to activate mutagens inside the cells (Wang *et al*.,1989) ,

The Plant aqueous extract at high concentrations and doses decreased MI and induced CAs and SCE. This might be related to the flavonoids, found in the plant, even low concentration of flavonoids can stabilizes DNA duplex, whereas helix destabilization can occur when DNA incubated for a long time with high flavonoid concentration (Kanakis *et al*.,2005). Geranillol ,citronellol and saponins were showed no toxic or carcinogenic effects at low or high dose levels in rats and mice (Timothy ,2001).

4.4: Interaction between Metronidazole and plant Aqueous Extract on Mouse Bone Marrow:

This experiment was designed to study the effect of plant extract on mutagenic effect of MTZ which show a high percentage of CAs and decrease MI in the bone marrow of mice.

4.4.1. Pre-Drug Treatment with Plant Aqueous Extract:

The results of this experiment were represented in table (4-8), which showed the ability of plant extract to reduce the effect on MI of the drug in the mouse bone marrow. This results may be attributed to the active compounds in the plant

extracts such as flavonoids and other chemical constituents in the crude plant extract (Kanakis *et al*.,2005).

MI of mice treated with plant extract before the drug administration increased to reach (5.00%). This result was significantly different from positive control (3.40%) .So plant extract provided (51%) protection against the genotoxic effect of MTZ on MI (Figure 4.10).

The percentage of CAs reduced significantly when plant extract was used before MTZ treatment, which reached (2.19%) in comparison with the positive control (3.837%) (Table 4.8).

The pre-treatment with plant extract provided a protection on CAs (51 %) against the effect of MTZ (Figure 4.10).

The plant water extract (crude extract) contains a combination of different chemical constituents in which they might act together to reduce the genotoxic effect of MTZ. Of these compounds, is the flavoniods, saponins, gerauillol and citronillol. Those active compounds may play an important role in reducing the genotoxicity of the drug, for example Flavonoids are strong antioxidants that prevent DNA damage at low concentrations and have the ability to scavenging the hydroxyl radicals, superoxide anions, and lipid peroxy radicals, e.g.Green tea, onions, apples, grapes, ginkgo, and silybum are just a few of the many thousands of plants that contain flavonoid antioxidants(Alan and Miller , 1996), whereas saponins are chemicals or compounds derived from plant sources and exhibit powerful antioxidant properties and the method of action of saponin appears to be by DNA repairing cytosolic proteins and the ability to decrease oxidative stress by up-regulating the powerful antioxidant glutathione (Pool-Zobel,1998).

Table (4.8): Interaction between MTZ and *P. odoratissimum* Aqueous Extract in Mouse Bone Marrow Cells(*in vivo*).

	Mitotic Index %m±SE	Chromosomal Aberrations % mean±S.E.							
		Ring	Gap	A centric	Dicenteric	Chromocome breaks	Chromatid breaks	Deletion	Total
Negative Control	6.53±0.04 A	0.0210±0.04 A	0.451±0.11 A	0.042±0.011 A	0.026±0.011 A	0.02±0.01 A	0.02±0.01 A	0.015±0.04 A	0.641±0.04 B
4days plant extract	6.28±0.06 A	0.025±0.01 A	0.40±0.003 A	0.037±0.003 A	0.00±0.000 A	0.045±0.08 B	0.030±0.09 A	0.026±0.04 A	0.588±0.01 A
4days drug	3.40±0.03 D	0.191±0.09 C	0.392±0.16 D	0.165±0.007 B	1.262±0.012 D	0.025±0.018 A	0.312±0.07 D	1.49±0.09 D	3.837±0.01 E
Simultaneous treatment	4.87±0.08 C	0.112±0.02 B	0.1210±0.09 B	0.165±0.017 B	0.681±0.06 B	0.015±0.09 A	0.154±0.09 B	0.891±0.005 B	2.139±0.21 C
Pre-drug treatment	5.00±0.03 B	0.158±0.002 B	0.1210±0.09 B	0.081±0.017 A	0.583±0.06 B	0.010±0.009 A	0.164±0.09 C	0.794±0.05 B	2.19±0.026 C
Post-drug treatment	4.35±0.02 CB	0.169±0.09 C	0.1770±0.10 C	0.102±0.010 B	0.654±0.03 B	0.025±0.013 A	0.133±0.06 B	1.083±0.02 C	2.689±0.011 D

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

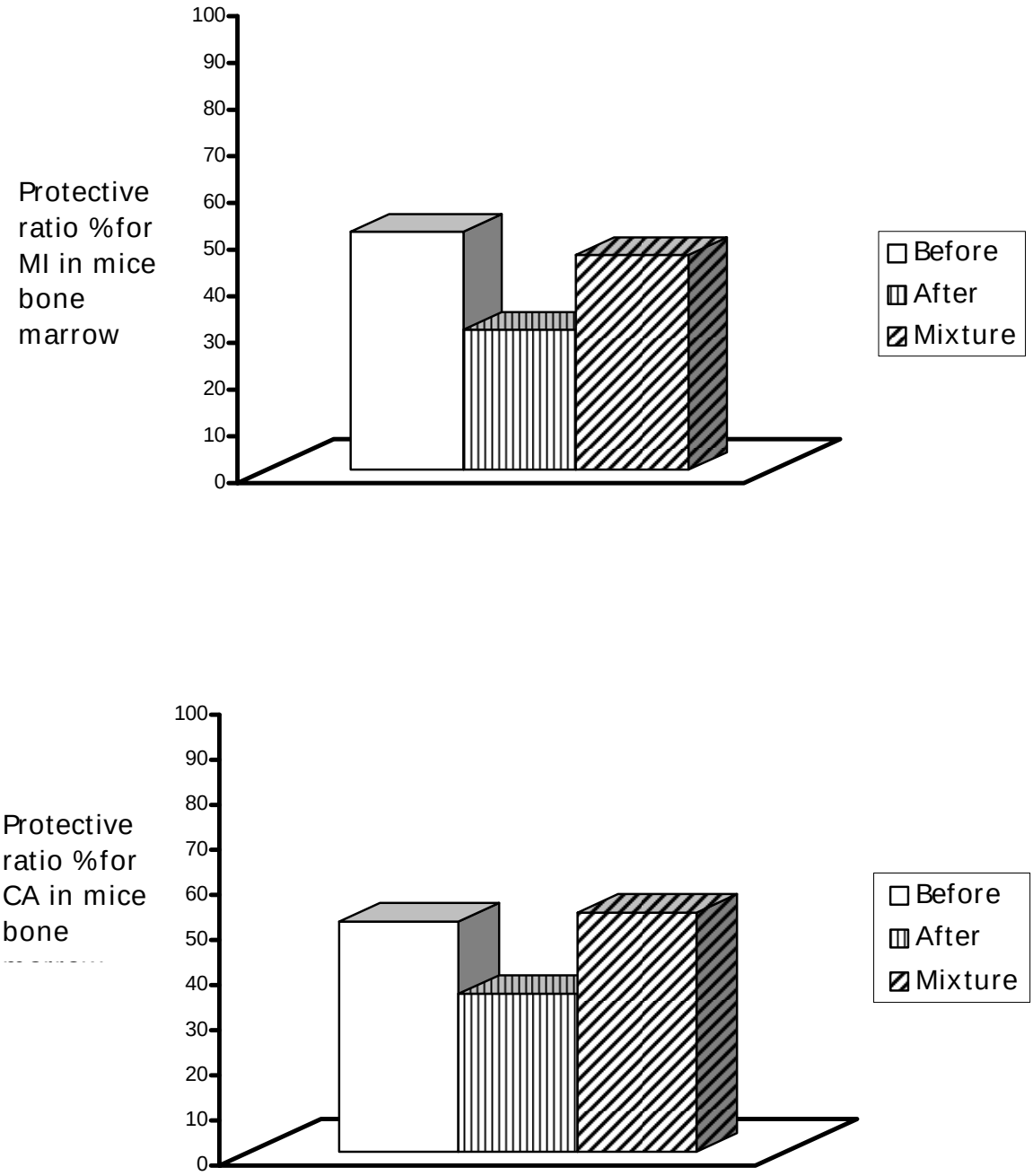


Figure (4.10): The protection ratios provided by *P. odoratissimum* when given before ,after and as a mixture *in vivo*.

These chemical constituents of plant aqueous extract may also linked to the drug or its metabolites to form non-absorbable complexes (Negishi *et al*. ,1998) or act to prevent activation of the drug by inhibiting cytochrom P 450 enzymes, while other suggested that there ingredients may inhibit the metabolic activation of the drug (Zhang *et al*.,1989).

4.4.2: Post-Drug Treatment with Plant Aqueous Extract

The MI of the positive control was shown in table (4-8) which was (3.40%) while the MI of post- drug treatment with plant extract was (4.35%). From these results it was found that the plant extract have the ability to reduce the effect of the drug, and its protective percentage provided by the plant aqueous extract was about (30%) (Figure 4.10).

The percentage of CAs after treatment with plant aqueous extract was (2.689%), which is significantly different ($p<0.05$) from the positive control (3.837%). All CAs decreased significantly in comparison with the negative control. This is also an indication that plant aqueous extract has the ability to repair the damage caused by the drug. The protective percentage for post treatment provided by the plant aqueous extract on CAs is (35 %) (Figure 4.10).

P. odoratissimum aqueous extract could be considered as bioantimutagen for its ability to decrease the effect of MTZ in post-treatment.

It was clear that post drug treatment with plant extract may activate the suppressing agent (Ramel *et al*., 1986) or activate the promoters of DNA repair mechanism (Kuroda and Hara, 1999), or may increase the error free repair fidelity in the cell (Bronzetti, 1994).

4.4.3: Simultaneous Treatment with Mixture of Plant Aqueous Extract and MTZ

Table (4-8) shows the ability of plant aqueous extract to reduce the effect of MTZ on MI, results showed that using the plant extract at the same time with drug can reduce the damage of the drug to reach (4.87%) while the result of positive control was (3.40%), the protective effect of the simultaneous treatment was (46%) (Figure 4.10).

The ability to reduce CAs was similar to the reduction ability of pre-treatment; the protection of the simultaneous treatment on CAs was (53%)(Figure 4.10), which means that they have similar mechanism to reduce genotoxicity of MTZ. Many plant extracts were considered as desmutagen e.g. garlic, onion, honey black seed. It is possible to consider *P. odoratissimum* aqueous extract as a desmutagens for its ability to decrease the effect of MTZ by chemical inactivaters, enzymatic inducers, mutagen scavenger or as antioxidants in simultaneous treatment.

Flavonoids of *P. odoratissimum* aqueous extract have the ability to increase the detoxifying enzymes in the body and therefore reduce the effect of these mutagenic materials and their metabolites (Kanakis *et al*.,2005).

Treatment with plant extract before the drug and as a mixture provided protection ratios for MI and CAs more than these ratios when given after drug. So, *P. odoratissimum* could be classified as desmutagen in the first order, and bioantimutagen in the second order.

Another plants contain flavonoids which can also be consider as desmutagen more than bioantimutagen in their ability to reduce the genotoxic effect of the drugs. *Hibiscus subdariffa* L. and *Glycyrrhiza ghlobra* L. in reducing the effect of MMC(AL-Kayat, 1999), Honey in reducing the effect of Tamoxifen

(AL-Sudany, 2005),*Thymbra spicata* L. can reduce the effect of cyclophosphamide(AL-Robiaay, 2000).

4.5: Interaction between Plant Aqueous Extract and MTZ on Human Blood Lymphocytes Culture

The concentrations of both drug and plant extract used in this experiment were selected according to the harmful concentration of drug and more useful plant extract concentration to human blood lymphocyte.

4.5.1: Pre-Drug Treatment

The mitotic index of normal human blood lymphocyte was (3.82%) as shown in table (4-9).

The pre-treatment showed that plant aqueous extract has the ability to reduce the effect of the drug on MI, when culturing human blood lymphocyte for 48 hr. with a plant aqueous extract at concentration 10µg/ml and then separated the plant aqueous extract and added a media with drug at concentration 80µg/ml , this pre-treatment significantly reduced ($p<0.05$) the effect of the drug to (2.5%) in comparison with the positive control which was (0.67%). Therefore the pre-treatment has a protection of (58%)on MI(Figure 4.11).

The blasto index of human blood lymphocyte pre –treated with the plant aqueous extract was (33.75%) compared with the positive control (10.75%). The protection of pre-treatment on BI was (86 %) (Figure 4.11).

RI of normal human blood lymphocytes was (2.01%) while in the human blood lymphocytes pre-treated with plant extract was (1.80%) this percentage increased in comparison with the RI of MTZ treated cultures (1.20%) and the result of plant extract in 48 hr. on RI was (1.97%) which was similar to the RI of negative control. The pretreatment with plant extract provided a protection for RI of (74%) against the effect of MTZ (Figure 4.12).

Table (4.9): Interaction between *P. odoratissimum* Aqueous Extract and MTZ in Human Blood Lymphocyte Culture(*in vitro*)

Type of treatment	Blasto Index %mean±S.E.	Mitotic Index %mean±S.E.	Cell cycle progression % (mean)			Replective Index %mean±S.E.	Sister chromatid exchange %mean±S.E.
			M1	M2	M3		
Negative Control	37.30±0.04 GF	3.82±0.012 E	34	31	35	2.01±0.01 E	6.40±0.02 A
Plant extract(72 hr.) 10 µg/ml	38.75±0.05 G	3.82±0.012 E	34	33	33	1.99±0.02 E	6.33±0.12 A
Drug(72 hr.) 80 µg/ml	10.75±0.15 A	0.67±0.02 A	79	20	1	1.20±0.02 A	30.32±0.03 D
Simultaneous treatment	31.62±0.87 EF	2.75±0.013 CD	36	36	28	1.92±0.01 E	16.00±0.5 C
Plant extract for (48 hr.)	35.30±0.07 DE	3.85±0.010 D	36	31	33	1.97±0.05 E	5.87±0.5 A
Drug for (48 hr.)	17.35±0.50 B	0.95±0.01 A	63	22	15	1.52±0.01 B	30.60±0.03 D
Pre-drug treatment	33.75±0.30 D	2.5±0.18 B	41	38	21	1.80±0.07 D	15.12±0.007 C
Post-drug treatment	23.95±0.70 C	2.27±0.02 B	48	31	21	1.73±0.04 C	14.39±0.02 B

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

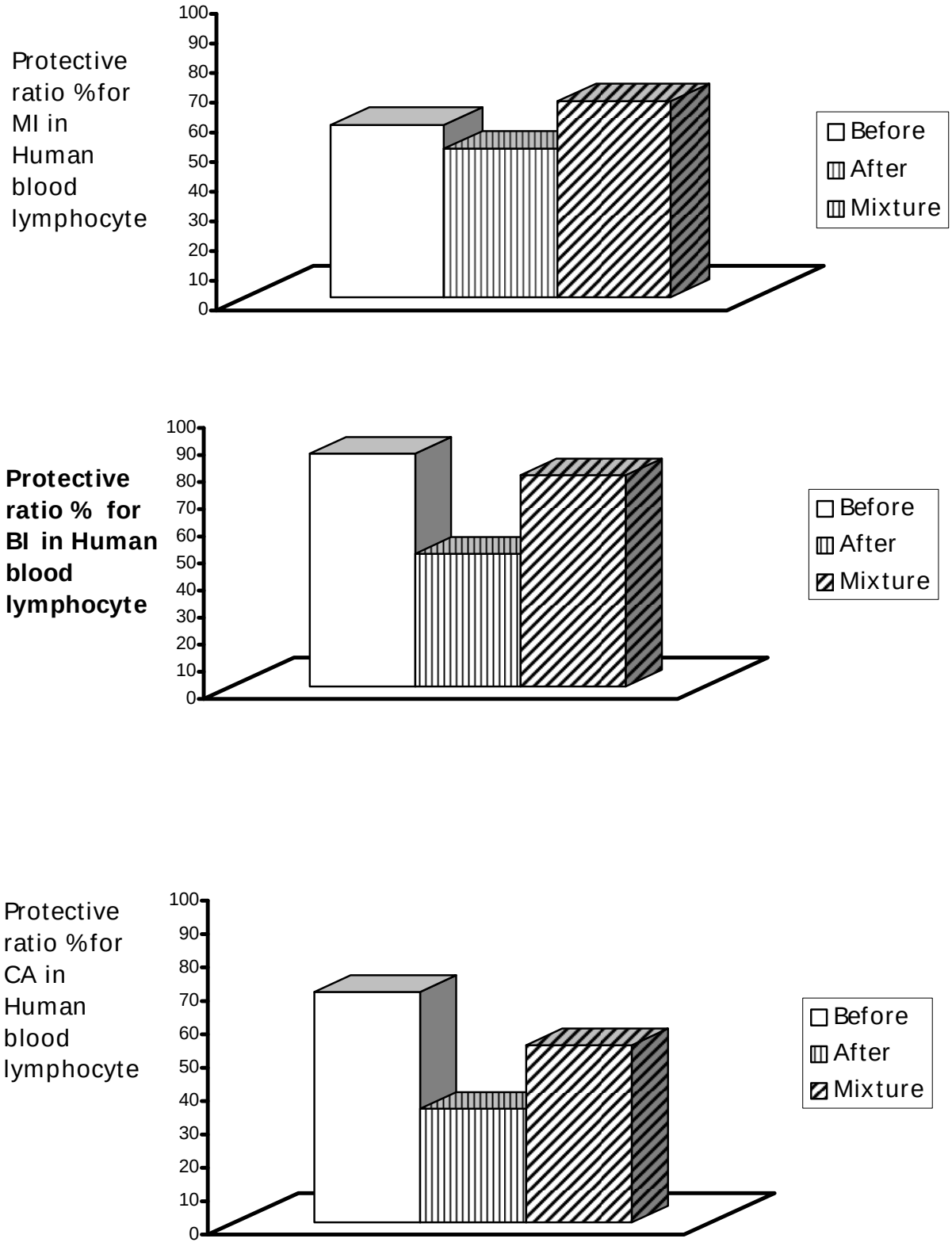


Figure (4.11): The protection ratios for MI, BI and CAs that provided by *P. odoratissimum* when given before, after and as a mixture *in vitro*.

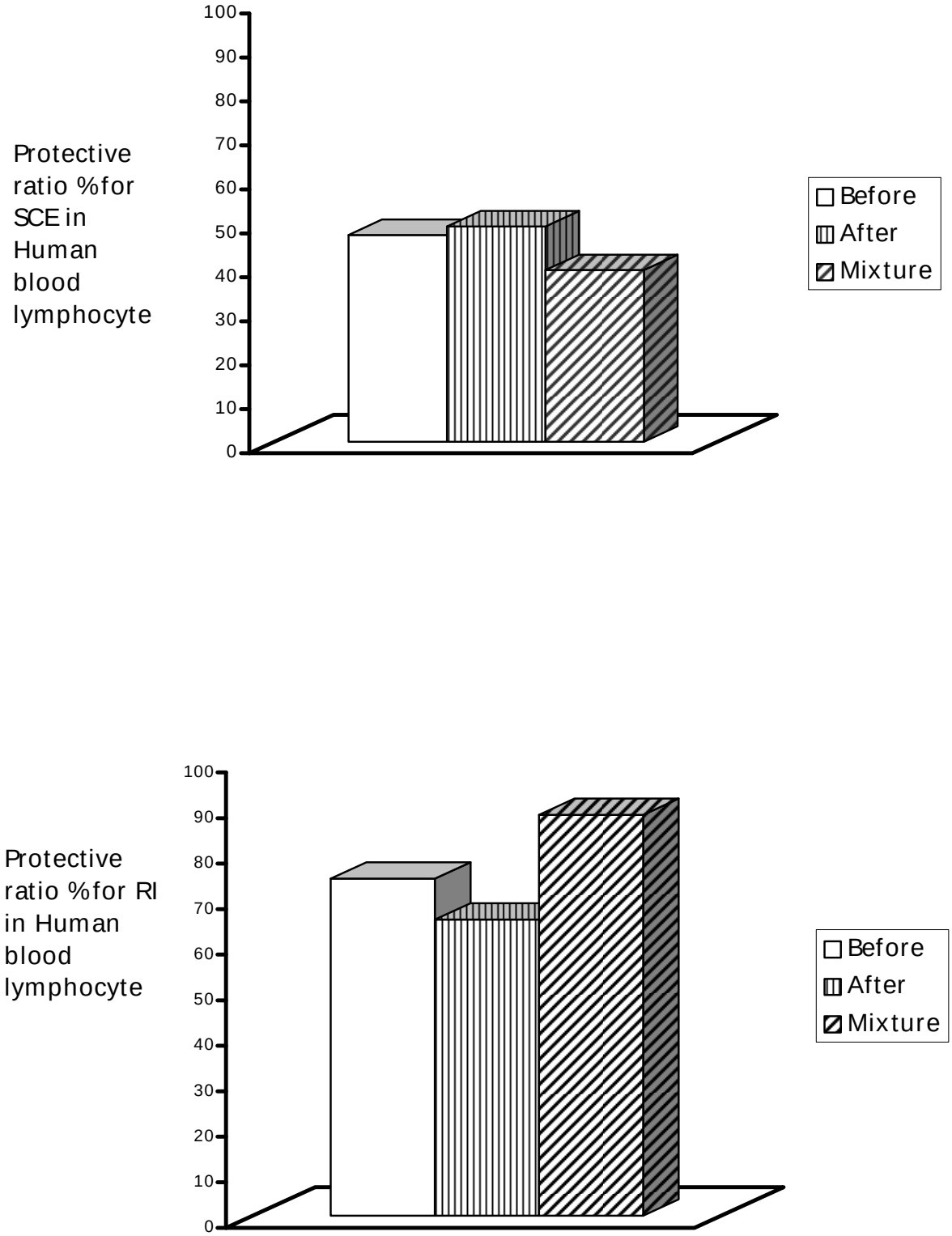


Figure (4.12): The protection ratios for RI and SCE that provided by *P. odoratissimum* when given before, after and as a mixture *in vitro*.

The cells in culture treated with MTZ for 72 hr. reached M3 in value of one cell while in the pre-treatment the cells reached 21 cells in M3 the plant extract has the ability to protect the cells from the toxicity of drug.

The SCE in normal blood lymphocyte culturing was (6.40%) this result was decreased significantly ($p<0.05$) to (30.32%) in the positive control of drug , in the pre-treatment the percentage was decreased to (5.12 %) in contrast to positive control, and the effect of plant extract in 48 hr. on SCE was decreased significantly ($p<0.05$) to reach (5.87%) in contrast to negative control. The pretreatment with plant extract provided a protection to SCE of (61.5%) against the effect of MTZ (Figure 4.12).

The CAs (Table 4.10) of MTZ (positive control) was (3.618%), the plant extract have shown to be effective in reducing significantly ($p<0.05$) the CAs to (1.27%). The protection of pre-treatment on CAs was (69%) (Figure 4.11).

4.5.2: Post- Drug Treatment

The results of this experiment were displayed in table (4.9). Post-drug treatment with plant extract caused significant increase in MI to (2.27%) in comparison with positive control (0.67%), while the percentage of cultures treated with MTZ for 48 hr. and 24 hr. normal media that MI was (0.95%) which was similar to positive control which mean that the MTZ will damage the cells in the first 48 hours and by removing MTZ in the last 24 hours the damage was not reduced. So the plant extract provided a protective effect against the effects of drug on MI in post-treatment (50 %) (Figure 4.11).

Post- treatment could reduce of damage of MTZ on BI to reach (23.95%) in comparison with the positive control (10.75%) while the percentage of culture treated with MTZ for 48 hr. and 24 hr. normal media, the BI was (17.35%). The protective percentage in the post- treatment was (49 %) (Figure 4.11).

Table (4.10): The Effect of Interaction between *P. odoratissimum* Aqueous Extract and MTZ on Chromosomal Aberrations of Human Blood Lymphocyte Culture.

Type of Treatment	Chromosomal Aberration % %mean±S.E.						
	A centric	Dicenteric	Chromosome breaks	Chromatid breaks	Deletion	Gap	Total
Negative Control	0.00 A	0.00 A	0.00 A	0.050±0.01 A	0.00 A	0.210±0.01 A	0.260±0.02 A
Plant extract (72 hr.) 10 µg/ml	0.00 A	0.00 A	0.00 A	0.015±0.5 A	0.00 A	0.245±0.01 A	0.260±0.03 A
Drug (72 hr.) 80 µg/ml	0.420±0.04 B	0.350±0.02 C	0.412±0.09 D	0.463±0.0010 C	0.400±0.013 C	1.573±0.012 D	3.618±0.026 E
Simultaneous treatment	0.12±0.09 B	0.137±0.07 B	0.021±0.09 B	0.023±0.05 A	0.10±0.01 B	1.314±0.03 C	1.835±0.09 B
Plant extract for (48 hr.)	0.00 A	0.00 A	0.047±0.01 B	0.010±0.01 A	0.00 A	0.22±0.02 A	0.277±0.05 A
Drug for(48 hr.)	0.390±0.005 B	0.340±0.02 C	0.370±0.09 D	0.411±0.10 C	0.400±0.012 C	1.370±0.026 C	3.281±0.15 D
Pre-drug treatment	0.50±0.002 C	0.00 A	0.130±0.02 C	0.230±0.06 B	0.110±0.05 B	0.300±0.01 B	1.27±0.09 B
Post-drug treatment	0.285±0.010 AB	0.230±0.08 C	0.217±0.01 D	0.293±0.05 B	0.05±0.01 B	1.387±0.06 C	2.462±0.09 CD

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

Another effect of MTZ was the reduction in the RI. There was a significant decrease in RI in contrast with untreated blood lymphocytes. The RI of normal human blood lymphocytes was (2.01%) while the result of human blood lymphocyte post-treated with plant aqueous extract was (1.73%). This was increased in comparison with the RI of MTZ (1.20%) ,and the result of drug treated for 48 hr. increased significantly ($p<0.05$) to reach a percentage of (1.52%) which mean that the cells have the ability to reproduce when the causative agent MTZ was removed. The post-treatment with plant extract provided a protective percentage to RI of (65%) against the effect of MTZ (Figure 4.12).

The cells in cultures treated with MTZ reached M3 one cell while in the post-treatment cells reached to 21 cells in M3. This indicated the ability of plant aqueous extract to protect the cells from the toxicity of drug, also the cultures treated with MTZ for 48 hr. had cells with the ability to reach M3 in value of 15 cells.

The SCE of normal blood lymphocyte culturing was (6.40%) which decreased significantly to (30.32%) in the positive control (Table 4.9).

In the post-treatment, the percentage was decreased to (14.39%) in contrast to positive control, and the percentage of SCE of culture treated with MTZ for 48 hr. was (22.60%) which means that the cells could repair the damage of SCE that produced by MTZ when the drug was removed. The post-treatment with plant extract provided a protective percentage to SCE of (66.5%) against the effect of MTZ (Figure 4.12).

The abnormalities in chromosomes of normal control and in post- treated cultures were examined in well separated metaphases of blood samples, the percentage of negative control was (0.26%) ,which observed in 100 lymphocytes and in the positive control was (3.618%), this result was decreased significantly ($p<0.05$) to (2.462%) in comparison with positive control , while the percentage of culture treated with MTZ for 48 hr. and 24 hr. normal media the CAs was

(3.281%)which indicate the inability of infected cells to repair the damage of MTZ on chromosomes .The post- treatment provide protective percentage of (34%)(Figure 4.11).

4.5.3 Treatment with a Mixture of *P. odoratissimum* Extract and MTZ:

This mixture has shown the ability to decrease the mutagenic activity of MTZ on MI .MI of positive control was (0.67%) while the mixture have a percentage (2.75%) which was similar to pre-treatment percentage(Table 4.9). Thus the mixture provided protective percentage of (66%) figure (4.11).

The effect of the mixture on BI appeared to be increased significantly ($p<0.05$) to(36.62%) in comparison with the result of positive control(10.75%), the protective ratio was (78%) as shown(Figure 4.11).

The RI results of human blood lymphocyte treated with the mixture was (1.92%) this percentage was high in comparison with the RI of MTZ (1.20%). The protective percentage of simultaneous treatment was (88%) (Figure 4.12).

The SCE of simultaneous treatment in blood lymphocyte culturing was (16.00%). This result was decreased significantly ($p<0.05$) to (30.32%) in the positive control. The protective percentage of simultaneous treatment was (59.8%) (Figure 4.12).

The mixture was able to reduce the damage of MTZ in the chromosomes, the reduction was in the total of CAs, The protective percentage of simultaneous treatment in CAs was (53%) which was similar to the protective percentage of pre-treatment level.

This might be related to the chemical constituent like saponins and flavonoids in the plant which can modulate the mutagenic effects of MTZ (Chung *et al*., 1992; Pool-Zobel,1998).

Both *Hibiscus subdariffa* L. and *Glycyrrhiza glabra* L. contain flavonoids which have the ability to reduce genotoxic effect of MMC. These plant extracts can be considered as desmutagen in the first order and bioantimutagen in the second order (AL-Kayat,1999).

4.6: Effect on Mice Leukocyte Cells:

4.6.1: The Effect of MTZ in Total and Differential Account of Leukocyte in Mice Blood:

Table (4-11) shows that there is no effect of MTZ on the animal leukocyte as a result of counting the differential and total animal blood leukocyte at different doses of drug. Certain drugs can damage bone marrow. Generally, this damage expected to affect on the quantity of WBC to decrease in range between 7 to 20 days following drugs administration .This decrease was depending on the dose, duration and type of the drug .Each drugs affects the bone marrow in different ways (Internet , 2001).

4.6.2: The Effect of *P. odoratissimum* Extract in Leukocyte of Mice:

From table (4-12) it seems that *P. odoratissimum* aqueous extract has no effect on the mice blood leukocyte on differential and total counting in comparison with the negative control. High dose of flavonoids can damaged mouse bone marrow, this damage expected to affect on the quantity of WBC to decrease in range between 7 to 20 days following plant extracts administration (Internet , 2001).

4.7.3: The Effect of plant drug interaction in Mice Leukocytes:

Table (4.13) shows no effect on leukocyte of mouse in the three types of treatment, pre-treatment, post-treatment and simultaneous treatment. The damage effect of both MTZ and high dose of *P. odoratissimum* extracts expected to affect on the quantity of WBC to decrease in range between 7 to 20 days following oral administration (Internet , 2001).

Table (4-11): The Effect of MTZ on Differential and Total Count of Blood Leukocyte.

Doses	mean± S. E. (cell/cu. mm. bloods)					
	Total	lymphocyte	Neutrophil	Monocyte	Basophil	Eosnophil
Control negative	8700±0.13 A	4335±0.49 A	2516±0.52A	1675±0.061 A	87±0.02 A	87±0.02 A
1mg/kg	8762.5±0.25 A	4367±0.11 A	2504±0.11 A	1675±0.04 A	131±0.05 A	87±0.03 A
100mg/kg	8700±0.13 A	4270±0.6 A	2516±0.5 A	1696±0.07 A	131±0.05 A	87±0.02 A
200mg/kg	8775±0.7 A	4362±0.3 A	2545±0.3 A	1646±0.05 A	131±0.06 A	87±0.03 A
400mg/kg	8762.5±0.25 A	4367±0.11 A	2504±0.11 A	1675±0.04 A	131±0.05 A	87±0.03 A

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

Table (4.12): The Effect of Plant Aqueous Extract on Differential and Total Count of Blood Leukocyte.

Doses	mean± S. E. (cell/cu. mm. bloods)					
	Total	lymphocyte	Neutrophil	Monocyte	Basophil	Eosnophil
Control	8780±0.2 A	4293±0.11 A	2585±0.9 A	1684±0.4 A	131±0.5 A	87±0.02 A
100mg/kg	8754±0.13 A	4290±0.11 A	2576±0.15 A	1693±0.9 A	109±0.4 A	87±0.02 A
200mg/kg	8710±0.14 A	4318±0.4A	2476±0.9 A	1742±0.9 A	87±0.02 A	87±0.02 A
400mg/kg	8748±0.17 A	4270±0.3A	2496±0.6 A	1786±0.8 A	109±0.4 A	87±0.9 A
800mg/kg	8780±0.2 A	4293±0.11 A	2585±0.9 A	1684±0.4 A	131±0.5 A	87±0.02 A

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

Table (4.13): Interaction between Drug and Plant Aqueous Extract and their Effect on Differential and Total Count of Blood Leukocyte

Doses of treatment	mean± S. E. (cell/cu. mm. bloods)					
	Total	lymphocyte	Neutrophil	Monocyte	Basophil	Eosnophil
Control	8780±0.2 A	4293±0.11 A	2585±0.9 A	1684±0.4 A	131±0.5 A	87±0.02 A
Post-treatment	8748±0.17 A	4270±0.3A	2496±0.6 A	1786±0.8 A	109±0.4 A	87±0.9 A
Pre-treatment	8700±0.13 A	4270±0.6 A	2516±0.5 A	1696±0.07 A	131±0.05 A	87±0.02 A
Simultaneous treatment	8748±0.17 A	4270±0.3A	2496±0.6 A	1786±0.8 A	109±0.4 A	87±0.9 A
Drug	8762.5±0.25 A	4367±0.11 A	2504±0.11 A	1675±0.04 A	131±0.05 A	87±0.03 A
Plant extract	8754±0.13 A	4290±0.11 A	2576±0.15 A	1693±0.9 A	109±0.4 A	87±0.02 A

Differences A, B, C, D, E are significant (P< 0.05) to compression rows.

CHAPTER ONE

Introduction

CHAPTER TWO

Literature Review

CHAPTER THREE

Materials and Methods

CHAPTER FOUR

Results and Discussion

CHAPTER Five

Conclusions And Recommendations

References

الإهداء

إلى مقام المصطفى و اله عليهم السلام

إلى الغائب الحاضر إلى الراحل الذي لن يرحل عن قلبي أبدا...والدي وأخي الحبيب

إلى شلال العطاء الذي لاينضب ...والدتي الحبيبة

إلى من رافقني في كل خطواتي ...أخي وعائلته الحبيبة

إلى من يوم وجعي تكون راحتي ...أخواتي الحبيبات

إلى كل من أوقد شمعة أنارت طريق حياتي

أهدي بحثي هذا

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5.1. Conclusions

1. The presence of flavonoids and saponins. in *P. odoratissimum.*
2. Metronidazole is highly genotoxic to mouse bone marrow cells (*in vivo*) and human blood lymphocytes (*in vitro*).

3. *P. odoratissimum* was genotoxic at high doses to mouse bone marrow cells (*in vivo*) and at high concentrations to the human blood lymphocytes culture (*in vitro*).
4. *P. odoratissimum* at all concentrations induced blasto index in human blood lymphocytes culture (*in vitro*).

5. *P. odoratissimum* aqueous extract was a promising plant extract at low dose that could be used for reducing the genotoxicity caused by MTZ in mouse bone marrow cells and human blood lymphocytes culture, for that *P. odoratissimum* was considered as desmutagen in the first order and bioantimutagen in the second order.
6. Metronidazole and *P. odoratissimum* have no effect on mouse leukocyte under the circumstances of the current experiments.

5.2: Recommendations

1. Further studies are needed to study the effect of MTZ on the chromosomes of other organs such as liver, kidney and spleen.
2. Further studies are required on other types of *P. odoaratissimum* extracts such as ethanol and aceton extracts.
3. Further studies are required on other plant species that can inhibit the MTZ genotoxicity.
4. Further studies are needed to study the effect of MTZ on the immune system.

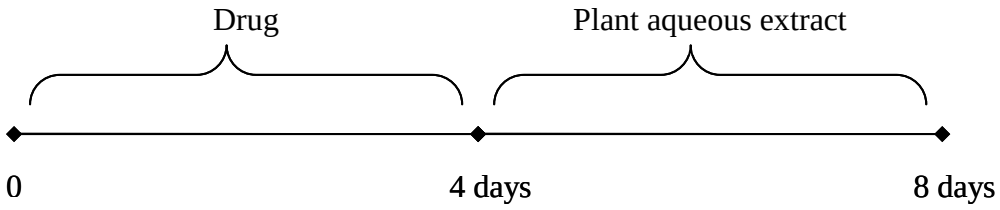
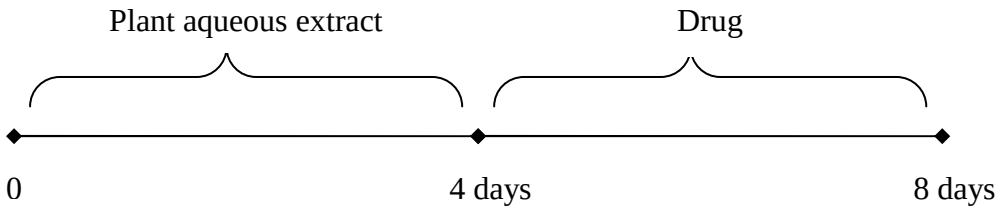


Fig. (3.1)



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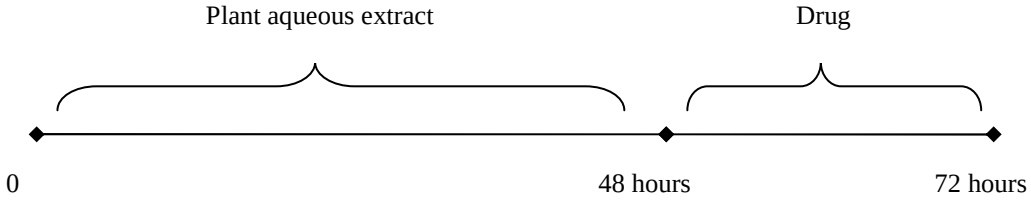

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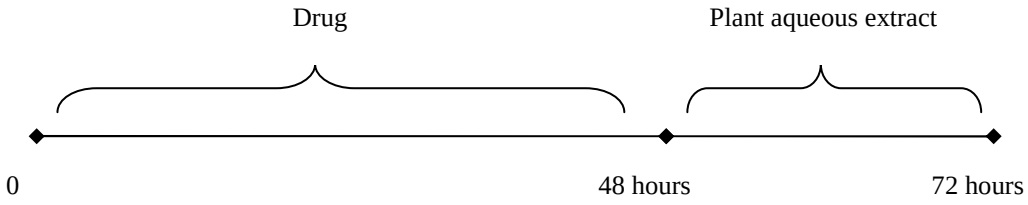

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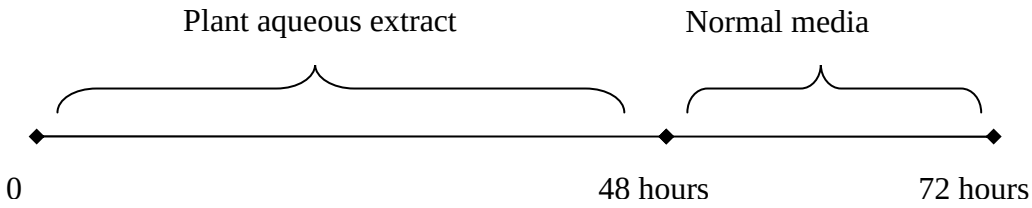

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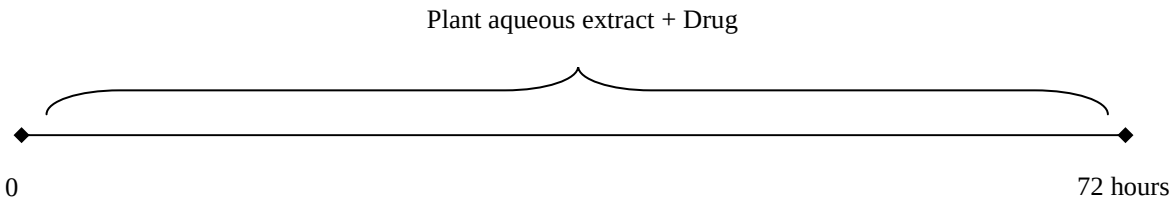
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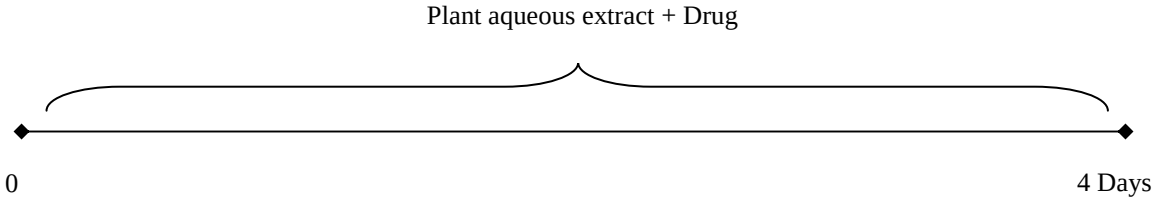
72 hours


Plant aqueous extract + Drug
0
4 Days

Republic of Iraq
Ministry of Higher Education
and Scientific Research
Al-Nahrain University
College of Sciences
Biotechnology Department

Inhibitory Effect of *Pelargonium odoratissimum* (L.) Soland Extracts on the Genotoxicity of Metronidazole in Mammalian cells

A thesis

**Submitted to the College of Science of AL-Nahrain University
As partial fulfillment of the requirements for the degree of Master of Science in Biotechnology**

By

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List Of Contents

List of Tabels

List of Figures

List of abbreviations

B.	Bacteriodes
BI	Blastogenic Index
BudR	5-Bromodeoxyuridine
CCP	Cell cycle progression
CNS	Central nerveous system
CAs	Chromosomal Aberrations
CP	Cyclophosphamide
D.W.	Distilled water
FDA	Food and Drug Adminstration
IP	Intraperitoneally
IV	Intravenously
MTX	Methotrexate
MTZ	Metronidazole
MMC	Mitomycin-C
MI	Mitotic Index
PBS	Phosphate Buffer Saline
PHA	Phytohaemoagglutinin
KCl	Potassium Chloride
RI	Replecative Index
SCE	Sister chromatid exchange
TMA	Tamoxifen
UV	Ultraviolet
WBCs	White blood cells
UPW	Ultra pure water

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المصادر العربية

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Summary

The present study was designed to shed light on a) the cytogenetic effects of metronidazole and aquatic extract of *Pelargonium odoratissimum* (L.) soland on laboratory mice (*in vivo*)and in human blood lymphocytes (*in vitro*) b)chemical analysis of *P. odoratissimum* for different active compounds .

It was also aimed to investigate the role of *P. odoratissimum* aquatic extracts in reducing the cytogenetic effects of metronidazole in mice and human blood lymphocytes. The examinations that conducted in mice, were mitotic index, chromosomal aberrations in bone marrow and study the total and differential count of leukocytes, while human blood lymphocyte culture studies (*in vitro*) were mitotic index , balsto index, replecative index, sister chromatid exchange and chromosomal aberrations.

The cytogenetic effects of the drug and plant aquatic extracts were investigated after four days of treatment in mice with four different doses from each. For metronidazole, they were (1, 100, 200 or 400mg/kg), while doses of *P. odoratissimum* used were(100, 200 , 400 or 800mg/kg). The concentrations for mteronidazole for human blood lymphocyte culture were (2.5 , 5, 10, 20, 40 or 80µg/ml), and for *P. odoratissimum* aqueous extract were (5, 10, 20, 40 80 or 100µg/ml).

An interaction between plant extract and metronidazole (high concentration) was carried out through three types of treatments (before, after and mixture of plant extract and drug treatment) to determine the activity of *P. odoratissimum* aqueous extract in preventing or reducing the drug side effects both *in vitro* and *in vivo*.

The following results were obtained:

1.Chemical investigation showed that *P. odoratissimum* contains two different classes of active compounds namely, flavonoids and saponins.

2.Metronidazole revealed clear effects, in reducing mitotic activity and increased spontaneous chromosomal aberrations in mouse bone marrow cells (*in vivo*) and in human blood lymphocytes (*in vitro*) causing a reduction in mitotic index, balsto index and replecative index and induction of sister chromatid exchange and chromosomal aberrations. These effects were proportional with the concentrations; a phenomenon, which suggested that drug, has a genotoxic effect.

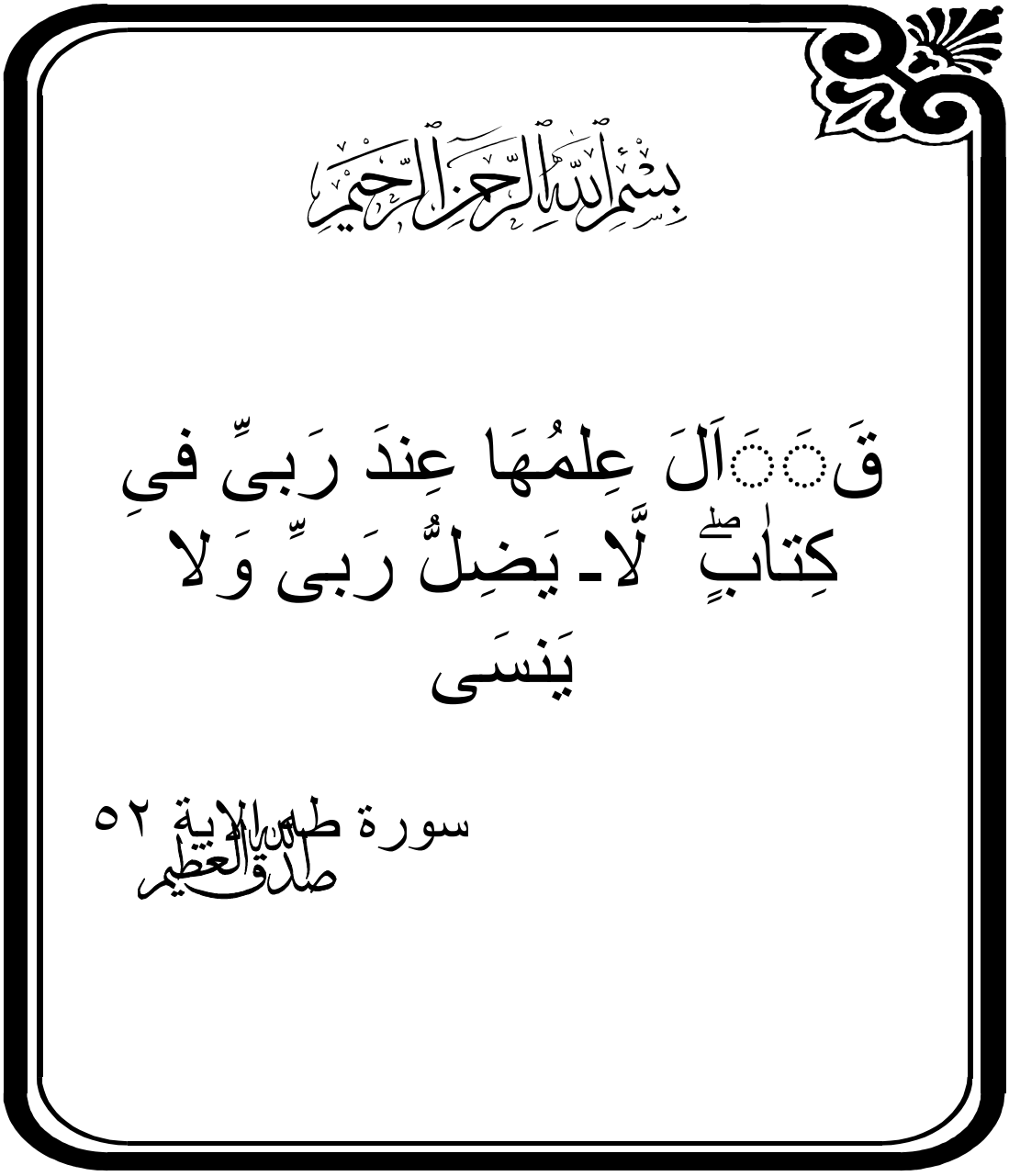
3. Aquatic extract of *P. odoratissimum* had a significant genotoxic effects at high doses on the mouse bone marrow cells (*in vivo*) and human blood lymphocytes culture (*in vitro*).

4. Aquatic extract of *P. odoratissimum* at low dose ,however, showed a protective value against the genotoxic effect of metronidazole. In moue bone marrow cells and human blood lymphocyte culture, this was more pronounced in pre-treatment and simultaneous treatment than in post-treatment.

So *P. odoratissimum* aquatic extract is consider as desmutagen in the first order and bioantimutagen in the second order, as a result for its ability to repair CA and increase MI in mouse system and in human blood lymphocyte culture system . It also had the ability to increase BI and RI and decease SCE in human blood lymphocytes culture (*in vitro*).

5. *P. odoratissimum* aquatic extract showed no effect on mouse blood leukocyte differential count.

6. Metronidazole also showed no effect on mouse blood leukocyte differential count.



بِسْمِ اللّٰهِ الرَّحْمٰنِ الرَّحِيْمِ

قَالَ عِلْمُهَا عِنْدَ رَبِّي فِي
كِتَابٍ لَّا يَضِلُّ رَبِّي وَلَا
يَنْسَى

سورة طٰهٰ الاية ٥٢
صدق الله العظيم

الخلاصة

الخلاصة

أجريت هذه الدراسة لمعرفة أ) تأثير عقار الميترونيدازول والمستخلص المائي لنبات العطرة *P. odoratissimum* في الصفات الوراثية الخلوية لخلايا نقي العظم في الفئران المختبرية وخلايا الدم الميحطي للانسان (خارج الجسم) ب)الكشف الكيميائي للمواد الفعالة في هذا النبات.

كما كان الهدف أيضا البحث في آلية نبات العطرة *P. odoratissimum* في تثبيط تأثير الميترونيدازول في الصفات الوراثية الخلوية في الفئران وخلايا الدم الميحطي للانسان (خارج الجسم). الفحوص التي اتبعت في الفئران كانت دراسة معامل الانقسام الخيطي و التغيرات الكروموسومية في خلايا نقي العظم ودراسة العد التمايزي في خلايا دم الفئران ،أما في خلايا الدم الميحطي للإنسان (خارج الجسم) دراسة الانقسام الخيطي،التغيرات الكروموسومية، معامل الأرومي، معامل التضاعف و التبادل الكروماتيدي الشقيقي.

درست التأثيرات الوراثية الخلوية للعقار والمستخلص النباتي المائي بعد أربعة أيام من المعالجة في الفئران لاربع جرع مختلفة لكل عامل للميترونيدازول كانت ١ و١٠٠ و٢٠٠ و٤٠٠ ملغم \ كغم بينما كانت لنبات العطرة ١٠٠ و ٢٠٠ و٤٠٠ و ٨٠٠ ملغم\كغم

فحص تأثير التراكيز للميترونيدازول في خلايا الدم الميحطي للإنسان (خارج الجسم)كانت ٥. ٢ و٥ و ١٠ و ٢٠ و ٤٠ و ٨٠ ميكروغرام\مل ولنبات العطرة ٥ و ١٠ و ٢٠ و ٤٠ و ٨٠ و١٠٠ ميكروغرام\مل .

أجري التداخل بين المستخلص النباتي والميترونيدازول (أعلى تركيز) من خلال ٣ أنواع من المعاملات (قبل، بعد، مع المعاملة بالعقار) لأجل اختبار فعالية *P. odoratissimum* في منع أو تقليل تأثير العقار خارج و داخل الجسم.

وقد توصلت الدراسة إلى النتائج الآتية:

١. ظهر بآن نبات العطرة *P. odoratissimum* يحتوي على مجموعتين من المواد الفعالة و هي الفلافينويدات و الصابونيات.

٢. ظهر بآن للميترونيدازول تأثيرات سلبية تمثلت بانخفاض معامل الانقسام الخيطي و زيادة في التغيرات الكروموسومية في خلايا نقي العظم في الفئران و في خلايا الدم المحيطي للإنسان بانخفاض في معامل الانقسام،معامل الآرومي و معامل التضاعف و زيادة في التبادل

الكروماتيدي الشقيقي و التغيرات الكروموسومية.وقد ازدادت جميع هذه التغيرات بزيادة الجرع والتراكيز مما يدل على ان للعقار تأثيرا سميا وراثيا.

٣.أظهرت النتائج أن المستخلص المائي لنبات العطرة يمتلك تأثيرا سميا معنويا في التراكيز العالية في خلايا نقي العظم في الفئران و في خلايا الدم المحيطي للإنسان(خارج الجسم).

٤.كما يمتلك المستخلص المائي لنبات العطرة وفي التراكيز الواطئة قابلية حماية ضد التأثير السمي للميترونيدازول في خلايا نقي العظم في الفئران و في خلايا الدم المحيطي للإنسان(خارج الجسم)،وهذا كان واضحآ عند المعاملة قبل ومع العقار اكثر من بعد العقار.
لذلك يعتبر المستخلص المائي لنبات العطرة من المثبطات المباشرة بالدرجة الاولى ومثبطات حيوية بالدرجة الثانية لقابليتها على إصلاح التشوهات الكروموسومية و زيادة معامل الانقسام الخيطي في خلايا نقي العظم في الفئران و في خلايا الدم المحيطي للإنسان(خارج الجسم)،كذلك له القدرة على زيادة معامل الأرومي و معامل التضاعف وخفض نسبة التبادل الكروماتيدي الشقيق في خلايا الدم المحيطي للإنسان(خارج الجسم).

٥.لم يؤثر المستخلص المائي لنبات العطرة في خلايا الدم البيض للفئران عند العد التمايزي.

٦. لم يظهر الميترونيدازول آي تأثيرات في خلايا الدم البيض للفئران عند العد التمايزي.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الاحيائية

دراسة القابلية المضادة للتطفير لنبات *Pelargonium odoratissimum* (L.) Soland على عقار الميترونيدازول في خلايا اللبائن

رسالة

مقدمة الى كلية العلوم جامعة النهرين

وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الاحيائية

من قبل

مياسة فاضل مهدي الروماني

بكلوريوس تقانة احيائية جامعة النهرين ٢٠٠٢

جمادي الثاني ١٤٢٧

تموز ٢٠٠٦