Hussam O. Al-Saffar ‼ H u s s a m

& M a t h e m a t i c s :S a f f a r -A l .O

.a p l l c o p u t e r

Acknowledgment

At the beginning thanks to great Allah who gave me the reality and strength to accomplish this work.

I would like to extend my thankful, respection and appreciation to my supervisor Professor Dr.Khulood Al-Samarrai and Dr.Esmail K.Shubber who have given me their advice and their important guidance through this work.

Also I would like to express my great thanks to the dean of College of Science Dr. Laith AL-Ani. and to the head of Biotechnology Department of Al-Nahrain University Dr.Nabeel Al-Ani for their support.

Also I would like to express my deep gratitude and appreciation to the Dr. Nahi Y. Yaseen chairman of Iraqi Center for Cancer and Medical Genetic Research for their support.

My sincere gratitude and deepest thanks and gratefulness go to Dr. Ahmed M. Al- Shamery in the Iraqi Center for Cancer and Medical Genetic Research for giving me his assistance.

I'm also grateful to the Dr. Hazim Al- Ahmed in the Biotechnology Research Center / Al-Nahrain University for the statistical analysis of this work, typing this thesis and for all his support and assistance

Very much thanks to the staff of Biotechnology Research Center/ Al-Nahrain University for their assistance. I would like to thanks the staff of Biotechnology Department/ College of Science /Al-Nahrain University for their assistance.

My deep thanks directed to Dr. Maysaa Ch. Al-yas, Dr. Mohammed A. Wahab , Rukia Mohammed, Niran Ala`a

Finally I wish to thank my family, mother , brother and sisters , may God bless them.

Supervisor Certification

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Chapter One

General Introduction

Chapter Two

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Chapter Three

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Conclusions & Future Work

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List of abbreviations

ADA	**Adenosine Deaminase**
AMN-3	**Ahmed Mohmmed Nahi,2003**
BI	**Blast index**
5-BudR	**5-Bromodeoxyuridine**
CA	**Chromosomal aberration**
CCP	**Cell cycle progression**
DHFR	**Dihydrofolate reductase**
DPX	**Distyrene Plasticizer Xylene**
FCS	**Fetal calf serum**
GI	**Growth Inhibition**
GOT	**Glutamic Oxaloacetate Transaminase**
GPT	**Glutamic Pyrovate Transaminase**

HEP-2	**Human Epedermoid laryngo Carcinoma**
HIV	**Human immunodeficiency virus**
HPLC	**High Performance Liquid Chromatography**
KCL	**Potassium chloride**
MI	**Mitotic index**
MMC	**Mitomycine C**
MN	**Micronucleus**
MTX	**Methotrexate**
NaHCO3	**Sodium bicarbonate**
NaOH	**Sodium hydroxide**
NCI	**National Cancer Institute**
PBS	**Phosphate buffer saline**
PHA	**Phytohaemoagglutinine**
Ref	**Rat embryo fibroblast**
RI	**Replicative index**
SCE	**Sister chromatid exchange**
SSC	**Salt sodium citrate**
UV	**Ultraviolet**
WBC	**White Blood Cell**
WHO	**World Health Organization**

Republic of Iraq
Ministry of Higher Education
and Scientific Research
Al-Nahrain University
College of Science

Biological Effects of *Capparis spinosa* and *Rumex acetosella* Extracts on Animal and Human Normal and Tumor Cells

A thesis
Submitted to the College of Science / AL-Nahrain University in a partial fulfillment of the requirements for the degree of Doctor of philosophy in Biotechnology

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March 2007

Introduction:

Herbal medicines are dietary supplements that contain herbs , either singly or in mixture . A herb also is a plant or plant part used for its scent, flavor, and/or therapeutic properties. Products made from botanicals that are used to maintain or improve health have been called herbal supplements ,botanicals, or phytomedicines (NCCAM, 2005).

Many herbs have a long history of use and of claimed health benefits. However, herbal supplements and botanicals have potent pharmacological activity and, consequently, contribute to potential adverse effects and drug interactions (NCCAM,2005).

Interest in a large number of traditional natural products has increased (Vilietinck *et al.*,1995; Taylor *et al*.,1996). It has been suggested that aqueous and ethanolic extract from plants used in allopathic medicine are potential sources of antiviral and anti tumor agents (Chung *et al*.,1995). Furthermore, the selection of crude plant extracts from screening programs has the potential of being more successful in its initial steps than the screening of pure compounds isolated from natural products (Kusumoto *et al.*,1995).

One of the current strategies for drug discovery involved the study of plant materials based on the ethnobotanical usage. The search for anticancer drugs, use of a plant or plant materials for the treatment of certain cancer-related disease can provide a guide for further studies , this includes; cancer treatment, immune disorders, infections diseases, parasitic diseases and viral diseases (Cordell *et al*.,1991).

However, natural products provide an inexhaustible source of anticancer drugs in terms of both variety and mechanism of action(Raghu *et al*.,2004).

The use of herbal supplements by cancer patients in the preoperative period is prevalent and consistent with the substantial increase in the use

of alternative medical therapies by cancer patients (Kumar *et al*.,2002). however from 25% to 85% of cancer patients are seeking alternative and complementary nutritional therapies for prevention or during cancer treatment. The use of these therapies is highest among patients with breast cancer (80% to 85%) (Morries *et al*.,2000),pediatric cancer (46%) (Sawter and Gannoini,1994), prostate cancer (27% to 43%) (Lippert *et al*.,1999), and head and neck cancer (25%). In a study of 820 cancer patients receiving chemotherapy or radiation therapy, 29.1% reported using complementary integrative nutritional therapies that were not prescribed by their physician (Kumer *et al*.,2002).

In recent years , nutrients as well as non-nutrient phytochemicals are being extensively explored for their potential preventive effects against cancer (Kelloff *et al*.,1999). In this regard, phytochemicals are shown to induce differentiation and apoptosis accompanied with growth inhibition in cancer cells (Zi *et al*.,1999 ; Bonnesen *et al*.,2001).

In ayurvedeic medicine *Capparis spinosa* recorded that contain considerable amounts of the antioxidant bioflavonoids rutin. The *in vivo* antioxidant /radical scavenger activity of *C.spinosa* was assessed by determining the ability of it to reduce UVB-induced skin erythema in healthy human volunteers (Bonina *et al*.,2002).

Sheep sorrel or *R.cetosella* was considered the most active herb in Essiac (original eight herb formula) for stimulating cellular regeneration, detoxification and cleansing and one of its properties as an anti-angiogenesis (Foster and Duke,1990).

There is a concern that antioxidants might reduce oxidizing free radicals created by radiotherapy and some forms of chemotherapy, and there by decrease the effectiveness of the therapy. The question is arisen whether concurrent administration of oral antioxidants is contraindicated during therapeutics (Conklin,2002).

On the strength of the following investigations, the aims of this study were proposed for :

1- Preparation of different extract and detection of some active compounds in four different plant species.
2- Studing antimicrobial activity of these active compounds against some species of bacteria.
3- *In vivo* study of the cytogenetic effects of the *C.spinosa* , *R. acetosella* extract on mouse bone marrow cells by using different parameter.
4- *In vivo* study of the cytogenetic effect of MTX as a positive control on mouse bone marrow cells, by using different parameters.
5- *In vitro* study of both *C.spinosa R..acetosella* extract and MTX drug as a positive control on human blood lymphocytes using different parameters.
6- *In vitro* study of the cytotoxic activity of both *C.spinosa* and *R.acetosella* extract on cancer and normal cell lines.
7- *In vitro* study of the cytotoxic activity of MTX and MMC drugs on cancer and normal cells lines.

2.1. History of Herbal Medicine

Medicinal plants are a valuable natural resource and regarded as potentially safe drugs. They have been playing an important role in alleviating human sufferings by contributing herbal medicines in the primary health care systems of rural and remote hilly areas where more than 70% of population depends on folklore and traditional system of medicines. The reason for their popularity is due to the high cost of allopathic medicines and side-effects which encouraged manufacturers of Greco-Arab and Ayurvedic systems of medicines to merge their orthodox medicine with local traditional medicines in order to spread health coverage at a reasonable price (Khan *et al*,1995).

All cultures have long folk medicine histories that include the use of plants. Even in ancient cultures, people methodically and scientifically collected information on herbs and developed well-defined herbal pharmacopoeias. Indeed, well into the 20^{th} century much of the pharmacopoeia of scientific medicine was derived from the herbal lore of native peoples. Many drugs, including strychnine, aspirin, vincristine, taxol, curare, and cryot, are of herbal origin (Farnsworth and Morries, 1976).

Recognition of the rising use of herbal medicines and other non-traditional remedies led to the establishment of the office of alternative medicine by the NIH in 1992. Worldwide herbal usage again became popular; in 1974 the WHO encouraged developing countries to use traditional plant medicine to " fulfill a need unmet by modern systems" (Travelyan, 1993).

There are multiple reasons why patients turn to herbal therapies often cited are a "sense of control and a mental comfort from taking action" (Brown and Marcy, 1991), which helps explain why many people who have

diseases that are chronic or incurable, such as diabetes, cancer, arthritis, or AIDS are taking herbs. In such situations, they often feel that conventional medicine has failed them when patients use home remedies for a cute, often self limited conditions, such as a cold, sore throat or bee sting. It is often because professional care is not immediately available, too inconvenient, costly or time- consuming (Brown and Marcy, 1991, Gill *et.al*., 1994).

In fact, studies have shown that patient who use herbs and other alternative therapies are more likely to abandon potentially beneficial conventional therapy when faced with an illness (Cassileth, 1989).

2.2. Cancer and Herbal Medicine

Cancer generally begins when a single normal cell converted into abnormal cell, cancer cells have three important properties that underlie the nature of the disease (Devita, *et al*., 1993).

1. Cancer cells grow divided with less restraint whereas growth and division of normal cells are closely regulated.
2. Cancer cells do not differentiate normally and therefore do not perform their normal functions in the body.
3. Cancer cells do not die on schedule.

Cancer results from disruption in the control normally exerted over production and differentiation. One key aspect of this abnormal differentiation is the greatly prolonged life spans of cancer cells compared with those of their normal counterparts. Cancer cells are essentially immortal. Another aspect is the failure of the cancer cells to develop the specialized functions of their normal counterpart, (La Vecchia and Tavani, 1998).

The first stage in the development of tumor cells is, the transformation of normal cell to a cell that reproduces and differentiate abnormal through cell division, a population of cancer cells is formed. The second stage is the spread of some cancer cells to other organs of the body, and eventually tumor may localized at different organs (Seizinger, 1993). Cancer, apparently, can result from exposure to a wide range of different agents, although cancers are known to be caused by a variety of chemicals including inorganic compounds and simple and complex organic compounds, ionizing radiation such as gamma x-rays, non ionizing radiation's such as ultraviolet rays, emissions from radioactive materials, and also by biological agents such as viruses (Bishop, 1991).

Carcinogenic processes involve multiple steps (Sugimura, 1992). It can be readily appreciated that, in the body of a healthy human, there are many cells which already have genetic alterations of cancer related genes caused by various genotoxic substances, including dietary carcinogenes. Moreover, genomic instability (Loeb, 1998; Lengauer *et al*., 1998), frequently resulting from mutation in genes, encoding proteins related to DNA repair would be expected to be produced by mutational events. If a mutation occurs in the genes, more rapid accumulation of additional gene alterations would be yielded in other cancer-related gene. Therefore, the potential contribution of minute amounts of mutagens/ carcinogenes present in the diet can not be over looked with regard to the significance for carcinogenesis (Takashi, 2000).

Boik, (1996), basis his approach to the use of natural agents for cancer on the growing understanding of the biological mechanisms by which cancer cell proliferate, maintain life and die: these include differentiation (the maturation process of cells), angiogenesis (the growth of new blood vessels

into tumors), apoptosis (programmed cell death), invasion (The spread of the tumor mass into adjacent tissues), metastasis (the spread of tumor cell to distant locations), mitosis (the proliferation of cells) and evasion of the immune system. As these mechanisms have become elucidated, their weak points have been identified and have become the targets of a new generation of less toxic anticancer drugs.

However, the component of individual herbal medicines is rather complicated and its effect is usually multiple and not single. So, beside the antineoplastic effect, the other effects are also presented. And although the active antineoplastic ingredient have been isolated from some of the herbal medicines, these ingredients may exert effects other than antineoplastic. This multiple effect phenomenon is also observed in some of the westren mono-component antineoplastic drugs. For example, cyclo-phosphamide can act on the various phases of proliferative cells causing degeneration of DNA, RNA, enzymes and protein and serves as a killer of tumor cells (Wang *et al*.,1985).

The herbal medicines achieves their antineoplastic effect through various ways. Moreover, some medicine can bring on several actions, for example, they may directly inhibit the growth of tumor as well as indirectly exert an antineoplastic effect by enhancing the bodily immunologic function. Generally, they elicit no significant adverse effect on the human body and this is a strong point of herbal medicine for antineoplastic treatment (Zhao and Huang,1992).

2.3. Description of Plants

2.3.1. *Capparis spinosa*

***Capparis spinosa* L. (syn. Capparis rupestris)**

Family : Capparidaceae (or Capparaceae)

Capers of commerce are immature flower buds which have been pickled in vinegar or preserved in granular salt. Semi-mature fruits (caperberries) and young shoots with small leaves may also be pickled for use as a condiment. In fact, the caper strong flavor comes from mustard oil: methyl isothiocyanate (released from glucocapparin molecules) arising from crushed plant tissues (Robert, 1990).

There is a strong association between the caperbush and ocean and seas. *Capparis spinosa* is said to be native to the Mediterranean basin, but its range stretches from the Atlantic coasts of the Canary Islands and Maracco to the black sea to the Crimea and Armenia, and eastward to the Caspian Sea and into Iran. Capers probably originated from dry regions in west or central Asia. Known and used for millennia, capers were mentioned by Dioscorides as being a marketable product of the ancient Greeks (Zohary, 1969).

2.3.1.1. Principles Constituents

The cortex and leaves contain stachydrine and 3-hydroxystachydrine. The root contains, gluconeoglucobrassicin and 4-methoxy-glucobrassicin. The crude extract of the flowerbuds contain the constituents of which isothiocyanates, thiocynates, sulphides and their oxidative products have identified as the major components. The seeds and leaves contain glucocapparin, root bark contains stachydrine, rutic acid and a volatile substance with garlic odour (Schraudolf, 1989).

2.3.1.2. Medicinal Uses

Capers are said to reduce flatulence and to be anti-rheumatic in effect. In ayurvedeic medicine capers are recorded as hepatic stimulants and protectors, improving liver function. Capers have reported uses for arteriosclerosis, as diuretics, kidney disinfectants, vermifuges and tonics.

Infusion and decoctions from caper root bark have been traditionally used for dropsy, anemia, arthritis and gout. Capers contain considerable amount of antioxidant bioflavinoid rutin (Hort, 1983).

Caper extracts and pulps have been used in cosmetics, but there has been reported contact dermatitis and sensitivity from their use (Barbera and Lorenzo, 1984).

2.3.2. *Rumex acetosella*

***Rumex acetosella* L. (Sheep Sorrel)**

Family: Polygonaceae

Sheep sorrel has alternate, arrowhead-shaped leaves 2 to 5 long, and long spike-like clusters of small red or orange flowers, with the plant 30 to 60 cm in height (Parker, 1972; Niering and Olmstead, 1988).

It is the most important herb in the Essiac formula. At least ten native tribes of Canada and the United States have used this plant. It is also known as sour grass or sour weed, as a food and medicine. Sheep sorrel is a popular ingredient of many folk remedies and the tea was used traditionally as a diuretic and to treat fever. Interestingly, even though it is not a legume, sheep sorrel contains significant level of phytoestrogens with notable estrogen receptor binding activity. Similar to the isoflavone phytoestrogens common to red clover, licoric and soy, all legumes are known for their strong health restorative properties (Duke, 1985).

Sheep or red sorrel, *Rumex acetosella*, was introduced from Europe and has wide distribution across Canada and the United States (Lewis *et al*., 1983).

In the 1868 Canadian pharmacy journal, the leaves of sheep sorrel *(Rumex acetosella*) were included in the list of Canadian medicinal plants. In 1926, the National Cancer Institute received a recipe from Canada citing an old Indian cure for cancer using a paste of this plant made with bread (Foster and Duke, 1990).

Sheep sorrel also has several traditional culinary uses, including the addition of the leaves to salad, egg dishes, soups, and stews; and as a juice (from the leaves) used to curdle milk to make cheese. This herb is known to add a lovely tart taste to food, and has been used to make cool, refreshing beverages (Turner and Kuhnlein, 1991).

2.3.2.1. Principle Constituents

Sheep sorrel dried aerial parts contain: rutin (0.53%), flavone glycosides (i.e. hyperoside or quercitin-3d-galactoside). 0.05%, and hyperin (12 mg/ 100g). Sheep sorrel also contains vitamins: C, A, B complex, D, E, K, P and U. Total vitamin C of leaves varies from 750-1200 mg/100g based on dry weight. It has a high mineral content, including calcium 20.0%, 13.9% phosphorus; 13.4% magnesium; 28.3% potassium, and 11.5% silicon, along with iron, sulphur, copper, iodine, manganese, and zinc. The leaves and stems contain beneficial carotenoids, chlorophyll, organic acids (i.e., malic, oxalic, tannic, tartaric and citric) and phytoestrogens. The plant also contains anthraquinones including emodin, aloe emodin, chrysophanol, rhein, and physcion (Yagi *et al*, 1997).

2.3.2.2. Medicinal Uses

Sheep sorrel, contains significant levels of phytoestrogens. Similar to the isoflavone phytoestrogens common to red clover, liquorice and soy, all legumes are known for their strong health restorative properties. Another reason, perhaps, it helps people with debilitating illness (Turner and Kuhnlein, 1991).

In China, sheep sorrel is given after giving birth to reduce the bleeding and also to prevent infection. It has been used for centuries to prevent the spread of contagious disease such as the plague and to cool the fever (diaphoretic) caused by cholera and malaria (Foster and Duke, 1990).

The high tannin content of the tea can also provide astringent action, which is useful for treating diarrhea and excessive menstrual bleeding. At higher doses, they are laxatives due to the presence of anthraquinones that directly effect the neuromuscular tissue, stimulate peristalsis, increase the mucous production of colonic mucosa cells and stimulate secretion of water into the intestinal lumen, thereby exerting a laxative effect (Fairbairn and Muhtadi, 1972).

It has anti-inflammatory properties, hence why it is suggested in the treatment of arthritis. Its many constituents related to functions of the blood, or circulatory system, indicate that it may be helpful for those who are suffering imbalances in that regard. Further, other constituents are present that would relax the nervous system, muscles, and decrease pain (Duke, 1985).

2.3.3. *Trifolium pratense*

***Trifolium pratense* L.(Red clover)**

Family:Fabaceae

Trifolium pratense or red clover, a wide plant used as grazing food for cattle and other livestock, has also been used medicinally to treat a wide array of conditions. These have included cancer, mastitis (inflammation of the breast), joint disorder, jaundice, bronchitis, spasmodic coughing, asthma, and skin inflammations such as psoriasis and eczema (Bradley,1992). Red clover is a perennial herb that commonly grows wild in meadows throughout Europe and Asia, and has now been naturalized to grow in North America (Dermarderosian, 2000).

Recently, specific chemicals in red clover known as isoflavones have been isolated and tested for their effectiveness in treating a variety of conditions. Although isolated isoflavone products are very different from the whole herb, they have shown promise in the treatment of a number of conditions associated with menopause, such as hot flashes (Baber *et al*.,1999),cardiovascular health and the bone loss associated with osteoporosis (Howes *et al*.,2000).

2.3.3.1.Principle Constituents

Red clover is a source of many valuable nutrients including calcium, chromium, magnesium, niacin, phosphorus, potassium, thiamine, and vitamine C, red clover is also considered to be one of the richest source of isoflavones, phenolic glycosides , cyanogenic glycosides ,genisteine salicylates ,lecithin,sitosterol, and coumarins (Husband,2001).

2.3.3.2. Medicinal Uses

Traditionally, red clover ointments have been applied to the skin to treat conditions such as psoriasis, eczema, and other rashes, red clover also has a history of use as a cough remedy for children (Kuhn and Winston, 2001) .

Menopause increases a woman's risk for developing cardiovascular disease. Supplementations with red clover isoflavones has been associated with a sizeable increase in high-density lipoprotein (HDL) cholesterol or good cholesterol in pre and postmenopausal women, leading some researchers to believe that these isoflavones may help protect against cardiovascular disease (Baber *et al* .,1999).

Some studies suggest that a proprietary extract of red clover isoflavones may slow bone loss and even boost bone mineral density in pre and per menopausal women (Nachtigali ,2001) .

2.3.4.*Solanum nigrum*

***Solanum nigrum* L. (Black night shade)**

Family :Solanaceae

The black nightshade is an annual branched herb up to 90 cm high with dull dark green leaves, juicy, ovate or lanceolate , toothless to slightly toothed on the margins . Flowers are small and white with short –pedicellate and five widely spread petals . It is frequently to be seen among growing crops and in damp and shady places , it is sometimes called the Garden Nightshade because it so often occurs in cultivated ground. *S.nigrum* are weeds of waste land , old field , ditches and roadsides , fense rows , or edges of wood and cultivated land (Frohne and Pfander , 1983).

The black Nightshade is a commonlly and generally distributed in the South of England. It is one of the most cosmopolitan of wild plants ,

extending almost over the whole globe (Grieve , 1995). On account of its berries , the Black Nightshade was called by older herbalists (petty Morel) to distinguish it from the deadly Nightshade often known as Great Morel (Grieve , 1995).

The main risk of *S.nigrum* are poisoning which occurs mainly from ingestion of plant, especially the un ripened fruits and the main target organs are the cardiovascular and central nervous system and the gastrointestinal tract (Bergers and Alink , 1980).

2.3.4.1.Principle Coustituents

Solanine a glyco – alkaloid is found throughtout the plant with the highest concentrations in the un ripened berries . The concertration of solanine increases in the leaves as the plant matures (Cooper and Johnson , 1984). When ripe , the berries are the least toxic part of the plant and are sometimes eaten without ill effects. Solanine is a mixture of two classes of glycosides , solanine and chaconines . These compounds contain the same basic alkamine aglycon and solanidine , but differ with respect to the composition of the sugar chain(Watt and Breyer – Brandwijk , 1962).

Other chemical contents of the plant is solanidine which obtained after hydrolysis of solanine , and is less toxic . Nitrates and nitrites are also occur in variable amounts in Black Nightshade and may contribute to its toxic effects in addition to alpha – solanine which is the main constituent of *S.nigrum* (Cooper and Johnson , 1984).

2.3.4.2.Medicinal Uses

The black nightshade has limited medicinal uses in liniments , poultices and decoctions for external use .It has also been used in folkloric medicine

as sedative and anticonvulsant. Solanine hydrochloride has been used as an agricultural insecticide (Hardin and Arena, 1974).

Solanine was compared with k- strophantidine by animal studies and has revealed similar effects .Bergers and Alink , (1980) pointed that if neonatal rat cells are exposed to solanine, they can caused an initial increased contraction rate followed by cessation .

Toxic intravenous doses of solanine cause ventricular fibrillation in rabbits . In addition , in rabbits , toxic intra – peritoneal (I.P)doses cause mild to moderate inhibition of both specific and non specific cholinesterase (Keeler, 1987). Many workers are investigated to find out whether solanidan alkaloids might be teratogenic or not, and although some effects were reported in chick embryos and hamsters, most workers are found not teratogenic in rats , rabbits and mice (Keeler and Tu , 1983).

2.4.Chemotherapy Drugs

2.4.1.Methotrexate

Methotrexate (MTX) is a chemotherapy drug that interferes with folic acid activation, preventing cell reproduction, methotrexate was first used in the treatment of childhood acute lymphocytic leukemia in 1984. Methotrexate has since been used in the treatment of various malignancies including osteosarcoma, non-Hodgkin’s lymphoma, Hodgkin’s disease ,head & nack cancer and lung cancer and breast cancer, MTX is used to treat disabling psoriasis, and severe, active rheumatoid arthritis (Huennekens, 1994) . At two stages in the biosynthesis of purines (adenine and guanine) and at one stage in the synthesis of pyrimidins (thymine,cytosine ,and uracil), one-carbon transfer reactions occur. which require specific coenzymes , these coenzymes are synthesized in the cell from

tetrahydrofolic acid. Tetrahydrofolic acid itself synthesized in the cell from folic acid with the help of an enzyme folic acid reductase (Morgan *et al*.,1994).

Dihydrofolate reductase is critically important because folate motecules are biochemically active only in their fully reduced form as tetrahydrofolates. The tetrahydrofolates are essential cofactors that donate one–carbon groups in the enzymatic biosynthesis of thymidylate and purine nucleotide precursors for DNA synthesis (Takimoto and Allegra, 1995).

Furthermore, DHFR inhibition leads to the accumulation of dihydrofolate polyglutamates within the cell, which can directly inhibit the folate dependent enzymes involved in the synthesis of thymidylate and Purine nucleotides . Thus, inhibition of DNA synthesis by the antifolates is amultifactorial process, resulting from the partial depletion of the intracellular reduces folate pool and from the direct inhibition of folate – dependent enzymes (Allegra *et al* ., 1987) .

Lethal DNA damage resulting from a drug – induced lack of essential nucleotides may occur because of ineffective DNA repair , or because of misinformation of uracil deoxynucleotides into DNA (Allegra , 1990). A secondary malignancy consisting of lymphoma may occur in patients receiving low – dose methotrexate. Teratogenesis is a serious concern if methotrexate is administered during pregnancy methotrexate crosses the placenta to the fetus and has been reported to cause fetal abortion , fetal death, congenital anomalies, chromosome abnormalities, and severe neonatal myelo- suppression when administered during pregnancy. Methotrexate antagonizes the effect of folic acid in neural tube development (Allegra , 1990) .

2.4.2.Mitomycin – C Drug

Mitomycin – C is an anti tumor antibiotics isolated from the fermentation of *Streptomyces capspitosus* that interrupts DNA replication and inhibit mitosis (Costes *et al* ., 1993) .

Mitomycin is a clinically active antineoplastic agent used to treat a variety of tumors and is regarded as the prototypical bioreductive drug (Sartorelli *et al* ., 1994).Its mechanism of action is complex involving several reductase enzymes, some of which have yet to be identified (Spanswick *et al*.,1996). It is generally believed that the enzyme DTD [NAD(P) H: quinone oxidoreductase] is the major enzyme responsible for bioreductive activation of MMC under normal oxygenated conditions , whereas under hypoxic conditions, other enzymes (Such as cytochrome p-450 reductase) assume a prominent role (Belcourt *et al*.,1996).

Attempts to elucidate the fine details of MMC activation in terms of identifying enzymes that determine cellular response have, however , generated conflicting and controversial results . This is particularly true in the case of DTD , where MMC has been shown to be a substrate for DTD at acidic PH , whereas under normal physiological PH conditions , MMC is not only a poor substrate but is also an inhibitor of enzyme activity (Schlager and Powis , 1988 ; Siegel *et al* ., 1993).

2.5. Cytogenetic Analysis

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 (Nakanishi and Schneider, 1979).

2.5.1. Mitotic Index

As a person develops from an embryo, through fetus and infant to an adult , cell divisions are needed to generate the large numbers of cells required. Additionally , many cells have a limited lifespan, so there is a continuous requirement to generate new cells in the adult. All these cell divisions occur by mitosis . Mitosis is the normal process of cell division, from cleavage of the zygote to death of the person. The M phase of the cell cycle consists of the various stages of nuclear division, and cell division (cytokinesis) , which overlaps the final stages of mitosis (Strachan and Read, 1999).

Mitotic index is the ratio of cells in metaphase divided by the total number of cells observed in a population of cells (1000 cells) (King *et al*., 1982 ; Shubber and AL-Allak , 1986).

Mitotic abnormalities often arise directly from defects of centrosome and/or mitotic spindles, which then induce prolonged mitotic arrest or delayed mitotic exit and trigger induction of apoptosis (Mollinedo and Gajabe, 2003). Recent reports have demonstrated that entry into mitosis in the presence of damaged DNA leads to inactivation of centrosomes , formation of aberrant spindles and blockage of chromosome segregation , which consequently delays mitosis progression and induces mitotic abnormalities (Takada *et al*., 2003). In addition, chemical or pharmacological inhibition of the DNA damage checkpoint at the G2 stage induces premature entry into mitosis and subsequent initiation of apoptosis (Sampath and Plunkett, 2001).

An additional evaluation of the potential of physical and chemical agents in producing effects on cells can be carried out by analyzing the proportion of mitotic cells and calculating a mitotic index. Depression of the

mitotic index is usually a consequence of a reduced rate of cell proliferation (mitotic delay) (Galloway *et al*., 1994).

Shubber and Juma, (1999) find that *Urtica dioica* herbs extract at high doses significantly increased the mitotic activity of bone marrow and spleen cells, although it reduced cell cycle progression and increased the spontaneous levels of sister chromatid exchange and total chromosome aberration.

2.5.2.Chromosome Aberrations

During the course of meiosis , portions of chromosome are often relocated , moving within the chromosome itself or between different chromosome. This process produces changes in the morphology of the chromosome itself, which are referred to as chromosomal aberrations (CAs) (Holland , 2005).

The type of chromosomal structural alterations produced by physical and chemical agents depend on the lesions induced in the DNA and therefore, upon the chemical structure of the genotoxic substance . Structural chromosome aberrations result from the breakage and rearrangement of whole chromosomes into abnormal forms .They are most efficiently induced by those substances that directly break the backbone of DNA (e.g.ionizing radiation and radiomimetic chemicals) or significantly distort the DNA helix (e.g.intercalating agents).Stractural aberrations can be classified as either unstable or stable ,indicating their ability to persist in dividing cell populations (Carrano,1986).

Unstable aberrations consist of dicentric ,rings ,deletion and other asymmetrical rearrangements. Previous studies have demonstrated that unstable aberrations will lead to death of the cell (Carrano,1973).Stable

aberrations consist of balanced translocations inversions , and other symmetrical rearrangements . They are considered stable because they can be transmitted to progeny cells without causing cell death (Carrano, 1986)

It is probable (Conforth and Bedford,1993) to see that most chromosome aberrations result from illegitimate reunion (misrejoining)of free ends from different DNA double –strand breaks (dsb). Sax (1940) find that aberration formation is influenced by proximity effects ,i.e. effects which occur because dsb free ends are more likely to undergo illegitimate reunion if the dsb are initially formed close together than if the dsb are formed far apart.

The *in vitro* chromosome aberration was used to identify agents that cause structural chromosome aberrations in cultured mammalian cells. Structural aberrations may be of two types , chromosome or chromatid .With the majority of chemical mutagens , induced aberrations are of the chromatid type ,but chromosome-type aberrations also occur (Galloway *et al*.,1987).

The clastogenic effect of *Ginger rhizome* has been studied by Mukhopadhyay and Muhherjee, (2000) ,they find that ginger oil gave a higher frequency of chromosomal aberrations .Another study by Hassan , (2002) indicates that the aqueous and alcoholic extracts of black seed reduce the spontaneous CAs in mouse bone marrow cells .

According to Preston ,(1997) ,all tumors contain chromosome alterations specific deletions and translocations are involved in different stages of the development of the tumors ,and unspecific alterations are common in genomic instability , which is a characteristic of tumors . So it is important to point out that high frequencies of chromosomal aberration individuals occupationally exposed to genotoxic and/or carcinogenic agent may be considered a relevant biological marker to demonstrate a future cancer case (Sorsa *et al.*,1990).

2.5.3.Micronucleus Assay

Micronuclei are cytoplasmic chromatin–containing bodies formed when a centric chromosome fragments or chromosomes lag during anaphase and fail to become incorporated into daughter cell nuclei during cell division . Because genetic damage that results in chromosome breaks , structurally abnormal chromosome, or spindle abnormalities leads to micronucleus formation , the incidence of micronuclei serves as an index of these types of damage (Armstrong and Galloway,1993). It has been established, essentially, that all agents that cause double strand chromosome breaks (clastogens) induce micronuclei (Shelby,1988).Because enumeration of micronuclei is much faster and less technically demanding that is scoring of chromosome aberration and because micronuclei arise from two important types of genetic damage (Clastogenesis and spindle disruption), the micronucleus assay has been widely used to screen for chemicals that cause these types of damage (Heddle,1973).

This guidance addresses the most widely used *in vivo* micronucleus assay: the mammalian erythrocyte micronucleus assay .This *in vivo* micronucleus test is used for the detection of damage induced by the test substance to the chromosomes or the mitotic apparatus of erythroblasts by analysis of erythrocytes as sampled in bone marrow and /or peripheral blood cells of animals, usually rodents (Heddle *et al.*,1983).

It was interesting that most of the human carcinogens give also a strong response in the MN assay (Shelby,1988) .This is most evident for the alkylating chemotherapeutic agents ,but is not constrained to them , as evidenced by the potent MN assay activity of agents as structrally diverse as aflatoxin , potassium arsenite , and hexavalent chromium compounds . The majority of the classical genotoxins (agents such as ethyl methane sulfonate,

benzo [a] pyrene , triethylenemelamine,etc.) also give a strong response in the MN assay (Heddle *et al*., 1983 ;Mavournin *et al*.,1990) .It is within the context of such carcinogen sensitivity that the mouse bone marrow MN assay was incorporated into regulatory guidelines for mutagenecity testing (Ashby, 1986).

Positive results in the micronucleus test indicate that a substances are micronuclei inducer, which are the result of chromosomal damage or damage to the mitotic apparatus in the erythroblasts of the test species . Negative results indicate that , under the test conditions , the test substance does not produce chromosomal or spindle damage leading to the formation of micronuclei in the immature erythrocytes of the test species (Schlegel and MacGregor , 1982) .

2.5.4.Sister Chromatid Exchange

Sister chromatid exchang (SCE) represents reciprocal interchange of DAN between homologous chromatides observable in metaphase chromosome (Latt,1981). In recent years , SCE analysis has been considered to be a sensitive method for detecting DNA damage.

There is a clear relationship between a substances ability to induced DNA damage, mutate chromosomes and cause cancers (DqWu and Wu,1995). The SCE frequency in the human body in peripheral blood lymphocytes is very steady and dose not vary with age or sex, so, any increase of the SCE frequency is primarily due to chromosome damage (Tsutsui *et al.*,1994).

Sister chromatid exchanges are known to be a manifestation of damage to the genome (Latt *et al.*,1981). SCEs are seemingly related to mutagenesis (Carrano *et al.*,1979), morphological transformation induction in mammalian cells by viruses, physical and chemical carcinogens (Nichols *et*

al.,1978 ; Popescu and Dipaolo ,1982) and the teratogenicity of many chemical and physical agents (Kram ,1982).

Sister chromatid exchange was reported to be significantly higher in lymphocytes of patients with acute lymphoblastic leukemia (Otter *et al.,* 1979),chronic myeloid leukemia (Shirishi and Sandberg,1980) ,and patients with schistosomiasis (Shubber,1987).The mutagenic and carcinogenic agents have the ability to increase the frequency of SCE *in vivo* especially the agents that interact with metabolism and DNA repair system or the drugs that cross link with DNA like CP (Deen *et al*.,1989) , MMC drug (Evans and Vijayalaxmi,1981),and ionized irradiation(Shubber and Shaikhly, 1989).

The incidence of SCE and micronuclei have been reported to be increased in peripheral blood lymphocytes of women using oral contraceptive (steroids) (Dutkowski *et al.*,1983;Murthy and Prima ,1983) in the same field Shubber *et al*.,(1998) had reported that the results of chromosomal analysis of blood lymphocytes from the forty woman IUCD (Intra Uterine Contraceptive Devices) users indicated chromosomal aberrations in the first metaphase cells and a high frequency of sister chromatid exchanges in the second metaphase lymphocytes.

Sister chromatid exchange assay was performed on human lymphoblast cell line (GM-7254) treated with 6TG,8AA,BUDR and MMC in parallel with the expression period by Shubber *et al.*,(1999) and he found that MMC, 6TG and BUDR effectively increased the SCE frequencies. It is also suggested that defective DNA – repair mechanisms may lead to abnormal SCE frequencies and an increased risk of neoplasia (Shubber *et al.,* 1991).

2.5.5. Cell Cycle Progression

Traditional methods for studying cell cycle progression and sister chromatid exchange relied on the incorporation of tritiated thymidine into DNA followed by either autoradiography or liquid scintillation counting (Taylor *et al*.,1957).

The BrdUrd-Giemsa technique is a useful method for identifying , with accuracy , the percentage of cells which have gone through one, two or three divisions *in vitro* and *in vivo* (Bianchi and Lezana, 1976).*In vitro* CCP was found to be affected by the medium, BrdUrd levels and colchicine (Shubber and Al-Allak, 1986). The nomenclature utilized for the evaluation of CCP pattern, according to the number of cell cycles was:

1-First Cell Division (M1)

These groups of cells have no incorporated BrdUrd or are able to incorporate BrdUrd during a single DNA replication phase . The chromosomes of this phase all appear bright under the light microscope.

2-Second Cell Division (M2)

This group contain cells, which incorporated BrdUrd during two "S" phases and display a typical differential staining of sister chromatides (One dull and one bright).

3-Third Cell Division (M3)

These metaphases incorporated BrdUrd during three "S" phases and contain BrdUrd –substituted DNA in both sister chromatid.

(Becher *et al.,* 1984).

Many halogenated Pyrimidine analogues have been assessed for the labeling of DNA including bromodeoxyuridine, chlorodeoxyuridine, iododeoxyuridine, bromodeoxycytidine, chlorodeoxycytidine and iododeoxycytidine (Dufrain, 1974; Kubbies *et al*., 1985). Cell labeling with

BrdUrd can be accomplished not only *in vitro* but also employed as an anti-cancer drugs (Danova *et al*.,1988 ; Jumae *et al.*, 1999).

2.5.6. Replicative Index (RI)

Cell cycle progression (CCP) may be defined using a parameter that consider , at the same time, the number of M1, M2, M3 metaphase cells. Many indicators were considered, but the most suitable was the RI. This index is formulated using the following equation :

$$RI = \frac{(1 \times M1\%) + (2 \times M2\%) + (3 \times M3\%)}{100}$$

(Lamberti *et al*., 1983).

2.5.7.Blastogenesis (Lymphocyte transformation)

It is synonymous term first used by Nowell (1960) to describe the morphological changes that resulted when small resting lymphocyte were transformed into lymphoblast on exposure to non specific mutagen or antigen, the competence of lymphocyte to enter the blastogenesis was considered a result of immunological recognition (Mills,1966). Soren,(1973) define the blastogenic index as a term to measure the cellular response to mitogens in the terms of the induction of proliferation .

Most thymic derived T-lymphocytes are thought to posses surface receptor for PHA (phytohaemagglutinin) mitogen (Borbery *et al*.,1968). Many of known lymphocyte activators can be classified into specific and non – specific activators for example PHA stimulate sizable proportion of lymphocytes of all animal individual and induce detectable biochemical and physiological changes (within one hour) after addition of lymphocyte suspension (Wait and Hirschhorn,1978), *in vitro* technique is commonly

used to assess cellular immunity in patient with immunodeficiency , autoimmunity ,infections disease and cancer (Stites, 1976; Dimolfett *et al.*,1995).Nowell , (1960) demonstrates that (PHA), an aqueous extract of the kidney bean *Phaseolus vulgaris*, was able to produce large dividing blast like cell in cultures of human peripheral blood .This mitogenic lectin acts as non specific stimulating agent for T-lymphocyte(Greaves *et al.*, 1974).

2.5.8.Sperm Morphology

Male germ cells or spermatozoa are produced in the testis by the process know as spermatogenesis. They are first formed at puberty, but they represent the culmination of events that begin early in embryonic life (Austin and Short, 1982). Spermatogenesis may be subdivided into three phases: the first phase concerns the mitotic multiplication and maturation of spermatogonia, the second phase called meiosis, and the third phase, called spermiogenesis or spermateleosis, involves the transformation of spermatids into spermatozoa. The various cell stages of spermatogenesis are distributed from the periphery to the center of somniferous tubule according to their age (Austin and Short, 1972).

The spermatozoon consists of a head and a tail like other cells and it is spermatozoon is enclosed within the plasma membrane. The shape of the sperm head is characteristics of the species, in mouse it is hook-shaped, and it is composed of two parts, the nucleus and the acrosome. The nucleus contains a highly condensed chromatin, the acrosome is surrounded by the acrosomal membranes and covers the anterior part of the sperm nucleus, it contains enzymes, important in penetration of the egg in the fertilization process (Austin and Short, 1982; Saladin and Porth, 1998). The tail is

divided into a neck, middle piece, principle piece and the end piece. The neck connects the tail to the head of spermatozoon (Austin and Short, 1972).

Sperm abnormalities might provide a rapid in expensive mammalian screen for agents that lead to errors in the differentiation of spermatogenic stem cells *in vivo* and thus indicate agents which might prove to be mutagenic, teratogenic or carcinogenic (Wyrobek and Bruce, 1975).

Abnormal sperm can be classified under abnormal heads (primary abnormalities), abnormal tails (tertiary abnormalities) and cytoplasmic droplets (secondary abnormalities). Abnormal heads that have been observed include asymmetrical, tapering, pyriform, giant, micro and double heads. Abnormal tails include enlarged, broken, bent, filiform, truncated, and double mid-pieces, along with coiled, looped and double tails. Most spermatozoa with tail abnormalities will not be motile, and the remainder exhibit abnormal motility. Cytoplasmic droplets formed on the neck of spermatozoa during spermatogenesis, are usually lost during maturation in the epididymis. If they are still present when spermatozoa are ejaculated, they are considered an abnormality and, as with other abnormalities, too high a percentage reduce the fertility of the semen (Bearden and Fuquay, 1992)

Tripchloride isolated from *Triptergium wilfordii* has a decreasing effect on cauda epididymal sperm density and motility with an increase in the percentage of abnormal sperms in male rats (Wong *et al*., 1989).

Chatterjee *et al.*, (2000) had reported that chemotherapeutic drugs such as fludarabine , cyclophosphamide and busulphan can cause testicular damage as manifested by reduce testicular volume , oilgozoospermia , and increase sperm abnormalities .

2.6. Enzymatic Assay

2.6.1.Liver Functional Enzyme(GOT,GPT)

Enzymes are proteins that act as biological catalysts to speed up the rates at which chemical reaction occur by lowering the activation energy. Without assistance of enzymes most of the chemical reactions of metabolism would barely proceed at all (Atlas, 1995).

Transamination means the process of transferring an aminogroup from an aminoacid to a keto-acid. Enzymes which catalyze this type of reaction are named Transaminases and the most important transaminase enzymes in diagnostics are Glutamic Oxaloacetic Transaminase (GOT) and Glutamic Pyruvic Transaminase (GPT) (Ricci and Federici, 1982) .

Glutamic Oxaloacetic Transaminase (GOT), also known as aspartate aminotransferase, catalyzes the following reaction :

$$\text{L-Asp} + \alpha\text{- Oxoglutrate} \xrightarrow{GOT} \text{Oxaloacetate} + \text{L-Glu.}$$

The enzyme is widely distributed in plants and animals, but it occurs in concentrate form in mammalian heart and liver (Bergmeyer, 1974).

Glutamic Pyruvic Transaminase (GPT), also known as alanine aminotransferase, catalyzes the following reactions:

$$\text{L-Ala} + \alpha\text{ - Oxoglutrate} \xrightarrow{GPT} \text{Pyruvate} + \text{L-Glu.}$$

GPT activity is particulary higher in the liver cells, kidney and skeletal muscle in decreasing order (Charles, 2003).

Many reports are concentrated on studying the effect of different drugs and chemical compounds on the level of liver enzymes, since the liver is the major organ in the body in which the metabolism and detoxification of different compounds occur (Mathur and Dive, 1981).

From another side, the effect of different plant extracts on detoxifying these chemical compounds and protecting liver cells from damaging by

treating with these compounds have been studied. It has been found that the water extract of green tea adds a protection for liver cells against aflatoxin B by activating the release Glutathion-S-Transfase (GST) enzyme in the rats and metabolize the aflatoxin into a non-toxic compound (Qin *et al*., 1997).

In a study done by Tasaduq and his partners showed that HP-1, a polyherbal phytomedicine comprising extract of five herbs have a hepatocurative and antioxidant profile against carbon tetrachloride (CCl_4) which increase the activities of GOT and GPT. He showed that HP-1 reversed the leakage of LDH, GOT, GPT and have attenuated the serum toxicity as manifested in elevated levels of GOT and GPT (Tasaduq *et al.*, 2003).

Hassan,(2002) notices that treatment of mice which had been previously exposed to Cp and MTX drugs by different high concentration from water and alcoholic extracts of Nigella, Lime, and Cardamom have added protection for GOT, GPT enzyme levels in liver cells in comparison with untreated mice.

2.6.2. Adenosin Deaminase Enzyme (ADA)

Adenosine deaminase (ADA) is an essential enzyme of purine catabolism that is responsible for the hydrolytic deamination of adenosine and 2`-deoxyadenosine to inosine and 2`- deoxyinosine (Frederiksen, 1966).These biochemical pathways for maintaining homeostasis, as both ADA substrates have substantial signaling properties. Adenosine engages G protein – coupled receptors on the surface of target cells to evoke avariety of cellular response (Olah and Stiles ,1995), whereas 2`-deoxyadenosine is cytotoxic via mechanisms that interfere with cellular growth and

differentiation (Hershfield *et al.*,1979 ; Benvenista *et al.*,1995) or the promotion of apoptosis (Benvenista and Cohen,1995; Liu *et al.*,1996).

Adenosin deaminase is present in all tissues but has much higher activity in lymphocyte development perhaps due to its direct association with CD26, which is exhibited on activated T-cell (Tsuboi *et al.*, 1995). ADA activity is particularly high in thymocytes of the thymic cortex , but drops off rapidly in the medulla (Resta *et al.*, 1997).

Adenosine deaminase deficient mice developed a combined immunodeficiency that was linked with profound disturbances in purine metabolism (Blackburn *et al.*,1998).In addition to immunodeficiency , ADA-deficient mice developes other phenotypes noted in ADA –deficient humans (Ratech *et al.*,1985) ,including bony and renal abnormalities and pulmonary insufficiency (Blackburn *et al.*,1998) .

Also ,there are many *in vitro* studies that implicate adenosine as a modulator of inflammatory processes that are central to asthma. These include adenosine's ability to enhance or directly evoke mediator release from mast cells (Fozard *et al.*,1996),and to influence eosinophil function (Knight *et al.*,1997).

2.7. Tissue Culture Applications for Establishing the Effect of Secondary Metabolites on Tumors (*In vitro*)

In order to study the effect of some compound , there are some principles which should be taken into consideration to detect the inhibitory effect on cancer cells ,thus numerous cancer cell lines need to be available (Grafone *et al.*,2003). In 1990 the National Cancer Institute in USA established a new idea for detecting effect of various compounds upon cancer cells *in vitro* by

providing different cancer cell lines to many tumors in a reliable and easy manner ways to get reasonable effect upon these cells (Boyd , 1998).

In general , these compounds might be cytotoxic or non-cytotoxic on cells , so many items have been established for their cytotoxic activity and they are (Wilson , 2000) :

1- Identify the antitumor effect of compounds .
2- Understand the mode of action of these compounds upon cancer cells.
3- Detect the effect of compound on the target cells .
4- Determine the optimal concentration .
5- Determine the relation between the concentration and exposure time (exposure cells to the effect of compound).

The main idea over all from determine the cytotoxic assay is cell death or inhibition over growth due to exert cytotoxic effect on these cells .In last years it has become important and essential to determine or identify the cytotoxic assay for these active compounds. it was found that the cytotoxic effect of unknown compound on cancer cells either reversible or irreversible and its effect could occur immediate or after several weeks (Freshney , 2000). The importance of cancer cell line arises from the facts of the biology of cancer cells *in vitro* , since these cells have uniform arise from pure cell population uncontaminated with fibroblast or epithelial cells (Tom *et al.*,1976).

The cytotoxic assay have several advantages. It can easily analyzed statically thus no average could be need. The relation between concentration and time of exposure can be controlled *in vivo* with the viability to control the physical , chemical and physiological effect of environment beside many experiment might be done in one experiment with little cost by micro titration system. On the other hand, cytotoxic assay have some limitation,

one of these are the difficulty of pharmacokinetics action of compounds *in vitro* as its *in vivo*. This depends on regulating of the effect of secondary metabolites *in vitro* , while *in vivo* its depend on distributing the activity over many cells in accurate way. Also the log phase for cancers *in vitro* is less than that *in vivo* , this will be negatively affect on the mode of action of these compounds . Further, the permeability of cancer cells *in vitro* is varied from that *in vivo* and this means that the effect of these compounds will differe between *in vivo* and *in vitro* (Freshney 2001) .

2.8. Tumor Cell Lines

2.8.1. HEP -2 Cell Line (Human epidermoid laryngocarinoma)

The HEP-2 cell line was established in 1952 by Moore and his partner from tumors that had been produced in irradiated cortisonized weanling rats after injection with epidermoid carcinoma tissue from the larynex of 56 – year – old male (Toolan , 1954). A hardy cell line , HEP-2 resists temperature , nutritional , and environmental changes without a loss of viability (Toolan , 1954).

2.8.2. AMN-3 Cell Line (Ahmed – Mohammed –Nahi , 2003)

This line were applied from passage 50 that represent mammary adenocrcinoma of female mice Balb /c have *in vitro* spontaneous mammary adenocarinoma . This line has been proposed by Al – Shamery , 2003 in Iraqi Center for Cancer and Medical Genetic Research. It was cultured on RPMI-1640 medium supply with 10% fetal calf serum .

3.1.Materials

3.1.1. Equipments and Apparatus

The following equipments and apparatus were used throughout the study :

Apparatus	Company
Analytical balance	Sartorius (Germany)
Analytical oven	Memmert (Germany)
Autoclave	Webeco Gmbh (Germany)
Centrifuge	Beckman (England)
ELISA reader	Ovganon Teknika
Incubator	Memmert (Germany)
Microfuge	Eppendorf (Germany)
Microscope	Motic(Japan)
PH-Meter	Radiometer (Denmark)
Rotary evaporator	Heidolph (Germany)
Soxhlet	Electrothermal (England)
Spectrophotometer	Cecil (France)
Vortex Mixer	Griffin (England)
Water bath	Gallenkamp (England)

3.1.2. Chemical Materials

Materials	Company
Acetic anhydride	BDH (England)
Ammonia	BDH (England)
Adenosine	BDH (England)
Ammonium Sulphate	BDH (England)
Bovin serum albumin	Sigma (USA)
Brain Heart infusion media	Biomerieux
5-Bromodeoxyuridine	BDH (England)
Chloroform	BDH (England)
Colchicine	BDH (England) Ibn Hayan(Syria)
$CuSO_4.5H_2O$	Riedrel-Datton(Germany)
Di Methyl Sulpha Oxide(DMSO)	Sigma (USA)
Eosin	Redel- DE Haen AG seelze-Hanno-ven
Ethyl acetate	BDH (England)
Ethanol	BDH (England)
Ferric chloride	BDH (England)
Fetal Bovin Serum	Sigma (USA)
Giemsa stain	Fluka(Switzerland)
Glacial acetic acid	Fluka(Switzerland)
Glycerin	Fluka (Switzerland)

Glutamic Oxaloacetate Transaminase (GOT)	Randox (England)
Glutamic Pyruvic Transaminase (GPT)	Randox (England)
HCL	Fluka(Switzerland)
Heparin	Sigma (USA)
Hexan	BDH (England)
Hoechst stain	BDH (England)
KCL	Fluka(Switzerland)
KH_2PO_4	Fluka(Switzerland)
Lead acetate	BDH (England)
Lieshman Stain	Merck-Germany
Methanol	BDH (England)
Methotrexate (MTX)	Hexal (German)
Mitomycin C (MMC)	Kyowa (Japan)
Methylene Blue	Fluka(Switzerland)
NaCl	Fluka(Switzerland)
NaOH	Fluka(Switzerland)
Na_2HPO_4	Fluka(Switzerland)
N-2-hydroxyethyl piprazine-N-ethanesulphonic acid (Hepes)	Sigma (USA)
Neutral Red Dye	Sigma (USA)

Penicillin	Sigma (USA)
Phenol	BDH (England)
Phytohaemagglutinin (PHA)	Ministry of Science and Technology (Iraq)
Potassium tertarate	BDH (England)
Potassium Iodide	BDH (England)
RPMI	Sigma (USA)
Sodium Bicarbonate	BDH (England)
Sodium Citrate	Fluka(Switzerland)
Sodium hypochlorite	BDH (England)
Sodium nitroprusside	BDH (England)
Streptomycin	Sigma (USA)
Tri-Sodium Citrate	Sigma (USA)
Trypan Blue stain	Pharmaciafine chemical Uppsala (Sweden)
Trypsin	Sigma (USA)
Versene	BDH(England)

3.2.Plants Used in This Study:

The plants(*C.spinosa, R.acetosella, T.pratense, S.nigrum*) in figure(3-1) was collected from Agriculture College (Baghdad, Abu-Graib)in June 2004 and was identified by Dr.Ali AL - Mosawy, Biology Department, College of Science, Baghdad University.The plant parts(aireal and root part) were air dried at room temperature and grinded into powder form.

A

B

C

D

Figure (3-1):Plants that used in this study(A.*Capparis spinosa,* B.*Rumex acetosella,* C.*Trifolium pratense,* D.*Solanum nigrum*).

3.3.Chemicals and Solutions Preparation:

3.3.1.Ferric Chloride Solution :

Prepared by dissolving (lg) of ferric chloride in (100ml) distilled water(Patton ,1967).

3.3.2.Sodium Hydroxide Solution :

This solution was prepared by dissolving (40gm) of NaOH in (1000ml) distilled water(Patton ,1967).

3.3.3.Potassium Hydroxide Solution:

Prepared by dissolving (50g) of potassium hydroxide in (100ml) distilled water(Patton ,1967).

3.3.4.Sodium Citrate solution :

Sodium citrate (2.9g) was dissolved in (100ml) distilled water stored at 4°C until use (Patton ,1967).

3.3.5.Sodium Bicarbonate solution:

Sodium bicarbonate (4.4gm) was dissolved in (100ml) distilled water.This was stored at 4° C until use (Allen *et al* ., 1977).

3.3.6.Phosphate Buffer Saline (PBS) :

The solution was prepared by dissolving the following chemicals in (1000ml) D.W.

Sodium chloride (NaCl)	8gm
Potassium chloride (KCl)	0.2gm
Sodium phosphate hydrate (Na_2HPO_4)	1.15gm
Potassium phosphate dihydrate ($KH_2 PO_4$)	0.2gm

The pH was adjusted to (7.2) .the solution was sterilized by autoclaving and stored at (4° C) (Hudson and Hay ,1989).

3.3.7.Colchicine (Ibn Hayan /Syrian):

Colchicine was prepared by dissolving (0.5mg) of colchicine in (1ml) of PBS to be used for mice injection . Each animal was injected with (0.25ml) of this solution in the intraperitonial membrane (IP),

while for human blood culture , one gram of colchicine was dissolved in (20ml)of sterile D.W to make a stock solution . This solution was stored at (-20ºC) until used (Allen *et al* ., 1977) .

3.3.8. Potassium Chloride (KCl) (Hypotonic solution) :

A concentration of (0.075M) was prepared by dissolving (5.75gm) of KCL salt in (1000ml)D.W. the solution was sterilized by autoclaving and stored at (4ºC) (Patton ,1967).

3.3.9.Fixative Solution :

The solution was freshly prepared by mixing (3) parts of a absolute methanol with (1) part of glycial acetic acid (Hungerford,1965) .

3.3.10.Bromodeoxyuridine (Brdu):

5-bromo- 2-deoxyuridin powder (50 mg) was suspended in (37.3 ml) of D.W. The solution was sterilized by filtration through (0.22 µm) millipore filter, distributed in sterile tubes with final concentration (1.33 mg/ml) and stored at (-20ºC) until use (Palmer,1970).

3.3.11. Leukocyte Diluent Solution:

Prepared by adding (2 ml) of glacial acetic acid and the volume was completed to (198 ml) D.W., then drops of methylene blue was added and stored at (4ºC) until used (Sood,1994).

3.3.12. Phytohaemagglutinin:

Phytohaemagglutinin was supplied by Ministry of Science and technology/Iraq. (1ml) from stock of PHA was added to 19ml D.W, sterilized by 0.22µm millipore filter unit and (0.1-0.2ml) was added to each (2.5ml) culture.

3.3.13. Trypsin Stock Solution:

Trypsin (2.5g) was dissolved in (100ml) of 0.85% NaCl and stirred for 1hr at room temperature, sterilized by filtration ,and dispensed into 10ml aliquots, stored at(-20ºC).The stock was diluted 1:10 in PBS for using (Freshney, 2000).

3.3.14. EDTA (versene) solution:

Diamine ethylene tetra acetic acid (EDTA) (0.2g) was dissolved in (400ml) PBS and sterilized by autoclaving (Freshney, 2000).

3.3.15. Trypsin-Versine Solution:

Equal volumes of trypsin solution and versene solution were mixed thoroughly and used (Freshney, 2000).

3.3.16. Hanks Balanced Salt Solution (HBSS):

$CaCl_2$ (anhydrous)	0.14 g/L
KCl	0.4 g/L
K_2HPO_4	0.06 g/L
$MgCl_2.6H_2O$	0.1 g/L
$MgSO_4.7H_2O$	0.1 g/L
NaCl	8.0 g/L
$NaHCO_3$	0.35 g/L
Na_2PO_4	0.09 g/L
D-glucose	1.0 g/L
Hepes	2.08 g/L

Each of the above constituents was dissolved separately. $CaCl_2$ was added last, and made up in 1L. pH adjusted to 7,the solution was sterilized by filtration (Freshney,2000).

3.3.17.Antibiotic Solution

Penicillin (1000000 IU) and (1g /ml) streptomycin were dissolved in 10ml D.W, sterilized by filtration. Dispensed into (1ml) aliquots and stored at (-20ºC).(Freshney, 2000).

3.3.18.RPMI-1640 Medium

This medium contained the following:

RPMI-1640 medium base	10gm
Fetal bovine serum	10%
Penicillin	1000000IU

Streptomycin	1g
Heps	4g
Sodium bicarbonate	1%
Brdu	1%

The volume was completed with sterile D.W. to (1000ml), and the pH was adjusted to 7.2 and sterilized by filtration using 0.22 µm size filter. The (2ml) of the medium was transferred into sterile test tubes and kept at (4ºC) until use. (Nara and McCulloch, 1985).

3.3.19. Leishman Stain:

Leishman kit was supplied from Institute of Sera and Vaccine / Baghdad.

3.3.20. Giemsa Stain:

Giemsa stock solution was prepared by dissolving (1g) of Giemsa powder in (33ml) glycerin and put it in water bath at (60ºC) for two hours with continuous shaking, then left it for (30min) at room temperature, then (66ml) of absolute methanol was added with continuous shaking. The stock solution was kept in dark bottle at room temperature (Allen *et al*,1977).

For slide staining, Giemsa solution was prepared as follow:

Giemsa stain stock	1ml
Absolute methanol	1.25ml
Sodium bicarbonate solution	0.5ml
D.W	40ml

3.3.21. Eosin Stain (Watery solution):

Prepared by dissolving (1g) of yellow eosin in (100ml) D.W.(Wyrobek and Bruce, 1975).

3.3.22. Hoechst 33258 Stain:

This fluorescent stain was prepared by dissolving (0.025 gm) from Hoechst in (100ml) PBS (stock) , and taken (2ml) from the stock solution and added to (100ml) D.W.(Freshney ,2000).

3.3.23. Neutral Red :

Neutral red dye (10mg) were dissolved in (100ml) PBS (pH 7.2) (Abdul-Majeed, 2000).

3.3.24. The Eluent:

The eluent was prepared by mixing equal amounts of 0.1M NaH_2PO_4 [prepared by dissolving (0.156 g) of NaH_2PO_4 in (10ml) distilled water] with absolute ethanol (Abdul-Majeed, 2000).

3.3.25. Trypan Blue Stain:

Trypan blue powder (1g) was dissolved in (100ml) Hank´s solution. The solution was filtered by Watman filter paper stored at 4ºC until use then diluted 1:10 in Hank´s solution for using (Freshney, 2000).

3.3.26. Adenosine Deaminase And Total protein solutions:

The solution was prepared according to (Giusti, 1981) as follow:

1-phosphate Buffer (50mmol):

Hydrated sodium dihydrogen phosphate $NaH_2PO_4.H_2O$ (4.73g) and (5.62gm) from $Na_2HpO_4.H_2O$ in(1000ml)D.W and pH was adjusted to 6.5, stored at (4° C).

2-Buffered Adenosine solution (5ommol):

Adenosine (140mg) was dissolved in (150ml) of phosphate buffer solution and put it in water bath at (70ºC) for (10min). The volume was completed to 25ml by phosphate buffer, pH adjusted to (6.5).

3-Ammonium Sulphate stock :

Ammonium sulphate $(NH_4)_2SO_4$(1.982 g) was dissolved in (1000ml) D.W..

4-Ammonium Sulphate Standard (75mmol):

Ammonium sulphate stock (0.5ml) was diluted in (1000ml) D.W.

5-Phenol/Nitroprusside solution(106mmol phenol,0.17mM sodium nitroprusside):

Sodium nitroprusside (50g) and (10g) from phenol was dissolved in (1000ml) D.W..

6-Sodium Hydroxide Solution (2N):

Sodium Hydroxide (40g) was dissolved in (1000ml) D.W.

7- Alkaline Sodium Hypochlorite Solution:

(125mmol / NaOH,11mmol NaOCl) 125 ml from sodium hydroxide mixed with 16.4 from 5% sodium hypochlorite and the volume was completed with deionized D.W..

8-Biurate Solution:

This solution was prepared by dissolving (9g) from potassium tarterate $KOOC(CHOH)_2$ COOKI/2 H_2O and (2g)from potassium iodide in one of 0.2N sodium hydroxide

9-Standard Protein Solution:

Prepared by dissolving (0.5g) from Bovin serum albumin in (100ml) D.W , and dispensed into (1ml)aliquots, stored at -20ºC.

10-Sodium Hydroxide:

This prepared by dissolving (8gm) from NaOH in(1000ml) D.W.

3.3.27. Preperation of Drugs

3.3.27.1. Preperation of Methotrexate:

Methotrexate was obtained from (Hexal company) at concentration of (50mg /2ml), methotrexate stock solution was (25mg/ml),and from this stock solution the concentration (50µg/ml)were prepared to be used *in vivo* study. In human blood culture study ,the stock solution (25mg/ml)was used to prepared the following concentration (0.4,2,4)

µg/ml, which was sterilized by filtration and kept at (4ºC) until being used. In cell line cytotoxicity assay, the following concentrations (1,2,4,6,8,10)µg/ml were prepared from the stock solution of methotrexate .

3.3.27.2. Preperation of Mitomycin :

Mitomycin was obtained from (Kyowa company) at concentration (10mg/ml),from this which is used as a stock solution the concentration (1,2,4,6,8,10)µg/ml was prepared to be used in the cell line cytotoxicity.

3.4.Preparation of Different Plant Extracts

3.4.1.Extraction of steroidal compound (Hexan Extract):

(50g) of plant powder was extracted with hexane (250 ml) by Soxhelt apparatus for 6 hours at 40-60ºC, then the cooled solution was evaporated to dryness by rotary evaporator at 40ºC and kept until used (Al-Jeboory, 1994).

3.4.2.Extraction of Terpenes (Chloroform Extract):

(50 g) of plant powder was extracted with chloroform (250 ml) by Soxhelt apparatus for 6 hours at 40-60ºC,then the cooled solution was evaporated to dryness (Rois *et al*.,1987).

3.4.3.Extraction of Glycoside(before Hydrolysis):

(50 g) of plant powder was extracted with ethyl acetate (250 ml) by Soxhelt apparatus for 6 hours at 40-60ºC, then the cooled solution was evaporated to dryness by rotary evaporator at 40ºC .

3.4.4.Extraction of Aglycon(after hydrolysis of Glycoside):

To get the aglycon part from the plant material, (50g) was hydrolysis with 2M hydrochloric acid for 30-40 minutes at 100ºC, then the cooled solution was extracted twice with ethyl acetate and the combined extract taken to dryness (Harborn, 1973).

3.4.5. Extraction of Flavonoids (Methanolic Extract):

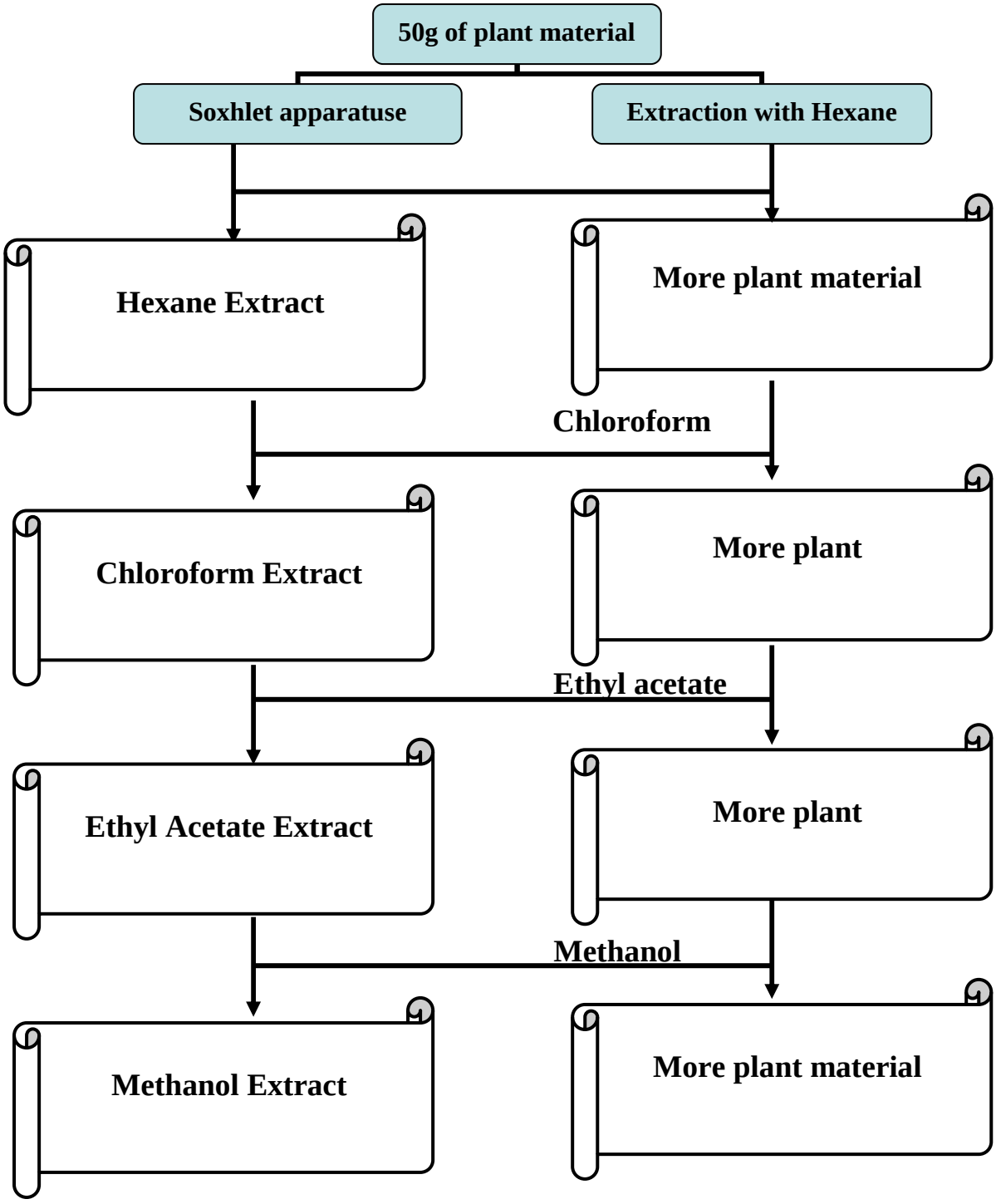
(50 g) of plant powder was extracted with 250 ml of 70% methanol by Soxhlet apparatus for 6 hours at 40-60ºC, then the solvent was removed under reduced pressure by rotary evaporator at 40ºC, and the crude extract was kept until used (Sabahi *et al*.,1987).

3.4.6.Ethanolic Extract:

(50 g) of plant powder was extracted with 250 ml of 80% ethanol by Soxhelt apparatus for 6 hours at 40-60°C,then the cooled solution was evaporated to dryness (Harborn.,1973).

3.5.Fractionation of Plant Ingredients According to Polarity

Plant material (50 g) was extracted according to the polarity of the solvent, starting with the less polar solvent hexane, chloroform, then with semi polar solvent ethyl acetate and finally with the polar one methanol by using Soxhelt apparatus for 6 hours to each solvent. Each fraction was collected and evaporated to dryness under reduced pressure at 40°C and kept until used (Harborn,1973), as shown in the figure (3-2).



Figure(3-2):Method for extraction of plant ingredients according to polarities(Fractionation).

3.6. Detection of Some Active Compounds:

3.6.1. Detection of Tannins:

(10 g) of the plant powder was mixed with 50 ml distilled water in a magnetic stirrer.The mixture was boiled in a boiling water bath for few minutes. Then filtered, and the filtrate was treated with few drops of 1% lead acetate solution.The development of greenish-blue precipitate is an indicator for the presence of tannins(Shihata, 1951).

3.6.2. Detection of Saponins:

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

Saponins were detected by two methods:

3.6.2.1. Aqueous extract of plants powder was shaken vigorously with D.W. in a test tube. The formation of foam standing for a time indicate a positive result.

3.6.2.2. Five milliliters of aqueous extract of the plant was added to 1-3 of 3% ferric chloride solution, a white precipitate was developed which indicates a positive result.

3.6.3. Detection of Glycosides:

This method done according to the method described by Harborn, (1973).

3.6.3.1. Non Hydrolysed Extract:

Equal a mount of the aqueous extract of the plants was mixed with Fehling reagent in a test tube, then boiled in a water bath for 10 minutes. The development of red precipitate indicates a positive result.

3.6.3.2. Hydrolysed Extract:

Few drops of dilute HCl was added to 5ml of the aqueous extract of the plant,then left it in a boiling water bath for 20 minutes, the acidity was neutralized by NaOH solution, equal volume of fehling reagent was added. The development of red precipitate indicates apositive result .

3.6.4. Detection of Terpenes and Steroids:

(1ml) of ethanolic extract was participated in a few drops of chloroform, then a drop of acetic anhydride and drop of concentrated sulphuric acid were added, brown precipitate appeared which representing the presence of terpene.,which the appearance of dark blue color after few minutes would represent the presence of steroids (Al-Abid, 1985).

3.6.5. Detection of Flavonoids:

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

3.7. Effect of Plants Extracts on Bacterial Isolates:

The microorganism used in this study includes:

No.	Microorganism	Origin
1	***Escherichia coli***	**Biotechnology Dept.,AL-Nahrain University**
2	***Klebsiella pneumonia***	**Biotechnology Dept.,AL-Nahrain University**
3	***Staphylococcus aureus***	**Biotechnology Dept.,AL-Nahrain University**

3.7.1. Preparation of Extracts Concentrations :

Stock solution of plants extracts was prepared by dissolving (1g) of plants extracts powder in 0.1% ethanol and then the volume was completed to 10ml sterile D.W.

The plant extracts were prepared at different concentration started with 5mg/ml,10mg/ml,15mg/ml,20mg/ml and finally 25mg/ml.

Extracts preparation was sterilized by filtration by using Millipore filter 0.22µm size filter .Then each concentration was poured into plates in addition to control plates .

3.7.2.Procedure:

The effect of plant extract on bacterial isolates ,was determined by agar dilution method according to (NCCL-2000)

Agar dilutions were prepared using brain heart infusion agar supplemented with (*Capparis spinosa,Rumex acetosella, Triflium pratense and Solanum nigram*) extracts in different concentrations as prepared in (3.7.1)ranging from (5-25mg/ml)

Exponentially, growth for each *S.aureus ,E.coli and K.. pneumonia* isolates were inoculated in sterile BHI –broth then incubated at 37ºC for 24 hr., then (0.1ml)of each inoculam was delivered into agar dilution plate . All plates were incubated at 37ºC for 24 hr..

3.8.Cytotxicity Assay of Plant Extracts on Mouse :

3.8.1. Laboratory animals :

Balb /C mice were obtained from Biotechnology Research Center/University of Al-Nahrain. Their age ranged between (8-12) weeks and weighting (23-25)gm. They were divided into groups ,each group was put in a separated plastic cage. The cages were put in a room

with optimal temperature . The animals were given water and fed with a suitable quantity of water and complete diet .

3.8.2. Treated groups:

According to the effect of *C.spinosa* and *R.acetosella* on bacterial isolates so four main types of experiments were carried out for these two plants to assess the cytogenetic ,immunological and enzymatic effects of ethanolic extract of *Capparis spinosa* and methanolic extract of *Rumex acetosella* and methotrexate drugs.

3.8.2.1. The first experiment:

This experiment was carried out to assess the cytogenetic analysis of plants extract in two different concentrations for *Capparis spinosa* (500,700)mg/kg ,*Rumex acetosella* (100,250)mg/kg and methotrexate drug as apositive control in a concentration (50µg/kg).

The total number of animals used in this experiment (36 mice).

The animals were divided to

Group1: As negative control (3mice).

Treated with (0.1ml)PBS.

Group2: As positive control (3mice).

Treated with (0.1ml) of methotrexate (50µg/kg).

Group3: Ethanolic extract of *Capparis spinosa* treatment (3mice).

Treated with (0.1ml) of extract (500mg/kg).

Group4: Ethanolic extract of *Capparis spinosa* treatment (3mice). Treated with (0.1ml)of extract (700mg/kg)

Group5: Methanolic extract of *Rumex ascetosella* treatment (3mice).

Treated with (0.1ml) of extract (100mg/Kg).

Group6: Methanolic extract of *Rumex acetosella* treatment (3mice). Treated with (0.1ml) of extract (250mg/Kg).

The doses were given intraperitonially (I.P) for seven successive days, and then the mice were sacrificed (spinal dislocation), samples were taken and cytogenetic analyses were carried out as described latter (3.8.3.1,2,3).

3.8.2.2.The second experiment:

This experiment was carried out to asses the abnormalities in sperms (head and tail) percentage for *Capparis spinosa* (500, 700) mg/ Kg, *Rumex acetosella* (100, 250) mg/kg and methotrexate drug (50µg/kg).The animals grouped into three main groups for three periods of time (7,21,35) days

The total number of animals used in this experiment (54mice).

The animals were divided into:

Group1: As negative control (3mice) .

Treated with (0.1ml)PBS**.**

Group2: As positive control (3mice).

Treated with (0.1ml) of methotrexate (50µg/kg).

Group3: Ethanolic extract of *Capparis spinosa* treatment (3mice) treated with (0.1ml) of extract (500µg/kg).

Group4: Ethanolic extract of *Capparis spinosa* treatment (3mice).

Treated with (0.1ml) of extract (700mg/kg).

Group5: Methanolic extract of *Rumex acetosella* treatment (3mice). Treated with (0.1ml) of extract (100mg/kg).

Group6: Methanolic extract of *Rumex acetosella* treatment (3mice). Treated with (0.1ml) of extract (250mg/kg) .

The doses were given intraperitonially for 7,21 and 35 days, and then the 1st groups were sacrificed after 7 days, 2nd groups were sacrificed after 21 days and 3rd groups were sacrificed after 35 days, then sample were taken and the assessment of percentage was carried out as described later in (3.8.3.4).

3.8.2.3.Thired experiment:

This experiment was carried out to assess the effect of plant extracts on liver functional enzymes (GOT, GPT) and Adenosine deaminase enzyme at doses of *Capparis spinosa* (500, 700) mg/kg , from *Rumex acetosella* (100,250) mg/ kg and from methotrexate drug (50µg/kg).

Total number of animals used in this experiment were (18 mice)
The animals was divided as follow

Group1: As negative control (3mice).
Treated with (0.1ml)PBS.

Group2: As positive control (3mice).
Treated with (0.1ml) of methotrexate (50µg/kg).

Group3: Ethanolic extract of *Capparis spinosa* treatment (3mice). Treated with (0.1ml) of extract (500mg/kg).

Group4: Ethanolic extract of *Capparis spinosa* treatment (3mice). Treated with (0.1ml)of extract(700mg/kg).

Group5: Methanolic extract of *Rumex acetosella* treatment (3mice). Treated with (0.1ml)of extract (100mg/kg)

Group6: Methanolic extract of *Rumex acetosella* treatment (3mice). Treated with (0.1ml) of extract (250mg/kg).

The doses were given intraperitonially for seven successive days then, the blood were taken by heart puncher and the serum was separated from the blood and used for enzymatic assay analysis as described later in (3.8.4).

3.8.2.4.Fourth experiment :

The aim of this experiment is to evaluate the immunological effects of plants extract *Capparis spinosa* , *Rumex acetosella* in two different concentrations and methotrexate drugs on the total number of white blood cells and number of each type of white cells by applying total and differential counts of leukocyte .

Total number of animals (18 mice).

The animal was divided as follow

Group1 : As negative control (3mice).

Treated with (0.1ml)PBS.

Group2: As positive control (3mice).

Treated with (0.1ml)of methotrexate (50µg/kg).

Group3: Ethanolic extract of *Capparis spinosa* treatment (3mice) Treated with (0.1ml) of extract (500mg/kg).

Group4: Ethanolic extract of *Capparis spinosa* treatment (3mice).

treated with (0.1ml) of extract (700mg/kg).

Group5: Methanolic extract of *Rumex acetosella* treatment(3 mice). Treated with (0.1ml)of extract(100mg/kg).

Group6: Methanolic extract of *Rumex acetosella* treatment (3mice). Treated with (0.1ml)of extract (250mg/kg).

The doses were given intraperetonially for seven successive days, the blood was collected by heart puncher by hepirinazed syringe and the blood film was carried out as described later in (3.8.5).

3.8.3. Cytogenetic Experiments:

3.8.3.1.Chromosme Preparation from Somatic Cell of the Mouse Bone Marrow:

The experiment was done according to (Evan's *et al.*,1964) as follow:

1- The animals were injected with (0.25ml)of colchicine with concentration of (1mg /ml)intraperitonially (I.P) for 2 hours before sacrificing the animals.

2- The animals were sacrificed by cervical dislocation .

3-The animal was fixed on tergal side on the anatomy plate and the abdominal side of the animal and its thigh region was swabbed with 70% ethanol.

4-The femur bone was taken and cleaned from the other tissues and muscles, 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 was injected so as to wash and drop the bone marrow in the test tube.

5-The test tubes were spinned at 2000 rpm for (10min).

6-The supernatant was removed and (5ml) of potassium chloride (KCI) was added as a hypotonic solution at (0.075M),then the tubes were put in incubator at (37ºC) for 1 hour with shaking from time to time .

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

8-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 shaken well .

9-The tubes were kept at (4ºC) for (30min) to fix the cells .

10- The tubes were spinned at 2000 rpm for (10min). The process was repeated for 3 times and the cells were suspended in 2ml of the fixative solution.

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

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

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

3.8.3.2.Micronuleus Test in Mouse Bone Marrow cells:

The experiment was done according to (Schmid, 1979)as follow :

1-The femur bone cleaned from tissues and muscles ,then gapped from the middle with a forceps in a vertical position over the edge of a test

tube by a sterile syringe . (1ml) of fetal bovine serum (heat inactivated) was injected so as to wash and drop the bone marrow in the test tube .

2-The test tubes were centrifuged at speed of 1000 rpm (5min.).

3-The supernatant was removed, and one drop from the pellet was taken to make a smear on a clean slide.The slides were kept at room temperature for (24hours).

4-The slides were fixed with absolute methanol for (5min),then stained with Giemsa stain for (15min),then washed with D.W. and left to dry.

5-Two slides for each animal were prepared for micronucleus test.

3.8.3.3.Chromosome Preparation from Germ Cells of Mouse Testis:

The experiment was done according to (Evans *et at.*, 1964)as follow:

1-The testis taken from the same animals which is used in (3.8.3.1) .

2-The testis was cut and placed in a petridish containing (5ml)of 1% sodium citrate (20 min at room temperature) and minced by using microsurgical scissor and forceps and then transferred in to test tube.

3-The test tubes were taken and put in the centrifuge at speed of 2000 rpm for (10min).

4-The supernatant was removed and (5ml)of potassium chloride (KCl) was added as a hypotonic solution at (0.075M),then the tubes were put in incubator at 37ºC for 1 hour with shaking from time to time .

5-The tubes were centrifuged at 2000 rpm for (10min).

6-The supernatant was removed and the fixative was added (as drops) on the inside wall of the test tube with continuous shaking, the volume was fixed to (5ml) and the content were shaken well.

7-The tubes were kept at (4ºC) for (30min) to fix the cells.

8-The tubes were centrifuged at 2000 rpm for (10min). The process was repeated for 3 times and the cells were suspended in 2 ml of the fixative solution .

9-By a Pasteur 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 .

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

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

3.8.3.4.Experiment for Assessment the Percentage of Abnormalities in Sperms Head and Tail:

The experiment was done according to (Wyrobek and Bruce ,1978)

1-The animals were sacrificed by cervical dislocation ,and fixed on tergal side on the anatomy plate and the abdominal side of the animals was swabbed with 70% ethanol .

2-The caudal epididymis was cut and placed in a petridish containing (2ml) of PBS and minced by using microsurgical scissor and forceps.

3-Three drops of eosin stain was placed on the cleaned and dried slide.

4-One drops of diluted semen was added on the slide and mixed with the stain for 10 sec.

5-The mixture was left to stand for about 50sec.

6-The mixture , semen + colorants, was spread under a second slide or cover slide by drawing a film of the mixture as thinly and regularly as possible.

7-The slide was left in the warm place to dry and then was examined by the microscope.

3.8.4. Enzymatic Assay

3.8.4.1. Liver Enzymatic Assay:

This assay involved the evaluation of the activity of liver enzymes (GOT, GPT).

3.8.4.1.1.GOT Enzyme:

The Glutamic Oxaloacetic Transaminase enzyme activity was determined in mouse serum using enzymatic colorimetric kit method produced by (Randox company) and according to Rietman and Frankel, (1957) by calculating oxaloacetate which produced from the L-Aspartate according to the following equation.

α- oxoglutarate + L-aspartate $\xrightarrow{GOT}$ L-glutamate + oxaloacetate.

The absorbency was measured at 546 nm by using spectrophotometer .

The kit composed from the following:

1-GOTsubstrate reagent (R1) composed from

100 mmol/L phosphate buffer

200 mmol/L L-Aspartate

2.0 mmol/L 2-oxoglutarate

2-GOT color reagent (R2) composed from

1.0 mmol/L DNPH

2.0 mmol/L HCI

3-Sodium hydroxide (R3)

4mmol/L NaOH

Procedure:

Two test tubes were used and the above solutions was added as shown in table (3-1).

Table(3-1) Method for measuring the GOT activity

Tubes / reagents	Blank reagent (ml)	Sample
Blood serum	–	0.1
Reagent 1 (R1)	0.5	0.5
D.W	0.1	_
1-Mixed well and put in water bath at 37º C for 30 min.		
Reagent 2 (R2)	0.5	0.5
2-Mixed well and put at room temperature for 20 min.		
Reagent 3 (R3)	5	5
3-Mixed well and left for 5min, then the absorbency for sample was measured at 546 nm.		

The activity of GOT enzyme was calculated by down the absorbency value on the standard enzyme curve on the kit and the GOT activity was measured by (Unit/ml).

3.8.4.1.2. GPT Enzyme:

The activity of this enzymes was determined in blood serum depending on colorimetric method using enzyme kit produced from (Randox company) according to Reitman and Frankel, (1957), through calculating the free pyruvate produced from the substrate (L- Alanine) and as shown in the following equation.

α-oxoglutarate + L-alanine $\xrightarrow{GPT}$ L-glutamate + pyruvate

The absorbency was measured at wave length of 546 nm .

The kit composed from the following solutions:

1-GPT substrate reagent (R1) composed from

100 mmol/L phosphate buffer

200 mmol/L L-alanine

2 mmol/L α -oxoglutarate

2-GPT color reagent (R2)

1.0 mmol/L DNPH

1.0 mmol/L HCL

3-Sodium hydroxide

4 mmol/L NaOH

Proceduce:

Two test tubes were used and the solutions was added in same way as shown in table (3-1).

The GPT activity was measured on the same way as the GOT activity was measured, the absorbency was down on the standard enzyme curve as shown in the enzyme kit and the enzyme activity was measured by (Unit/ml).

3.8.4.2.The Immune Enzyme Adenosine Deaminase (ADA):

The ADA assay depend on measuring the free ammonia from adenosine substrate using colorimetric method and as shown in the following equation.

$$\text{Adenosine} \xrightarrow{ADA} \text{Inosine} + NH_3$$

According to Giusti, (1981) the activity of ADA enzyme was evaluated in mouse serum and the absorbency was measured at 628 nm.

Procedure:

The solutions preparation mentioned previously in the (3.3.27) and the procedure for measuring the ADA activity was shown in table(3-2).

Table (3-2) Method for Adenosine Deaminase Measuring Activity

Tubes / Reagents	Reagent Blank ml	Standard ml	Adenosine Blank ml	Sample Blank ml	Sample ml
Phosphate buffer solution	1.0	_	_	1.0	_
Buffered adenosine solution	_	_	1.0	_	1.0
Ammonium sulphate standard	_	1.0	_	_	_
Samples	_	_	_	0.05	0.05
D.W	0.05	0.05	0.05	_	_

All the tubes mixed well and put it in water bath at (37ºC) for (60 min) and then 3ml from phenol nitroprusside solution (3.3.27.no.5)and 3ml from alkaline sodium hypochlorite solution (3.3.27.no.7) were added to each tubes, mixed well and put it in water bath at (37ºC) for (30min). The absorbance read at wave length 628 nm, and the enzymes volume activity was calculated according to the following equation:

Volume activity = $\frac{A-B}{C}\times 50$

A = Sample – Sample blank

B = Adenosine blank – Reagent blank

C = Standard – Reagent blank

After that, the total protein was estimated and from it the specific activity for ADA was calculated according to the following equation:

Specific activity = $\frac{VolumActivity}{Total\ \mathrm{Pr}otein} = U/mg$

(Giusti,1981).

3.8.4.3.Total Protein Determination:

The method for total protein estimation (Biurat method) was done according to Bradford,(1976).The estimation depended on the presence of potassium iodide in alkalin biuret solution ,the absorbency was measured at 550 nm .

Procedure: The solutions preparation mentioned previously in the (3.3.27. no.8,9,10),and the procedure for measuring was shown in table(3-3).

Table (3-3) Method for total protein estimation in serum

Tubes / Solution	Reagent blank ml	Standard ml	Sample
D.W	2.0	_	1.8
Protein standard solution	_	2.0	_
Sample	–	–	0. 2
Biurat solution	5.0	5.0	5.0

All the contents mixed well , put it in water bath at (37ºC) for (10min) and the absorbency was measured at wave length 550 nm and the total protein was calculated according to the following equation:

$$\text{Total protein} = \frac{Sample A - Blank}{S\tan dard - Blank} \times 5gm$$

3.8.5. Blood Film Methods:

This methods was done according (Catalavo, 2002)

3.8.5.1. Total Count of Leukocytes:

1-The blood was taken by heart puncher and put into heperinazed tube.

2-Adiluting solution (190µL) was pipetted into test tube.

3-The heperinazed blood (10µl) was pipetted and mixed well with diluting fluid for at least 2 minutes.

4-The hemocytometer was seted up with its cover glass in position and by a pasture pipette, both sides of the hemocytometer were filled with the diluted blood.

5- The cells were allowed for two minutes to be settled.

6-The cells were count in the four large squares on both sides of chamber using the 40 X objectives and subdued light.

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

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

3.8.5.2. Differential Count of Leukocytes:

1- A small drops of heparinized blood which drawn from mouse was put on the end of clean, and dry slide. A pusher slide was place at an angle of 30º to 45º to the slide and then moved 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 Leishman stains, after 3 min., the slides were washed gently and then examined under light microscope and by applying the following equation :

No. of cells (cells/mm^3 blood) = (Total no. of leukocytes %) / 100.

3.9. Cytogenetic Analysis of Human Blood Lymphocytes:

The experiment was done according to (Shubber, 1987) which adapted from Nowells *et al* (1960).

3.9.1.Chromosomal Preparation of Human Blood Lymphocytes:

1-Human blood was collected in heparin coated syringe.

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

3-PHA (0.25ml) was added. The components were mixed very well.

4-Different concentration of extracts (200, 400, 800)µg/ml were added to each test tube (0.1ml) . Also (0.1ml) of different concentration of methotrexate (0.4, 2, 4)µg/ml were added to other test tube after 24hr of incubation and this considered to be a positive control. Also (0.1ml) of PBS was added and this considered to be a negative control.

5- Test tube was put back in the incubator at (37ºC)and shaked gently each (24 hours) one try at least. The incubation period was completed to (72 hours).

6-Colchicine (0.1ml) was added to each tube 1/2 hours before harvesting the cells, and gently shacked each (10 min).

7-The test tubes were spinned at 2000 rpm for (10 min).

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

9- The tubes were spinned at 2000 rpm for (10 min).

10-The supernatant was removed and the fixative solution was added as drops on the inside wall of the test tube with the continuous shaking, and then, the volume was fixed to (5ml), and the contents were shake well.

11- The tubes were kept at (4ºC) for (30min) to fix the cells.

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

13-By a Pasteur pipette, few drops from the tube were dropped vertically on the chilled grease-free slide from a height of 3 feet at a rate

(4-5) drops to give the chance for the chromosomes to spread well. Later, the slides were kept to dry at room temperature.

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

15-Two slides for each concentration were prepared for cytogenetics assays.

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

3.9.2.Hoechst (33258) Staining:

(Freshney, 2000)

1-The slides were immersed in Hoechst 33258 at a concentration of (20 µg/ml) for (10min) in a coplin jar.

2-The slide were transferred to a slide rack, and drop (500µl) of 2 x SSC.

3-The slides were covered with a 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. A distance of approximately (4cm) between the slides was maintained and the UV source. The longer the pale chromatid will become, expose the slides for about (24-60min).

5-The cover slips were removed from the slides, and the slides were washed three times in water , for 5 min. per wash. The slide 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 PBS buffer (PH 6.8) for (3-5min).

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 (1hour) and dipped into xylene. Four drops of DPX mountant were dropped onto the slide and a 22-mm x 50-mm cover slip was lowered, expressing any air bubbles with tissue.

10-The slides were air dried in a fume hood overnight.

3.9.3.Micronucleus Test in Human Blood Lymphocytes Culture :

(AL-Sudany, 2005).

1-Human blood was collected in a heparin coated syringe.

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

3-PHA (0.25ml) was added. The components were mixed very well and transferred to (37ºC) incubator.

4-Different concentrations of extracts (200,400,800)µg/ml) were added to each test tube (0.1ml). Also (0.1ml) of different concentration of methotrexate (0.4,2,4)µg/ml was added to other test tube after 24 hr. of incubation and this considered to be a positive control. Also (0.1ml) of PBS was added and this considered to be a negative control.

5-The testes tube were put back in the incubator at (37ºC), and gently shaken each (24hr) one try at least. The incubation period was completed to (72hr).

6-The test tubes were spinned at speed of 800 rpm for (5min).

7-The supernatant was removed and (5ml) of potassium chloride (KCl) (0.1 M) was added as a hypotonic solution, then the test tubes were left for (30 min) in water bath at (37ºC) and the tubes were shaken from time to time.

8-The tubes were spinned at 800 rpm for (5 min).

9-The supernatant was removed and the fixative solution was added as drops on the inside wall of the test tube with continuous shaking, and then, the volume was fixed to (5ml), and the contents were shaken well.

10-The tube were kept at (4ºC) for (30min) to fix the cells.

11-The tubes were transferred into the centrifuge at 800 rpm for (5min). The process was repeated 3 times and after that, the supernatant was discarded and one drop of the pellet was smeared on a clean slide and left 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 concentration were prepared for micronucleus assay.

3.10. Cytogenetic Analysis Test

3.10.1. Mitotic Index (MI)Assay:

The slides were examined under high dry power (40x)of 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:

$$MI = \frac{\text{Number of dividing cells}}{\text{(Number of dividing cells + number of non-dividing cells)}} \times 100$$

(Shubber and AL-Allak , 1986).

3.10.2. Blastogenic Index (BI) Assay:

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

$$BI = \frac{\text{No. of the blast cells}}{\text{Total no. of the cells (1000)}} \times 100$$

(Nowell *et al*,1960)

3.10.3. Chromosomal Aberration (CA) Assay :

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

3.10.4. Replicative Index (RI) Assay:

The replicative index (RI) was determined by counting the number of cells at the first, seconed and the third metaphase in ١٠٠ successive dividing cells at metaphase, the RI was calculated according to the following equation:

$$RI = \frac{(1 \times M1\%) + (2 \times M2\%) + (3 \times M3\%)}{100}$$

(Lamberti *et al*., 1983).

3.10.6. Micronucleus Test (MN):

The number of MN in (2000) cells of polychromatic erythrocytes (PCE) in mice and in human blood lymphocyte were scored under the oil immersion lens and the percentage of MN was calculated.

3.11. Anti –Tumor Cells Activity:

Cell line study of the plants *Capparis spinosa* and *Rumex acetosella* was carried out in Iraqi Center for Cancer and Medical Genetic

Research . In this study, the preliminary screening on cytotoxic activity of Capparis and Rumex was carried out .

The screening involved the investigation of cytotoxicity of plant extract *C. spinosa* roots extracted by 80% absolute ethanol in a soxhlet for 6 hours, and *R. acetosella* aerial parts extracted by 80% methanol in a soxhlet for 6 hours ,then the extracts were evaporated until complete dryness,with two types of chemotherapeutic drugs as a positive control (Methotrexate and Mitomycin-C).

The screening of cytotoxiciy was carried out on two tumor cell liens : HEP-2 (Human epidermoid laryngocarcinoma) and AMN-3 (Ahmed-Mohammed-Nahi,2003 mammary adenocarcinoma), and normal cell line Ref (Rat embryo fibroblast).

The investigation included the comparative study between the cytotoxic activity of the plants extract and the drugs.

The percentage of growth inhibition was calculated according to (Phuangsab *et al*.,2001) ,and according to following equation :

$$\text{Growth inhibition \%} = \frac{Control - Treatment\,cell}{Control} \times 100$$

Cell line procedure:

This was applied according to the method adopted by Abdul-Majeed (2000) .

1- Human epidermoid laryngocarcinoma cells from a 57- years old male and mammary adenocarcinoma cells from a female mice and normal cell line (Ref) were cultured in culture bottles (falcons) then stored at 20ºC.

2- The cultured cells were washed with phosphate buffer saline (PBS). The trypsine –versine solution was added with gentle shake , then the final mixtures were poured to another culture bottles (Sub-culturing) and incubated at 37ºC for 1 minutes .

3- Counting of viable cells was carried out using trypan – blue dye (0.4%).Dead cells take up the dye and appear blue under microscope while living cells exclude the dye and appear white.

4- Cytotoxic assay of plants extract and chemotherapeutic drugs were done using neutral red cytotoxic assay .The plant extract was dissolved in dimethylsulfoxide, then six concentrations (62.5, 125,250,500,750,1000) µg/ml of each plant extract and six concentrations of each drug (1,2,4,6,8,10) µg/ml were prepared.

5- The 0.2 ml of cultured cells (HEP-2, AMN-3 , Ref) were transported to 96-well micro plates ,so each well contained 10^5 cell, followed by addition of 0.2 ml of prepared concentrations of plant extract and drugs, leaving some wells contained cultured cells but without any treatment by plants extract or drugs to be considered as negative control.

6- The cells were incubated for 72 hours at 37º C ,and then washed with PBS followed by addition of neutral red solution (0.8ml) to each well re-incubated at (37ºC) for 2 hours .

7-After incubation, the medium was discarded, and the wells were washed with PBS. Viable cells would take the dye, while the dead cells would not take dye.0.1ml of phosphate and buffered-ethanol(0.1M NaH_2PO_4-ethanol; 1:1), were added to elluent the dye from the viable cells.

8-The plate was read by micro ELISA reader at an optical density of (492nm).

3.12. Statistical Analysis :

A one-way analysis of variance was performed to test whether group Variance was significant or not. The comparison between groups were used analysis of variance test at significant value($P \leq 0.05$) (ANOVA) (AL-Mohammed *et al*, 1986).

5.1 Conclusion :

1- *C.spinosa* and *R.acetosella* contained different active compounds including flavonoids(Rutin ,Quercetin) and glycosides.

2-The *C.spinosa* and *R.acetosella* have antibacterial effects on pathogenic strain *E.coli, K.pneumoniae* and *S.aureus* ,while *T.pratense* and *S.nigrum* were exhibited low antibacterial activity .

3-Both plants extracts have neither genotoxic nor clastogenic effects in mouse bone marrow cells (*in vivo*) or on human blood lymphocytes (*in vitro*).

4-Both plants extracts *C.spinosa* and *R.acetosella* reduces the spontaneous frequency of CAs, MN, and increase MI in mouse bone marrow and reduced SCE, MN, CAs with the increase in MI, BI, and RI in human blood lymphocytes.

5-Both plants extract enhance the immune system by increasing the total and differential counts of leukocytes and ADA enzyme activity in mouse serum .

6-*In vitro* study proves that *C.spinosa* and *R.acetosella* has significant cytotoxic activity on two types of cell lines (HEP-2 and AMN-3) , and has no cytotoxic effect non tumor cell line (Ref) . This cytotoxic activity of plants extract is comparable with that of MTX and MMC drugs and are greater in both cell lines than both drugs .

5.2 Recommendation :

1-Qualitative and quantitative study of different active constituent present in *C.spinosa* and *R.acetosella* .

2-Further studies are needed on the effect of plants extracts on the chromosomes of other organs such as liver, spleen, and kidney.

3- Further studies are required for the plants extracts on the inhibition of MTX genotoxicity (Interaction study).

4- Using another parameters for detection the effect of *C.spinosa* and *R.acetosella* on the immune system like phagocytosis.

5-*In vivo* study of *C.spinosa* , *R.acetosella* on tumor bearing animals.

6-Identification of plant extract targets in tumor cells .

References

A

- **Abdul- Majeed, M.R. (2000) .** Induction and characterization of SU99 plasmacytoma cell line and its effects on mice immune response , Ph. D. Thesis, College of Science , AL-Nahrain University.
- **Acosta, A.; Ochninger,S.; Morshed,M; Swanson, R.; Scott ; R.; and Irianni ,F. (1988) .** Assisted reproduction in the diagnosis and treatment of the male factor . Obstet. and Gynecol . Surrey. , 44:1-17.
- **AL-Abid, M.R.(1985).** Zur zusammem setrung der Absschlu B memtrame in phoenix dactily frawuzbury univ. Wurzburg F.R.of Germany.
- **AL-Amiry, E.W.(1999)**. Enzymatic, cytogenetic , and drug resistance studies on blood from patients with breast cancer. M.Sc. Thesis, College of Education for Women , University of Baghdad .
- **AL-Fayadh, B.S. (1997).** Study of cytogenetic and enzymatic effects of water on mammalian cells .M.Sc. Thesis , College of Education for Woman , University of Bagdad.
- **AL-Janabi, A.M.(2004).** A study of some plant extract on lymphocytes of chronic myelogenous leukaemia patients. Ph.D.Thesis, Collage of Science, AL-Mustansiriya University.
- **AL-Jeboory, A. (1994).** Natural pharmacology , the future of medical plant in drug and medicine industry 1st ed . Baghdad . Dar AL-Huria press, (Arabic edition) .p.75
- **Allegra, C.J.(1990).** Antifolate, in Chamner, B.A; Collins, J.M.(eds):Cancer chemotherapy: Principles and practic, pp.110-153. Philadephia, Lippincott.
- **Allegra, C.J.; Hoang, K.; and Yeh, G.C.(1987).** Evidence for direct inhibition of de novo purine synthesis in human MCF-7 breast cancer cells as a principal mode of metabolic inhibition by methotrexate. J. Biol. Chem., 262:520-526.

References

- **Allen, J.W.; Shuler , C.F.; Mendes, R.W.; and Latt, S.A. (1977).** Asiplified technique for *in vivo* analysis of sister chromatic exchange using 5- bromodeoxy –uridine tablets . Cytogenetics ,18:231-237.
- **AL-Mohammed, N.T.; AL-Rawi, K.M .; Younis, M.A and AL-Morani, W.K.** (1986) .Principles of statistics . AL-Mosil University .
- **AL-Qadhi,D.J.K. (1997).**The cytogenetic effects of Gossypol on the albino mice (Mus musculus). M.Sc.Thesis, Collage of Education, (IBn-ALhatham), University of Baghdad .(In arabic)
- **AL-Rubaie,F.A.A.(2000).** Astudy of the mutagenic and anti mutagenic ability of some Iraqi medicinal plants in albino mice.M.Sc. Thesis ,College of Education, (Ibn-AL-hatham), University of Baghdad . (In arabic)
- **AL-Shamery, A.M.H. (2003) .** The study of Newcastle disease virus effect in the treatment of transplanted tumors in mices.M.Sc.Thesis, College of Veterinary medicine, University of Baghdad.
- **AL-Sudany, A.M.A.(2005).** Inhibitory effects of *Nigella sativa* oil and honey on the genotoxicity of tamoxifen in mice.M.Sc. Thesis,College of Science, AL-Nahrain University.
- **Arab, Y.M.; AL-Flaugy, S.N.; and Karmasha, F.N.(1989).** Anatomy and physiology , Ministry of High Education and Scientific Research , Baghdad University , AL-Hekma house .
- **Armstrong, M.J.; and Galloway, S.M.(1994).** Micronuclei induced in peripheral blood of mu-pIM-1 transgenic mice by chronic oral treatment with 2- Acetylaminofluorene or benzene but not with diethynitrosamine or 1,2 – Dichloroethane. Mutat . Res ., 302:61-70.
- **Ashby, J. (1986).** The prospects for a simplified and internationally harmonized approach to the detection of possible human carcinogens and mutagens. Mutagenesis, 1:3-16.

- **Atkin,N.B.; and Backer,N.C.(1979).**Nuclear protrusions in malignant tumours with large abnormal chromosomes:observations on –C banded preparation.Experientia,35:899-901.
- **Atlas,R.M.(1995).** Principles of microbiology.1st ed.,Robert J.Callanan (ed). Chapter 4,P.122.
- **Austin, C.R.; and Short, R.V. (1972).** Reproduction in mammals . Book 1: Germ cells and fertilization,1stedition,Cambridge University press.
- **Austin,C.R.; and Short,R.V.(1982).**Reproduction in mammals (book1):Germ cells and fertilization2ndedition.Cambridge University press.
- **Ayllon, F.; and Garcia-Vazque, E.(2000).**Induction of micronuclei and other nuclear abnormalities in European minnow *phoxinus phoxinus* and mollie *Poecilia latipinna*: an assessment of the fish micronucles test. Mutat .Res ., 467:177-186.

B

- **Baber, R.; Bligh, P.C; Fulcher, G.; Lieberman, D., Nery, L.;and Moreton, T.(1999).**The effect of an isoflavone dietary supplement (P-081) on serum lipids, forearm bone density and endometrial thickness in post menopausal women . Mononpause , 6:326.
- **Banerjee, S.; Sharma,R.; Kale, R.K.; and Rao, A.R.(1994) .** Influence of certain essential oils on carcinogen metabolizing enzymes and acid soluble sulphydryls in mouse liver . Nutr. Cancer, 21:263-269.
- **Barbera, G.; and Lorenzo, R.D. (1984).** The caper culture in Italy. Acta Horticulturae, 144.
- **Bearden, H.J.; and Fuquay, W.J. (1992).** Applied animal reproduction 3rd edition . Mississippi state University. Pp.139-140.
- **Becher,R.; Scmidt,C.G.; and Sandberg ,A.(1984).** SCE and cell cycle studies in leukemia .In sister chromatid exchange,25 years of experimental

research. Tice,R.R. ;and tolleander,A. (ed.),part B.New York,U.S.A.,pp:839-853.

- **Becker,C.; Barbulescu,K.; Hildner, K.; Meyer Zum Budschenfelde, K.H.; and Neurath, M.F.(1998).** Activation and methotrexate –mediated suppression of the TNF alpha promoter in T cells and macrophages . Ann.N.Y.Acad. Sci.,859:311-314.
- **Belcourt, M.F.; Hodnick ,W.F.; Rockwell , S.; and Sartorelli , A.C. (1996).** Differential toxicity of mitomycin C and porfiromycin to aerobic and hypoxic Chinese hamster ovary cells over expressing human NADPH: cytochrome p- 450 reductase .Proc. Natl. .Acad .Sci .USA , 93:456-460.
- **Beneke,E.S.; and Rogers,A.L.(1980).** Medicinal mycology manual,with human mycosis monograbh, 4th ed. Minneabolis-burgess Publishing Comp.,P:20-75.
- **Benveniste, P; Zhu ,W; and Cohen, A .(1995)**.Interference with thymcyte differentiation by an inhibitor of S-adenosylhomocysteine hydrolase .J.Immunol ., 155:536-544 .
- **Benveniste, P.; and Cohen, A . (1995).** P53 expression is required for thymocyte apoptosis induced by adenosine deaminase deficiency .Proc. Natl . Acad .Sci., 92:8373-8377.
- **Beresford, T. (1999) .** *Glycyrrhiza glabra* and treatment of respiratory disorders . Nature , 73:91-101.
- **Bergers, W.A.; and Link, G.M.(1980).**Toxic effects of glycoalkaloids solanine and tomatine on cultured neonatal rat heart cells . Toxicology Letters , 6 : 29-32.
- **Bergmeyer,H.U.(1974).**Methods of enzymatic analysis Bergmeyer. H.U.(ed). Vol I,6,Academic Press,New York.
- **Bertino, J.R.; Goker, E.; Gorlick, R.; Li, W.W.; and Banerjee, D.(1990).** Resistance mechanism to methotrexate in tumors. The Oncologist; 1:223-226.

- **Bianchi,N.; and lezana,E.A.(1976).** Kinetics of lymphocyte division in blood culture studies with the BUdR-Giemsa technique. Experimentia,32:1257-59.
- **Bishop, J.M. (1991).** Molecular themesin oncogensis. Cell, 64:235-243.
- **Blackburn, M.R.; Datta, S. K.; Kellems, R.E .(1998).** Adenosine deaminase-deficient mice generated using a two stage genetic engineering strategy exhibited a combined immunodeficiency . J . Biol. Chem . , 273:5093-5100.
- **Blijianski,M.(2002).**The anticancer agent PB-100 selectivity active malignant cell line inhibits multiplication of sixteen malignant cell lines ,even multidrug resistant .Genet.Mol.Biol.,23:224-235.
- **Bonina,F.; Puglia,C.; Ventura, D.; Aquino, R.; Tortora, S.; Sacchi, A.; Saija .A;Tomaino,A.;Pellegrino, M.L.; and deCaprariis, P.(2002) .** *In vitro* antioxidant and *in vivo* photoprotective effects of a lyophilized extract of *Capparis spinosa* L. buds .Journal of Cosmet .SCi ., 53:321-335.
- **Bonnefoi, M.; Hasim ,M.;Sauvagnac ,P.; Burgat, V.; and Braun, J.P. (1989) .** Liver enzyme changes in a Guinea-pig model of facial eczema (sporidesmiotoxicosis) . Enzyme , 42:39-46.
- **Bonnesenn,C.; Eggelston ,I.;and Hayes,J.(2001).** Dietary indoles and isothiocyanates that are generated from cruciferous vegetables can both stimulate apoptosis and confer protection against DNA damage in human colon cell lines .Cancer Res., 61:6120-6130.
- **Borberg ,H.; Yenser,Y.; Gesner, B.; and Silber, R .(1968)**. The effect of N-acetyle O-Galactosamin and other suger on mitogenic activity and attachment of PHA to tonsil cells. Blood, 31:747.
- **Boik.(1996).** Cancer and natural medicine:A textbook of Basic Science and Clinical Research (Princeton, NN:Oregon Medical Press),105

- **Boyd , M.R. (1998).** The NCI *In vitro* anti cancer drug discovery screen from: Anticancer development guide: preclinical screening, Clinical trialls and approval .B. Tricher Humana press Inc. Totowa , N.J.. U.S.A.
- **Bracke,M.E.; Depypere,H.T.; Boterberg,T.; Van Marck,V.L.; Vennekens, K.M.; Vanluchene,E.; Nuytinck,M.; Serreyn,R.;and Mareel, M.M.(1999).** Influence of tangeretin on tamoxifen´s therapeutic benefit in mammary cancer.J.Natl.Cancer Inst.,91:354-359.
- **Bradford,M.(1976).** A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein –dye binding . Anal. Biochem .,72:248-254.
- **Bradley, P.R. (1992) .** Red clover . In :British Herbal Compendium. Vol1 . Bournemouth , UK : British Herbal Medicine Association;183- 184.
- **Brannwald, D.E.; Isselbacher, K.; Petersdorf, R.G.; Wilson, J.; Martin, J.B.; and Fausi, A.S. (1987) .** Harrisons principles of internal medicine . 11th ed . McGraw Hill Company .U.S.A.
- **Brody, M.; Bohm ,I.; and Bauer,R.(1993).** Mechanism of action of methotrexate :experimental evidence that methotrexate blocks the binding of interleukin 1 beta to the interleukin 1 receptor on target cells .Eur. J.Clin .Biochem ., 31:667-674.
- **Brown, J.S; and Marcy, S.A.(1991).**The use of botanicals for health purposes by members of a prepaid health plan. Res. Nurs. Health,14:339-350.

C

- **Cappuccino,J.; and Shermany,N.(1987).**Microbiology a laboratory manual .2nd ed.Benjamin Cummiimgs Publiching,Inc.California,B.53-171.
- **Carrano,A.V.(1973).**Chromosome aberrations and radiation induced cell death:II. Predicted and observed cell survival .Mutat.Res.,17:355-366.
- **Carrano,A.V.; Thompson, L.H.; Stetka, D.G.; Mazirmal, G.A.; and**

Fong, S. (1979). DNA cross linking SCE and specific – locus mutation, Mutat. Res., 63:175.

- **Carrano,V.A.(1986).** Chromosomal alterations as markers of exposure and effect . J. of Occupational Medicine, 28:1112-1116.
- **Carter, S.K.; and Livingston, R.B.(1982).** Drugs available to treat cancer In:Carter, S.; Glatstein, E; and Livingston , R.B (Eds) principles of cancer treatment. McGraw-Hill, NewYork, pp.111-145.
- **Cassileth, B.R.(1989).** The social implications for questionable cancer therapies. Cancer, 63:1247-1250.
- **Catalavo, A.B.(2002).** White blood cell count with differential, in George –Gay, B. and Chernecky (eds). Clinical medical Nursing Sounders, pp.282-290.
- **Chang,T.H.; Kim,J.C.; Kim,M.K.; Choi,S.C.; Kim,S.L.; and Chung, J.M. (1995).** Investigation of Korean plant extracts for potential phytotherapeutic agents against B-virus Hepatitis. Phytotherapy Research, 9:429-434.
- **Charles,E.O.(2003).**Diagnostic serum enzymes.Virtual ChemBook, Elnhmrst College.
- **Chatterjee, R.; Haines, G.A.; Perera, D.M.; Goldston, A.; and Morris, I.D.(2000).** Testicular and sperm DNA damage after treatment with fludarabine for chronic lymphocytic leukemia.Hum. Reprod.,15:762-766.
- **Chew,B.P.(1995).**Antioxidant vitamins affect food animal immunity and health .J.Nutr ., 125 : 1804-1808.
- **Chlebowski,R.T.; Akman,S.A.; and Block,K.B.(1985).**Vitamin K in the treatment of cancer .Cancer Treat Rev.,12:49-63.
- **Chlebowski,R.T; Dietrich,M.; Akman,S.; and Block,J.B. (1985).**Vitamin K3 inhibition of malignant murine cell growth and human tumor colony formation.Cancer Treat Rev.,69:527-532.

- **Chu ,D.; Sun,Y.; Lin, J.; Wong , W.; and Mavligit, G. (1990) .** F3 , afractionated extract of *Astragalus membranaceus* , potenta by low. dose recombinant interleukin-2. Chung Hsil Chieh Ho Tsa Chih , 10: 34-6 .
- **Clark, S.C.; and Kamen, R.(1987).** The human hematopoietic colony stimulating factors. Science ,236: 1229-1237.
- **Clermont, Y.(1970).**Dynamics of human spermatogenesis in the human testis Rosemberg , E.; and Panlsen, C.A.(eds). New York, London : Plenum Press .pp. 47-61.
- **Cooper, M.R.; and Johnson, M.N.(1984).** Poisonous plants in Britain and other effects on animals and man Ministry of Agriculture,Fisheries and Food, London. 219-220.
- **Cordell,G.A.(1995).**Changing strategies in natural products chemistry. Phytochemistry, 40:1585-1612.
- **Cordell,G.A.; Beecher,C.W.; and Pezzento,J.M.(1991).**Can Ethanopharmacology contribute to the development of new anticancer drugs? J.Ethnopharmacol., 32:117-133.
- **Cornforth, M.N.; and Bedford,J.S.(1993).** Ionizing radiation damage and its early development in chromosomes. In Advances in Radiation Biology,17:423-496.
- **Costes,V.P.; Mostter, M.R. ; and Wilson, R. P. (1993).**Effects to topical mitomycin – C on primary trabeculecto – mies and combined procedures . Br . J . Ophthalmology , 77:693-697.
- **Cowan, M.M .(1999).**Plant product as antimicrobial agent. Clin. Microbiol.,12:564-582.
- **Cragg, G.M.; Newman, D.J.; and Snader, K.M.(1997).** Natural products in drug discovery and development .J. Nat .Prod . 60:52-60 .
- **Curley,B.J.; Gardner,S.F.; and Hubbard,M.A.(2000).** Content versus label claims in ephedra-containing dietary supplements. Am.J.Health.Sys.Pharma.57:963-969.

- **Cutolo,M.; Sulli,A; Pizzorni,C.; Seriolo,B.; and Straub,R.H. (2001).**Anti-inflammatory mechanisms of Methotrexate in rheumatoid arthritis.Ann.Rheum.Dis.,60:729-35.

D

- **D´ Mello,D.; Mehto, D.; Pereira, J.; and Rao, C.V.(1999).** A toxicity study of simultaneous administration of tamaxifen and Diazepam to female Wistar rats . Exp . Toxicol . Pathol ., 51:549-553.
- **Danova,M.; Riccardi,A.; Gaeyani,P.; Wilson,G.D.; Mazzini,G.; and Brugantelli, S. (1988).** Cell kinetics of human brain tumors in vivo study with bromo-deoxyuridine and flow cytometry.Eur.J.Cancer Clin.Oncol.,24:873-880.
- **David,G.;Jonas,B.;Mattias,H.;Fredrick,M.;Paola,D,C;Mans,A.; and Nils,M. (2001).** Abnormal nuclear shape in soild tumors reflects mitotic instability.American Journal of Pathology,158:199-206.
- **Davis,W.L.; Matthew,S.; and Brignall,N.D.(2000).**For more information on antioxidant and cancer.Alter.Med.Rev.,5:152-163.
- **Deen, D.D.; Morgan, W.F.; Tofilon, P.J.; and Barcelloschoff, M.H. (1989).** Measurement of Sister chromatid exchange and their relationship to DNA damage , repair and cell killing. Pharmacol. Ther., 42:349-360.
- **Deen,D.F.; Kwndall,L.E.; Marton,L.J: and Tofilon,P,J.(1986).** Prediction of human tumor cell chemosensitivity using the sister chromatid exchange assay.Cancer Res.,46:1599-1602.
- **Deflora, S.; and Ramel, C.(1988).**Mechanisms of mutagenesis and carcinogenesis, classification and over view. Mutat. Res., 202:285-306.
- **Dermarderosian,A.; Burnham, T.H.; and Short, R.M .(2000).** Red clover monograph , amongraph in the series the review of natural products .St.louis , Mo: Facts and Comparisons .
- **Devita,V.T; Hellman, S.; and Rosenberg,S.A.(1993).**

Cancer:Principles and practice of oncology. Chapter 1-4th. Ed.Philadelphia:Lippincott.

- **Dimolfett, L; Ericleson,K.L.; Channel, M.; Jeffrics, S.J; and Harvey, J.T.(1995)**. Blastogenesis and interleukin -2 recepter expression assay in Harborscal . J. of Wild Llife Disease , 31:150-158.
- **DqWu; and Wu, Y.(1995).** Micronucleus and sister chromatid exchange frequency in endemic fluorosis .J. of the International Society for fluoride Research, 28:125-127.
- **Dufrian,R.J.(1974).** Probing sister chromatid exchange formation with halogenated pyrimidines .In sister chromatid exchang ,25 years of experimental research. Tice,R.R. ;and tolleander,A. (ed.),plenum press, New York and London.
- **Duke, J.A. (1985).** *Rumex crispus* L. In handbook, of medicinal herbs. CRC press, Boca Raton, FL, pp.414-415.
- **Dutkowski, R.T.; Kevin, M.J.; and Jenkins, S.C.(1983).** The effect of oral contraceptives on SCE, blast transformation , mitotic index and micronuclei formation, Exp. Cell Biol., 51:115.

E

- **Evans, E.P.; Breckon, G.; and Ford, C.E.(1964).**An air drying method for meiotic preparation from mammalian testes. Cytogenetics , 3: 284-294.
- **Evans, W.C.(1999).** Trease and Evans pharmacognosy 4th ed ., Saunders , W.B(ed.) Saunders company Ltd . UK. PP:612.
- **Evans, H.J.; and Viagalaxmi, A.(1981).** Induction of 8-azaguanine resistance and sister chromatid exchange in human lymphocytes exposed to mitomycin C and X-ray *in vitro*. Nature, 292:601-695.

- **Eylar , E.; Bjaez, I.; Navas, J.; and Mercado, C. (1996).** Sustained levels of ascorbic acide are toxic and immunosuppressive for human T cells . Health Sci . J. , 15:21-6.

F

- **Fairbairn,J.W.; and Muhtadi,F.J.(1972).** Chemotaxonomy of anthraquinones in Rumex. Phytochemistry, 11:263-268.
- **Farnsworth,N.R.; and Morris,R.W.(1976).** Higher plants : The sleeping giant of drug development. Am. J Pharm ., 4:46.
- **Folkman,J.(2000).**Tumor angiogenesis In: Bast,R.C; Kufe, D.W.;Pollock,R.F.;Weichselbom,R.R.;Holland,J.F.;Ferri,E.;and Ganster, T.E(eds.).Cancer Medicine (5 th ed).BC.Decher Inc.Canada.
- **Foster, S.; and Tyler, Y.E.(1999).** Tyler's honest herbal .4th ed. Binghamton , New York
- **Foster, S.; and Duke, J.A.(1990).** Sheep Sorrel in medicinal plants. Houghton Mifflin co., New York, NY. P.214.
- **Fozard,J.R .; Pfannkuche, H.J.;and Schuurman , H.J. (1996).** Mast cell degranulation following a denosine A3 receptor activation in rats. Eur . J . Pharmacol.,298:293-297.
- **Francis, A.R.; Shetty, T.K.; and Bhattacharya, R.K.(1989).** Modifying role of dietary factors on the mutagenesity of aflatoxin Bl : *In vitro* effect of plant flavonoids . Mutat . Res ., 222: 393-401.
- **Frederiksen, S.(1966).**Specificty of adenosine deaminase toward adenosine and 2- deoxyadenosine analogues . Arch .Biochem., 113 :383-388.
- **Freshney, R.I.(2000).** Culture of animal cells :A manual for basic technique (4th ed) .Wiley –liss, Ajohnwiley and sons , Inc. publication , New York .

- **Freshney, R.I.(2001).** Application of cell culture to toxicology .Cell Boil .Toxical., 17:213-230
- **Frohne, D.; and Pfander, H.S (1983)**. A colour atlas of poisonous plants . Wolfe puld .LTd ., London .

G

- **Galloway, S.M.; Aardema, M.J. ;Ishidate, M.; Ivett , J L.; Kirkland, D.J.; Morita, T.; Mosesso, P.; and Sofunl, T.(1994).** Report from working group on *in vitro* tests for chromosomal aberrations. Mutat. Res. ,312:241-261.
- **Galloway,S.M.; Armstrong, M .J.; Reuben ,C.; Colman , S.;Brown , B.; Cannon, C.; Bloom, A.D.; Nakamura, F.; Ahmed, M.; Duk, S.; Rimpo, J.; Margolin, G.H.; Resnick,M.A.; Andarson , G.; and Zeiger, E.(1987).**Chromosome aberration and sister chromatide exchanges in Chinese hamster ovary cells :Evaluation of 108 chemicals. Environ . Molec . Mutagen . 10 :1-175.
- **Ganapathi, N.G.(1991).** Treatment of mice with a herbal preparation (Liv.52) reduces the frequency of radiation induced chromosomal damage in bone marrow. Mutat .Res ., 253 :123-126.
- **Ganong,W.F.(1991).** Review of medical physiology .Connecticut: Appleton and lauge , 401:94-95.
- **Gebhart,E.(1992).** Anticlastogenicity in cultures mammalian cells . Mutat.Res ., 267:211-220.
- **Gebhart,E.; Wagner, H.; and Behnsen, H .(1984).** The action of anti clastogens in human lymphocytes cultures and its modification by rat liver S9 mix.I.studies with AET and sodium fluoride. Mutal. Res.,129:195-206.
- **Genestier, L.; Paillot,R.; Fournel , S.; Ferraro ,C.; and Revillared , J.P.(1998).**Immunosuppressive properties of MTX:apoptosis and clonal deletion of activated peripheral T cells. J. Clin. Invest .,102:322-328.

- **Gey,K.F.(1998).** Vitamins E plus C and interacting conutrients required for optimal health . A critical and constructive review of epidemiology and supplementation data regarding cardiovascular disease and cancar . Biofactors , 7 :113 -74.
- **Ghaskabdi, S; Rajmachikar, S.; Agate,C.; Kapadi,A.H.; and Vaidya,V.G.(1992).**Modulation of cyclophosphamide mutagenicity by vitamin C in the *in vivo* rodent micronucleus assay .Teratogensis Carcinogenesis and Mutagenesis , 12:11-17.
- **Gill,G.V.; Redmond, S.; Garratt, F.; and Paisey, R.(1994)**. Diabetes and alternative medicine cause for concern. Diabet. Med., 11:210-213.
- **Giusti, G.(1981).**A denosine deaminase. In:Bergmeyer , H.V. methods of enzymatic analysis . 2^{ed} . Academic press.Vol 2 . pp.1092-1099.
- **Gonsebatt,M.E.; Vega,L.; Montero,R.; Garcia-Vargas,G.; DeL Razo, L.M.;Albores,A.; CebrianM.E.; and Ostrosky-Wegman,P. (1994).** Lymphocyte replicating ability in individuals exposed to arsenic via drinking water. Mutat.Res.,313:293-9.
- **Grafone,T. ; Marhinelli, C .; Gianning , B.;Terragna,C.; Soverini, S.;Amabile,M.; Bianchine,M.; Ferrari,S.; Palnerini, E.; and Baccarrani , M.(2003) .** *In vitro* study with FTIs in CML ph-positive cell lines .Proc . Am . Soc . Clin . Oncol.,22:902-908.
- **Greaves ,M.F.;Janossy , G .;and Doenhoff, M.(1974).** Activation of human T-lymphocyte by polyclonal mitogens . Nature , 248:648 .
- **Grieve,M.(1995).**Nightshade.,Black.Amodern herbal. Botanicial.com .

H

- **Hagelstrom,A.H.;Gorla,N.B.;and Larripa,I.B.(1995).** Chromosomal damage in workers occupationally exposed to chronic low level ionizing radiation . Toxicol . Lett., 76:113-7.

- **Handa , S.S ; Mundkinajedu, D. ;and Mangal , A.K. (1998) .** Indian herbal pharmacopoeia (volume I) . Indian Drug Manufactures Association and Regional Research Laboratory , Mumbai. , P. 50.
- **Haq,A.; Abdullatif , M.; Lobo, P.; Khabar, K.; Sheth ,K.; and AL-Sedairy, S.(1995).** *Nigella sativa* effect on human lymphocytes and PMN phagocytic activity . Immunopharmacology , 30: 147-155.
- **Harborne, J.B.(1973).** phytochemical methods. Science paper Backs , Chapman and Hall , New York .
- **Hardin,J.N; and Arena,J.M.(1974).**Humam poisoning from native and cultivated plants.2nded. DukeUniversity press,Durham, North Carolina.
- **Hassan, M.A.K. (2002).** The use of some plant extracts for inhibition of genotoxic effects for some anticancer drugs in mouse .Ph.D Thesis , College of Sciences, Babylon university .(In arabic)
- **Heddle,J.A.(1973).** A rapid *in vivo* test for chromosomal damage. Mutat. Res., 18:187-190.
- **Heddle, J.A.; Salamone, M.F.; Hite, M.; Kirkhart, B.; Mavournin, K.; MacGregor, J.G.; and Newell, G.W.(1983).** The induction of micronuclei as a measure of genotoxicity. Mutat .Res., 123:61-118.
- **Hershfield, M.S; Kredich,N.M.; Ownby, H.;and Buckley, R.(1979).** *In vivo* inactivation of erythrocyte S-adenosylhomocysteine hydrolase by 2`- dexyadenosine in adenosine deaminase –dificient patient. J. Clin . Invest ., 63: 807-811.
- **Hirschhorn,R.; Paageorgiou,P.S.; Kesarwala,H.H; and Taft,L.T. (1980)**.Amelioration of neurologic abnormalities after "enzyme replacement" in adenosine deaminase deficiency.N.Engl.J.Med.,303:377-380.
- **Holden,S.A.; Teicher,B.A.; Robinson,M.F., Northey,D.; and Rosowsky,A.(1995).** Antifolates can potentiate topoisomerase II inhibitors *in vitro* and *in vivo* . Cancer Chemother .Pharmacol ., 36:165-171.

- **Holland,D.F.(2005).** Chromosome analysis : Banding patterns and structural aberrations. The University of Texas Southwestern Medical Center at Dallas.
- **Hollman , P.C.; and Katan ,M.B.(1999).** Dietary flavonoids : intake , health effects and bioavailability . Food Chem . Toxicol., 37:937- 942.
- **Hollman , P.C.; and Katan, M.P. (1997).** Absorption , metabolism and health effects of dietary flavonoids in man . Biomed . Pharmacother ., 51:305-310.
- **Hort,J. (1983).** Germination of caper (*Capparis spinosa* L.) seeds. Sci., 58:262-270.
- **Hose, J.E.; Cross, J.N. ; Smith, S.G.; and Diehl, D. (1987).** Elevated circulating erythrocyte micronuclei in fishes from contaminated of southern California . Mar. Environ .Res ., 22:167-176.
- **Howes,J.B.; Sullivan, D; and Lai ,N .(2000) .** The effects of dietary supplementation with isoflavones from red clover on the lipoprotein profiles of postmenopausal woman with mild to moderate hypercholesterolemia . Atherosclerosis , 152:143-147.
- **Huang , S.; Apasov, S.; Koshiba, M.; and Sitkovsky,M.(1997).** Role of A2a extracellular adenosine receptor mediated signaling in adenosine mediated inhibition of T-cell activation and expansion. Blood.90:1600-1610.
- **Huang,S.; Aasov,S.; Kashiba,M.; and Sitkovsky,M.(1997).**Auto antibodies to ubiquitous intracellular antigen interact with living cells.The immunologist .612:71-75.
- **Hungerford,D.A.(1965).**Leukocytes cultured from small inocula of whole blood and the preperation of metaphase chromosomes by treatment with hypotonic KCL.Stain Tech.,40:333-338.
- **Hudson,I.; and Hay,F.(1989) .** Practical Immunology section 1 ; Black well scientific publication .pp. 116.

- **Huennekens, F.M. (1994).** The methotrexate story: A paradigm for development of cancer chemotherapeutic agents. Adv. Enzyme. Regul., 34:397-419.
- **Husband , A. (2001) .** Red clover isoflavone supplements : safety and pharmacokinetics . Journal of the British menopause Society , 1: 4-7 .

J

- **Jialal,I.(1993) .** The effect of antioxidant dietary micrantrients on LDL oxidation :Implications for atherosclerosis prevention. Canadian Journal of Cardiology, 9:11-13 .
- **Juan,C.C.; and Wu,F.Y.(1993).**Vitamin K3 inhibits growth of human hepatoma HepG2 cells by deacreasing activities of both P34CDC2 kinase and phosphatase. Biochem. Biophys. Res.Commun., 190:907-913.
- **Juma,A.S; Shubber,E.K.; and Al-Jeboori,T.I.(1999).** Cytogenetic analysis of bone marrow and spleen cells of mice expermintally infected with secondary hydatid disease.Cited by Juma,A.S.(1999 a).

K

- **Kasahara,Y.; Nakai,D.; Miura,K.; Yagi, K.; and Hirabayashi,T. (1992).** Mechanism of induction of micronuclei and chromosome a berrations in mouse bone marrow by multiple treatments of methotrexate. Mutat .Res ., 280:117-128.
- **Keeler,R.F.(1987).**Alkaloid teratogens from lupines, conium, veratrum and related genera .Lu: Effects of poisonous on livestock. Keeler,R.F; Van Kampen,K.R., James,L.F.(eds).Academic press New York and London , pp. 397-408.
- **Keeler, R.F; and Tu, A.T.(1983).** Handbook of Nortural Toxins.Vol., Plants and Fungal Toxins .New York , pp.176.
- **Kellof,G.; Sigman,C.; and Greenwald,P.(1999).** Cancer chemoprevention : Progress and promise .Eur.J.cancer.35:1755-1762.

- **Ketterer,B.(1988).** Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis;Mutat.Res., 202 :343-361.
- **Khan, B.A; Abraham,A.; and Leelamma, S.(1995).** Haematological and histological studies after carry leaf (*Murraya* koenigi) and mustard (*Brassica juncea*) feeding in rats . Indian J. Med . Res ., 102:184-186 .
- **King, M.T.; Wild,D.; Gocke,E.; and Eckhardt,K.(1982).**5-Bromodeoxy uridin tablets with improved depot effect for analysis *in vivo* of SCE_s in bone marrow and spermatogonial cells.Mutat.Res., 97:117-129.
- **Knight,D.; Zheng,X.; Rocchini,C.; Jacobson,M.; Bai,T.; and Walker,B.(1997)**. Adenosine A3 receptor stimulation inhibits migration of human eosinophils. J.Leukoc . Biol., 62:465-468.
- **Kola,I; Vogel,R.; and Spielmann,H.(1989).**Coadministration of ascorbic acid with cyclophosphamide (CPA) to pregnant mice inhibits the clastogenic activity of CPA in preimplantation murine blastocyst. Mutagenesis , 4:297-301.
- **Kram,D.(1982).** In utero analysis of sister chromatid exchange in: Sandberg,A.A (ed.).Sister Chromatid Exchange.Liss, New York,pp.205

Kubbies,M.; Schindler,D.; Hoehn,H.; and Rabinovitch,P.S.(1985).
Cell cycle kinetics by BUdR-hoechst flow cytometry:Analternative to the differential metaphase labeling technique.Cell Tissue kinetics,18:551-562.

- **Kuhn;M.N.; and Winston, D.(2001).** Herbal therapy and supplement .philadelphia , pa ,Lippincott :273 -277.
- **Kumar,N.B.;Hopkins,K.; and Allen,K.(2002).**Use of complementary inte-grative nutritional therapies during cancer treatment implications in clinical practice. Cancer Control ,9:236-243.
- **Kusumoto,I.T.; Nakabayashi,T.; Kida,H.;and Miyashiro,H. (1995).** Screening of various plant extracts used in a yurvedic medicine for inhibitory effects on human immunodeficiency virus type (HIV-1) protease.Phtotherapy Research,9:180-184.

ℒ

- **Lamberti,L.; Ponzetto,P.B.; and Ardito,G.(1983).**Cell kinetics and sister chromatid exchange frequency in human lymphocytes. Mutat. Res.,120:193-99.
- **Lamson,D.W.; and Plaza,S.M.(2003).**The anticancer effects of vitamin K. Alter. Med. Rev.,8:303-18.
- **Latt,S.; Allen,S.; Bloom,S.; Carrano,A.; Falke,F.; Kram, Schneider,E.; Scherch,R..; Tice,R.; Whitefield,B.; and Wolff,S. (1981).** Sister chromatid exchanges: A report of the Gene-Tox program . Mutat. Res., 87:17-62.
- **Latt,S.A. (1981).** Sister chromatid exchanges formation. Ann. Rev. Genet., 15:11-55.
- **La Vecchia,C.; and Tavani,A.(1998).** Fruit and vegetables and human cancer. Europ. J. Cancer Prev., 7:3-8.
- **Lengauer,C. ; Kinzler,K.W.; and Vogelstein,B.(1998).** Genetic instabilities in human cancers. Nature ,396:643-649.
- **Lewis,W.H.; Vinay,P.; and Zinger,V.E.(1983).** Airborne and allergenic Pollen of North America. Baltimore, MD: Jhons Hopkins University press, 167-170.
- **Li,J.C.; and Kaminskas , E. (1984).** Accumlation of DNA strand breaks and methotrexate cytotoxicity .proc . Natl. Acad . Sci ., U.S.A. 81:5694-5698.
- **Lialiaris,T.; Mourelators,D.; and Dozi-Vassiliades,J.(1988).** Enhancement of cytogenetic damage by chlorpromazine in human lymphocytes treated with alkylating antineoplastics and caffeine. Mutat.Res.,206:361-365.

- **Lippert,M.C.; McClain,R.; and Boyd,J.C.(1999).** Alternative medicine use in patients with localized prostate carcinoma treated with curative intent. Cancer .86:2642-2648.
- **Littlefield,L.; Colyer,S.; and Dufrain,R.(1980).** Comparison of SCE in human lymphocyte after exposure to MMC *in vitro,in vivo* Mutat. Res. , 67:191-195.
- **Liu,X.; Kim,C.N.; Yang, J.; Jemmerrson,R; and Wang, X. (1996).** Induction of apoptotic program in cell –free extract : requirement for dATP and Cytochrome C . Cell , 86: 147-157.
- **Liu Weijun .(1992).**Improvement of micronucleus test method in peripheral blood lymphocytes and preliminary observation in clinical use.Chinese Journal of Medical Testing.15:233-234.
- **Loeb,L.A.(1998).** Cancer cells exhibit a mutator phenotype. Adv. Cancer Res., 72:25-56.
- **Loeico,A.; Boiocchi, M.R.; Rappa,G.; Sen,S.; Erba, E.; and Incalci , M . (1990) .**Increase in topoisomerase II –mediated DNA breasks and cytotoxicity of VP16 in human U937 lymphoma cells pretreated with low doses of methotrexate. Int . J. Cancer , 45:156-162.
- **Loric,A.; Toffoli,G.; Boiocchi,M.; Erba,E.; Broggini,M.; Rappa, G.; and D'Incalci,M.(1988).** Early DNA damage induced in cells exposed to N10-propargyl 5,8-dieazofolic acid (CB3717) or methotrexate. Biochem . pharmacol ., 37:1875-1876.

M

- **Madrigal –Bujaidar, E.; and Diaz –Barriga,S.(1995).** Inhibitary effect of vitamin C against the frequency of sister chromatid exchanges produced by mitomycin C *in vitro*. J. Med.Sci. Res., 23: 63-64.

- **Mathur,V.; and Devi,P.(1981)**.Effect of MPG on the radiation induced changes in the intestinal activity of ALP in mice. Natl. Acad.Sic.Letters,4:183-185.
- **Mavournin,K.H.; Blakey,D.H.; Cimino, M.C.; Salamone, M.F.; and Heddle, J.A.(1990).** The *in vivo* micronucleus assay in mammalian bone marrow and peripheral blood. Mutat .Res., 239:29-80.
- **Melanie Nathen.(2002).** Herbs for cancer prevention. http://WWW. Shareguid.Com.
- **Michael,R.B; Jonathan,B.V; Janci,LT.; Hongyan,Z.; Jeff,R.C; James,J.L; and Rodney,E.K.(2000).** Metabolic consequences of adenosine deaminase deficiency in mice are associated with defects in alveogenesis, pulmonary inflammation ,and airway obstruction. J.of Experimental Medicine,192:159-170.
- **Mills,J.A.(1966).**The immunological significance of antigen induced lymphocytes transformation *in vitro* J.Immunol .,79 :239.
- **Miski,M.; Ulubele,A.; and Mabry,T.J. (1983).**6-Hydroxy flavones from *Thymbra spicata* .phytochemistry , 22 : 2093 -2094 .
- **Mollinedo,F.; and Gajate,C.(2003).** Microtubules, microtubule-interfering agents and apoptosis. Apoptosis, 8:413-450.
- **Moore,A.E.; Sabachewesky,L; and Toolen,H.W.(1955).** Culture characteristics of four permanent lines of human cancer cells, Cancer Res ., 15 : 598-605.
- **Morgan,S.L.; Bagott,J.E.; and Vaughn,W.H. (1994).** Supplementation with folic acid during methotrexate therapy for rheumatoid arthritis. A double-blind, placebo-controlled trial. Ann. Intern. Med.; 121:833-41.
- **Morris,K.T.; Johnson,N.; and Homer,L.(2000).** A comparison of complementry therapy use between breast cancer patients and patients with other primary tumor sites. Am.J.Surg.,179:407-411.

- **Morris,S.M.; and Heflich,R.H.(1984).**A comparison of the toxic and SCE-inducing effects of inhibitors of ADP-ribosyl transferase in chinese hamster ovary cells.Mutat.Res.,126-71.
- **Moteki,H.; Hibasmai,H.; Yamada,Y.; Katsuzaki,H.; Imai,K; and Komiya,T.(2002).**Specific induction of apoptosis 1,8-cineole in two human leukemia cell line ,but not in a human stomach cancer cell lines. Oncology Reports,9:757-760.
- **Mowrey,D.(1986).** The scientific validation of herbal medicine keats publishing, U.S.A.
- **Mukhopadhyay,M.J. ; and Muhherjee,A.(2000).** Clastogenic effect of *Ginger rhizome* in mice. Phytochemistry,14:555-557.
- **Murad,T.S.H.(2005).** Some physiological and immunological effect of tamoxifen on breast cancer patients and Albino femal rat(*Rattas norvegicus*).Ph.D. Thesis, College of Education, (Ibn-AL-hatham), University of Baghdad .
- **Murthy,P.B.; and Prima,K.(1983).** Further studies on SCE frequency in users of hormonal contraceptives, Mutat. Res. , 119:351.

N

- **Nachtigall, L.E. (2001).** Isoflavone in the management of menopause . Journal of the British menopause Society , 1:8-12 .
- **Nakanishi,Y.**; and **Schneider,E.L.(1979).** *In vivo* sister chromatid exchange: a sensitive measure of DNA damage. Mutat.Res.,60:329-337.
- **Nakatsuru,S.; Oohashi,J.; Nozaki,H.; Nakada,S.; and Imura,N. (1985).** Effect of mercurials on lymphocyte functions "*in vitro*".
- **Nara,N; and McCulloch,E.(1985).** The proliferation in suspension of the progenitors of the blast cells in a cute myeloblastic leukemia . Blood, 65:1484-1492.

- **National Center for Complementary and Alternative Medicine. (2005).** National Institutes of Health;Office of Dietary supplements.A vailable at:http://ods.od.gov/ factsheets /Botanical Backgrounds.
- **National Committee for Clinical Laboratory Standards (NCCLs). (2000).** Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard,5th ed .NCCLs documented M7-A5 .Wayna
- **Nichols,W.W; Bradt,C.I.; Toji,L.H.; Godley,M.; And Sigawa,M. (1978).** Induction of SCE by transformation with simian virus 40(SV-40). Cancer Res., 38:906-910.
- **Niering,W.A.; and Olmstead,N.C.(1988).** The Audubon society field guide to North America wildflowers. Eastern region. NewYork, NY: Alfred A. Knopf, 704-709.
- **Nowell,P.C.(1960).**Phytohemagglutinin in initation of mitosis in culture of normal human leukocytes .Cancer Res., 20:462

O

- **Ohta,T.; Watanabe,K.; Moriya,M.; Shirasu,Y.; and Kada,T. (1983) .** Anti –mutagenic effects of coumarin and umbelliferone on mutagenesis induced by 4-nitroquinolin 1-oxide or UV irradiation in E.coli. Mutat . Res ., 117:135-138 .
- **Okabe,M; Asano,M.; Kuga ,T.; Komatsu,Y.; Yamasaki ,M; Yokoo , Y.; Itoh,S.; Morimoto,M.; and Oka, T. (1990) .** *In vitro* and *in vivo* hematopoietic effect of mutant human granulocyte colony stimulating factor . Blood , 75: 1788-1793.
- **Olah,M.E.; and Stiles,G.L.(1995).** Adenosine receptor subtypes : characterization and therapeutic regulation .Annu. Rev.pharmacol.toxicol ., 35:581-606.
- **Otter,M.; Palmer,G.G.; and Bachner, R.L.(1979).** Sister chromatid

exchange in lymphocytes from patients with acute lymphoblastic leukemia. Hum. Genet., 52:185-192.

P

- **Palmer,C.G.(1970).**5-Bromodeoxyuridine induced constriction in human chromosome. Can.J.Genet.Cytol.12:816-830.
- **Parker,K.F.(1972).** An illustrated guide to Arizona weeds. Tucson, AZ: University of Arizona ; 86-93.
- **Patrick,Q.(2002).**The powerof nutritional synergism.Immunopower,8.
- **Patton,J.L.(1967).**Chromosome studies of certain pocket genus perogenathus (Rodentia :Heteromyidae) .J.Mamm ., 48 :27-37.
- **Phuangsab,A.; Lorence,R.M.; Reichard,K.W.; Peeples,M.E; and Walters,R.J.(2001).** Newcastle disease virus therapy of human tumor zenograftsi intitumor effects of local or systemic administration. Cancer Letters, 172:72-36.
- **Popescu,N.C.; and Dipaolo.(1982).** The relevance of SCE to the induction of neoplastic cell transformation, in : Sandberg, A.A. (ed)., Sister Chromatid Exchange . Liss, New York, pp.425-460.
- **Preston, R.J.(1997).** Short / medium term carcinogenicity tests and genetic and related effects. IARC Metting-Lyon.
- **Puck,T.T.; Johnson,R.; Webb,P.; Cui,H; Valdez,J.G; and Crissman,H. (2002).** Mutagenesis and repair by low doses of ά radiation mammalian cells.Proc.Natl.Acad.Sci.U.S.A.,99:12220-12223.

Q

- **Qin,G.; Gopalan,P. ; Su,J.; Ning,Y; and Lotliker,P.(1997).** Inhibition of aflatoxin B1-induced inhibition of hepatocarcinogenesis in the rats by green tea.Cancer Lett.,112:149-159.

- **Qiusheng,Z.; Xiling,S.; Meng,S.; and Changhai,W.(2004).** Protective effects of luteolin -7- glucoside against liver injury caused by carbon tetrachloride in rats . Pharmazie , 59:286-9 .

ℛ

- **Raghu,C.; Ashok,G.; Dhanaraj,S.A.; Suresh,B.; and Vijayan, P.(2004).***In vitro* cytotoxic activity of Lanatana camara Linn.Indian Journal of pharmacology ,36:94-95.
- **Ratech,H.; Greco,M.A.; Gallo,G.; Rimoin,D.L.; Kamino,H.; and Hirschhorn,R.(1985)**. Pathologic findings in adenosine deaminase-deficient sever combined immunodeficiency . I. Kidney , adrenal and chondroosseous tissue alterations . Am . J . Pathol . 120: 157-169 .
- **Rauha,J.P. ; Remes ,S.; Heinonen , M.; Hopia ,A.;and Kahkonen , M.; and Kujala,T.(2000)**. Antimicrobial effects of finnish extracts containing flavonoids and other phenolic compounds. Int. J. Food Microbiology, 56:3-12.
- **Rawat,A.K.; Mehrotra,S.; Tripothi,S.C.; and Shome,U.(1997).** Hepatoprotective activity of *Boerhaavia diffusa* L-roots a popular Indian ethnomedicine . J. Ethno .,56:61-66.
- **Recio,M.C.; and Villar,A.(1987).** Antimicrobal activity of selected plants employed in the Spanich Mediterranean area. J.Ethnopharmacology, 21: 139-152.
- **Reitman,S.; and Frankel,A.S.(1957).**A colorimetric method for the determination of serum glutamic oxaloacetatic and glutamic pyruvic transaminases .Amer .J.Clin.Path.,28:56-58.
- **Resta,R.; Hooker,S.W. ; Laurent,A.B.; Jamshedur Rahman,S.M.; Franklin,M.; Knudsen,T.B.; Nadon,N.L.; and Thompson,L.F. (1997) .** Insights in to thymic purine metabolism and adenosine deaminase

deficiency revealed by transgenic mice over experessing ecto-5-nucleotidase (CD73) .J . of Clinical Investigations , 99: 676-683.

- **Ricci,G.; and Federici,G.(1982).**Method for determination of transaminases and relative diagnostic kit,United States patent.
- **Robert,E.B.(1990).** The Caper Bush. In: The herbalist. 56:77-85.
- **Rois,J.L.; Recio,M.C.; and Villar,A.(1987).**Antimicrobial activity of selected plants employed in the Spanish Mediterranean area .J. Ethnopharmacology , 21:139-152 .
- **Roitt,I.M.; and Rabson,A.(2000).** Really essential medical immunology . Black Well Science London .pp :186.
- **Roitt,I.M.; Brostooff, J.; and Male, D.(1998).** Immunology . 5th ed . Mosby C.V.company.London .
- **Rosenberg,H.F.; and Gallin,H.I.(1999).** Inflammation, in: Fundamental Immunology. Paul,W.E(ed) lipincott, Raeni press., pp:1060.

S

- **Sabahi,M.; Mansouri,S.H.; Ramezanian,M.; and Gholam-hoseinian,A.(1987).**Screening of plants from the southeast of Iran antimicrobial activity . Int. J. Crude Drug Res .,25:72-76 .
- **Sahelian,R.M.(2004) .**The modulating effects of quercetin and rutin on the mitomycin C induced DNA damage .Toxicol Lett .,151:143 -9.
- **Saladin,K.S.; and Porth,C.M. (1998).** Anatomy and physiology , the unity of form and function. McGraw-Hill, USA.
- **Sampath,D.; and Plunkett,W. (2001).**Design of new anticancer therapies targeting cell cycle checkpoint pathways. Curr. Opin. Oncol., 13:484-490.
- **Sano,H.; Kubota,M.; Kasai,Y.; Hashimoto,H.; Shimizu,T.; Adachi, S.; and Mikawa,H.(1991).**Increased methotrexate –induced DNA strand

breaks and cytotoxicity following mutational loss of thymidine kinase . Int. J. Cancer , 48:92.95.

- **Sartorelli,A.C.; Hodnick,W.F.; Belcourt,M.F.; Tomaz,M.; Haffty, B.; Fisher,J.J.; and Rockwell,S.(1994).** Mitomycin C: a prototype bioredctive a gent .Oncol . Res ., :6:501-108.
- **Sawayer,M.G.; Gannoni,A.F.; and Toogood,I.R.(1994).**The use of alternative therapies by children with cancer .Med.J.Aust.,160:320-322.
- **Sax,K.(1940).** An analysis of x-rays induced chromosomal aberrations in Tradescantia. Genetics, 25:41-68.
- **Sayhood,Y.D.(2000).**Inhibiting the nematologic and cytogenetic effects of tamoxifen by garlic extract .M.Sc .Thesis,. Ibn AL-Hathem College , University of Baghdad .Iraq .
- **Schlager,J.J; and Powis,G.(1988) .** Mitomycin C is not metabolized by but is an inhibitor of human kideney NAD(P)H: (quinine accepter) oxidoreductase . Cancer Chemother Pharmacol ., 22 : 126-130 .
- **Schlegel,R.; and MacGregor,J.T.(1982).** A rapid screen for cummulative chromosomal damage in mice. Accumulation of circulating micronucleated erythrocytes. Mutat. Res., 113:481-487.
- **Schmid,W.(1979).** The cell micronucleus test for cytogenetic analysis . In : Holaender , A. (ed) . Chemical mutagenes principles and methods for their detection . Vol .4, plenum press , New York and London.
- **Schmid,W.(1982).** The micronucleus test : an *in vivo* bone marrow method .Hsu T.C. (ed) .Cytogenetic assays of environmental mutagens. Allanheld , Osmun Totowa,P. 221-229.
- **Schoenherr,W.D. ; and Tewell,D.E.(1997).** Nutritional modification of inflammatory disease . Semin .Vet .Med .Sury ., 12: 212-22.
- **Schraudolf. (1989).** *Capparis spinosa* . Phytochemistry, 28:259.
- **Seeley,R.R.; Stepheus,T.D.; and Tate,P.(1996).**Essentials of anatomy and physiology .2nd ed . Medical econmics company , U.S.A, pp.110.

- **Segal,R.; Mozes,E.;Yaron,M.; and Tartakovsky,B.(1989).**The effects of methotrexate on the production and activity of 1L-1. Arth. Rheum.,32:370-377.
- **Seizinger,B.R.(1993).** A prevalent cause of tumourgenesis in human cancer. Nature Genet . , 3:97-109.
- **Shelby,M.D.(1988).** The genetic toxicity of human carcinogens and its implications. Mutat. Res., 204:3-16.
- **Shen,F.; and Weber,G.(1997).**Synergistic action of quercetin and genistein in human ovarian carcinoma cells.Oncol.Res.,9:597-602.
- **Shihata,I.M.(1951).**A pharmacological study of *anagalis arvensis,* M.D.Vet.Thesis,Cario University,P.43.
- **Shirashi ,Y.(1978).**Chromosome aberration induced in germ cells of mice . Mutat . Res ., 57:313-324.
- **Shirishi,Y.; and Sandberg, A.A.(1980).** SCE in human chromosome including observations in neoplasia.Cancer Genet. Cytogenet.,1:363-380.
- **Shubber,E.K.(1981).** The genetic hazard of ten antiparasitic drugs compared to radiation . Ph.D. Thesis, Harvard University. U.S.A.
- **Shubber,E.K.(1987).** Sister chromatid exchange in lymphocytes from patients with Schistosoma hematobium . Mutat . Res ., 180 :93-99.
- **Shubber,E.K.; Amin,S.N.; and EL-Adhami,H.B.(1998).** Cytogenetic effects of copper –containing intrauterine contraceptive device (IUCD) on blood lymphocytes. Mutat. Res., 417:57-63.
- **Shubber,E.K.; and AL-Allak,B.M.(1986).**Spontaneous chromosomal aberrations and SCE_s in human lymphocytes . Effect of culture conditions. Nucleus.29:92-98.
- **Shubber,E.K.; and Al-Shaikhly,A.W.(1989).** Cytogenetic analysis of blood lymphocytes from X-ray radiographers. Int. Arch.. Occup. Environ. Health, 61:385-389.
- **Shubber,E.K.; and Juma,A.S.(1999).** Cytogenetic effects of plant

extract of *Uritica dioica* on mouse somatic cells. Nucleus, 42:182-187.

- **Shubber,E.K.; Auda,H.; Jafar,ZMT; and Abdul-Rahman,M.H. (1999).** Phenotypic expression of three genetic markers in human lymphoblastoid cells (GM-7254) treated with mitomycin C. The Nucleus, 42:122-130.
- **Shubber,E.K.; Hamami,H.A.; Allak,B.M.; and Khaleel,A.H. (1991).** Sister chromatid exchange in lymphocytes from infants with Down's syndrome. Mutat. Res., 248:61-72.
- **Siegel,D.; Beall,H.D.; Kasai,M.; Arai,H.; Gibson,N.W.; and Ross, D.(1993).** PH dependent inactivation of DT-diaphorase by mitomycin C and porfiromycin . Mol .Pharmacol ., 44:1128-1134 .
- **Sierens,J.; Hartley,J.A.; Camphell,M.J.; Leathem,A.J.; and Woodside,J.V.(2002).** *In vitro* isoflavone supplementation reduces hydrogen peroxide induced DNA damage in sperm in sperm . Teratogenesis Carcinogenesis and Mutagenesis, 22: 227-234.
- **Singh,R.P.; and Agarwal,R.(2006).**Natural flavonoids targeting deregulated cell cycle progression in cancer cells.Purchase Curr.Drug Targets,7:345-54.
- **Sood,R.(1994)**.Medicinal laboratory technology.4th ed. Jaypee Brother, India .
- **Soren,L.(1973).** Variabity of the time at which PHA-stimulated lymphocytes intiate DNA-synthesis.Exp.1.Cell Res.,78:201-208.
- **Sorsa,M.; Ojajarvi,A.; and Salomaa,S.(1990).** Cytogenetic surveillance of workers exposed to genotoxic chemicals :Preliminary from a prospective cancer study in a cytogenetic cohort. Teratog . Carcinog. Mutagen, 10:215-21.
- **Spanswick,V.J.; Cummings,J.; and Smyth,J .F .(1996).** Enzymology of mitomycin C metabolic activation in tumar tissue : Characterization of a novel mitochondrial reductase . Biochem. Pharmacol .,51:1623-1630

- **Stahle,E.(1969)**. Thin layer chromatography a laboratory hand book, 2nd ed., translated by Ashworth, M. Spring –verag, Berlin, heidelberty, New York .
- **Stites,D.P.(1976).** Laboratory methods of detecting cellular immune function "in Basic and Clinical immunology . Fundenberg , H.H.;Stites, D.P.; Galdwell J. L.; Wells , J. V .(ed). Lang medical publication Calfornia , pp.318-322.
- **Strachan,T.; and Read,P.A.(1999).** Human molecular genetics.2nd ed. John Wiley and Sons Inc., 605 Third Avenue , NewYork. pp. 38.
- **Sugimura,T.(1992).** Multiple Carcinogensis: a 1992 prespective. Science, 23:603-607.

T

- **Takada,S.; Kelkar,A.; and Theurkauf,W.F.(2003).**Drosophila checkpoint kinase 2 couples centrosome function and spindle assembly to genomic integrity. Cell, 113:87-99.
- **Tasaduq,S.A.; Singh,K.; Sethi,S.; Sharma,S.C.; Bedi,K.L.; Singh,J.; Jaggi,B.S.;Johri,R.K.(2003).**Hepatocurative and antioxidant profile of HP-1,a polyherbal phytomedicine.Human and Experimental Toxicology, 22:639-645.
- **Tates,A.; and Natarajan,A.(1976).** A correlation on the genetic damage induced by chemical mutagen in bone marrow and spermatogonia . Mutat . Res ., 37:267-278.
- **Taylor,J.; Wood,P.S.; and Huggen,W.(1957).**The organization and duplication of chromosomes as revealed by outoradiographic studies using tritiumlabeled thymidine .Proc Natt . Acad. Sci.U.S.A.,43:122-128 .
- **Taylor,R.S.; Manandhar,N.P.; Hudson,J.B.; and Towers,G.H. (1996).** Antiviral activites of Nepalese medicinal plant. J.Ethonopharmacol.,52:157:163.

- **Tennekoon,K.H.; Arundathif,S.J.; Asooriya,P.K.; and Karunanyake,E.H.(1991) .**Possible hepatotoxicity of *Nigella sativa* seeds and *Dregea volubilis* leaves .J. Ethno ., 31:283-289.
- **Tesarik,J.; Bault,A; and Testart,J.(1992).** Effect of pentoxifylline on sperm movement characteristics in normospermic and asthenozoospermic specimens, Hum. Rep., 7:1257 – 1263.
- **Theodore,M.; Theodore, L.; and Constantine, T. (1998).** Induction of cytogenetic damage in human lymphocytes *in vitro* and of antineoplastic effects in Ehrlich ascites tumor *in vivo* treated by methotrexate , hyperthermia and / or caffeine .Mutat .Res., 422:229-236.
- **Threlkeld,D.S.(1990).**Ed. Antineoplastic, antimetabolites, methotrexate. Infacts and comparisons drug information st. Louis, Mo: Facts and comparisons, 653-4.
- **Tilton,R.C.; Balows,A.; Hohnadel,D.; and Reiss,R.F.(1992).** Clinical Laboratory Medicine.Mosby.Year book,P.550.
- **Tom,B.H; Rutzky,L.P.; Jakstys.,M.M.; Oyasu,R; kaye,C.I; and kahan,B.D.(1976)**.Human chronic adenocarcinoma cells Establishment and description of a new cell line . *In vitro* . 12:180 -191
- **Toolan,H.W.(1954).** Transplantable human neoplasms maintained in cortisone treated laboratory animals :H.S. -1 , hep -1, hep-2 , hep-3. Cancer Res.,14:660-666.
- **Totes,A.; and Natarajan,A.(1997).**A correlation on the genetic damage induced by chemical mutagen in bone marrow and spermatogonia. Mutat . Res ., 37:267-278.
- **Travis,E.L.(1995).**.Primer of medical radiobiology year book medical publisher, Travis, E.L.(ed). U.S.A.
- **Tsuboi,I.; Sagawam,K. ; Shichijo,S.; Yokoyama,M.M.; Ou,D.W.; and Wiederhold,M.D.(1995).** Adenosine deaminase isoenzyme levels in patients with human T-cell lymphotropic virus type 1 and human

immunodeficiency virus type 1 infections . Clinical and Diagnostic Laboratory Immunology , 2 :626-630 .

- **Tsutsui,T.; Susuki,N.; and Ohmori,M.(1994).** Sodium fluoride-induced morphological and neoplastic transformation, chromosome aberrations, sister chromatid exchanges, and unscheduled DNA synthesis in cultured Syrian hamster embryo cells. Cancer Research , 44:938-941.
- **Turner,R.R.; Wakely,G.K.; Hannon,K.S; and Bell,N.H.(1988) .** Tamoxifen inhibits osteoclast mediated resorpition of trabecular bone in ovarian hormone deficient rats . Endocrinology , 122:1164-1160 .
- **Turner,N.; and Kulnlein,H.(1991).**Traditional plant foods of Canadian indigenous peoples. Nutritions, botany and use . In food and nutrition in history and anthropology Vol.8. Gordon and Breach Science Publishers, Philadelphia, PA, p.222.

V

- **Verschaeve,L.; Kirsch-Volders, M.; Hens,L.; and Susanne,C. (1985).** Comparative "*in vitro*" cytogenetic studies in mercury –exposed human lymphocytes. Mutat. Res., 157:221-226.
- **Vlietinck,A.J.; Vanhoof,L.; Totte,J.; Lasure,A. ; Vanden Berghe,D.; Rwangabo,P.C.; and Mvukiyumwami,J.(1995).** Screening of hundred Rawandese medicinal plants for antimicrobial and antiviral properties .J.Ethonopharmacol., 46:31-47.

W

- **Wait,W.I.; and Hirschhorn,K.(1978).** Lymphocyte response to activators in Handbook of experimental immunology. Vol II.Cellular immunology Weir , D.M. (ed). Blakwell scientific publication , Oxford London Edinbargh .

- **Wang,Z.Y; Chen,S.J.; Zhon,Z.C.; Athar,M.; Khan, M.A.; Bickers , D.R.; and Mukhtar,H.(1989).** Antimutagenic activity of green tea polyphenol . Mutat . Res . 223:273 -285.
- **Wang,B.; Cui,J.; and Lui,A.(1985).** The effect of Ginseng on immune response.. Advances in Chinese midicinal materials research (Singapore World Scientific).
- **Wang,X.; Yan Jin,D.; Wong,Y.C.; Cheung,L.M.; Chun,C.S.; Lo, K.F.; Liu, Y.; and Tsao,S.W.(2000).** Correlation of detective mitotic checkpoint with aberrantly reduced expression of MAD2 protein in nasopharyngeal carcinoma cells. Carcinogenesis , 21:2293-2297.
- **Wang, Z.P.; Zhi-ping,G.V.; Cao, L.; You,Y.; Mao,B.Y.; and Qian, S.Z. (1999).** Effect of tripchlorolide on the epididymides and tests of rats. Asian J. Androl., 1:121-125.
- **Wascher,T.C; Hermann,J.; Brezinschek,H.P.;Brezinschek,R.; Wilders–Truschnig,M.; Rainer,F.; and Krejs,G.J.(1994).** Cell-type specific response of peripheral blood lymphocytes to methotrexate in the treatment of rheumatoid arthritis . Clin . Invest ., 72: 535-540.
- **Watt,J.M.; and Breyer–Brandwijk,M.G.(1962).** The medicinal and poisonous plants of Southern and Eastern Africa E and S,Livingston Ltd., Edinbourgh and London UK , 996-1000
- **Weiss,D.J.; and Gurpide, E.(1988).** Non-genomic effects of estrogens and antiestrogens .J.Steroid Biochem ., 31:671-676.
- **Weiterg ,A.B.(1987).** Antioxidants inhibit the effect of vitamin –C on oxygen radical induced sister chromatid exchange.Mutat. Res.,191:53-56.
- **Wilson,A.P.(2000).** cytotoxicity and viability assay . In: masters , J.R.W. , Animal cell culture :a practical approach (3rd ed.) .published by United States by Oxford University Inc . New york .

- **Wong,C.K..; Ooi,V.E.; and Aug,P.O.(2000).** Protective effects of seaweeds against liver injiury caused by carbon tetrachloride in rats. Chemosphere , 41 : 173-6
- **Wong,Z.Y.;Chen,S.J; Zhon,Z.C.; Athar,M.; Khan,W.A. ; Bickers , D.R.; and Muthtar,H.(1989).** Antimutagenic activity of green tea polyphenols . Mutat. Res ., 223:273-285.
- **Wyrobek,A.J.; and Bruce,W.R.(1978).** Chemical induction of sperm abnormalities in mice. Proc. Natl. Acad. Sci. U.S.A., 72:4425-4429.

Y

- **Yagi,T.; Yamauchi,K.; and Kuwano,S.(1997).** The synergistic purgative action of aloe-emodin anthrone and rhein anthrone in mice. J. Pharm. Pharmacol., 49:22:25.
- **Yaseen, N.Y.(1990).** Cytogenetic study on human colorectal cancer. Ph.D.Thesis , Department of experimental and Clinical Microbiology University of Sheffield Medical School.

Z

- **Zdzienicka,M.; Hryniewicz,M.; and Pienkowska,M.(1982).** Thiram induced sperm head abnormalities in mice .Mutat .Res . ,102:261-264.
- **Zhao,Z.Z.; and Huang.(1992).** A SoS induction test screening study for vegetable inhibiting mutagenicity caused by antineoplastic drugs. Chinese Journal of Preventive Medicine . 26: 92-3.
- **Zi,X.; Grasso,A.; and Kung,H.(1999).** Aflavonoid antioxidant, silymarin,inhibits activation of erab B1 signaling and induces cyclin-dependent kinase inhibitors ,G1 arrest, and anticarcinogenic effects in human prostate carcinoma cells.Cancer Res.,58:1920-1929.
- **Zohary, M. (1969).** The species of capparis in the Mediterranean and the near eastern countries. Bull. Res. Counc. Israel, 8.

4.1. Detection of some active compounds in four species of plants :

Using different chemical reagents and solutions for detection of various active compounds found in the *Capparis spinosa, Rumex acetosella, Trifolium pratense* and *Solanum nigrum* were presented in table (4-1).

Results obtained by chemical detection indicated the presence of flavonoids, tannins, terpenes, steroids, and glycosides in the *C.spinosa* and *R.acetosella* while in other two plants only tannins , flavonoids, and glycosides were detected in *T. pratense* and only saponine and glycosides were detected in the *S.nigrum* .

The specific odor of the *C. spinosa* and *R.acetosella* extract were caused by the high percentage of terpenes .

Capparis contains considerable amounts of the antioxidant bioflavonoid rutin and the strong flavor comes from mustard oil methyl isothiocyanate released from glucocapparin molecule and this was in agreement with Schrandolf , (1989).

Fairbairn and Muhtadi , (1972) detected the presence of rutin , flavone glycosides , tannins , and anthraquinones including emodin , aloe emodin , chrysophanol , and physcion in the *R. acetosella* .

Isoflavonoids (Biochanin A , daidzein), and cyanogenic glycosids were detected in *T. pratense* by Foster and Tyler (1999).

Solanine and aglyco-alkaloid is found throughout the *Solanium nigrum* which represent a toxic compounds (Cooper and Johnson , 1984).

Table (4-1): Detection of some active compounds in four species of plants extracts.

Chemical compounds	Indication	Result of detection			
		C.spinosa	***R.acetosella***	***T.pratense***	***S.nigrum***
Tannins	Greenish blue	+ve	+ve	+ve	–ve
Saponin	Foaming	–ve	–ve	–ve	+ve
	White ppt.	–ve	–ve	–ve	+ve
Glycosides (befor hydrolysis)	Red ppt.	+ve	+ve	+ve	+ve
Glycosides (after hydrolysis)	Red ppt.	+ve	+ve	+ve	+ve
Terpense	Brown colour	+ve	+ve	–ve	–ve
Steroids	Blue colour	+ve	+ve	–ve	–ve
Flavonoids	Dark colour	+ve	+ve	+ve	–ve
Flavonoids	yellow colour	+ve	+ve	+ve	–ve

4.2.Examination of the Activity of Plants Extracts:

Active compounds of *Capparis spinosa, Rumex acetosella, Trifolium pratense,* and *Solanum nigrum* were extracted using different organic solvents (hexane, chloroform, ethyl acetate, methanol, and ethanol).

Activity of these plants extracts were examined againest the pathogenic bacteria (*E.coli, S.aureus, K.pneumonia*). Result showed that there are a significant effects ($P \leq 0.05$) of Capparis ethanolic extract and Rumex methanolic extract on the growth of these pathogenic bacteria.

The high inhibitory action of ethanolic extract of *C. spinosa* may be returned to that the alcoholic extract is a good solvents used in extraction method (Cowan , 1999) , for its ability to extract all or most of the active compounds found in the plant including glycosides and flavonoids especially rutin and quercetin which represent one of the most abundant natural flavonoids . A high significant inhibition of bacterial growth by *R..acetosella* extracts especially methanolic extract may be returned to the presence of rumicin a powerful antibacterial compounds which is effective against *E.coli,S. aureus* and *K. pneumonia* , in addition to the high content of tannins in the Rumex extracts (Foster and Duke , 1990)

The differences in the antibacterial activity of *T.pratense* and *S.nigrum* from other two plants *C.spinosa* and *R. acetosella* on its effects on three species of bacteria may be returned to that the first two plants have active constituent different from active constituent found in the second two plants and thus have a little effects on these three species of bacteria and may have effected or acts against another type of bacteria such as *Candida albicans* , *Pseudomonous aerogenosa , Micrococcus luteus* and other (Rauha *et al* ., 2000).

Qualitative and quantitative analysis using HPLC chromatography were done on these extracts , so cytogenetic , immunological, enzymatic of

ethanolic and methanolic extracts of *Capparis spinosa* and *Rumex acetosella* respectively were studied.

4.3. HPLC Analysis of *C. spinosa* and *R. acetosella* :

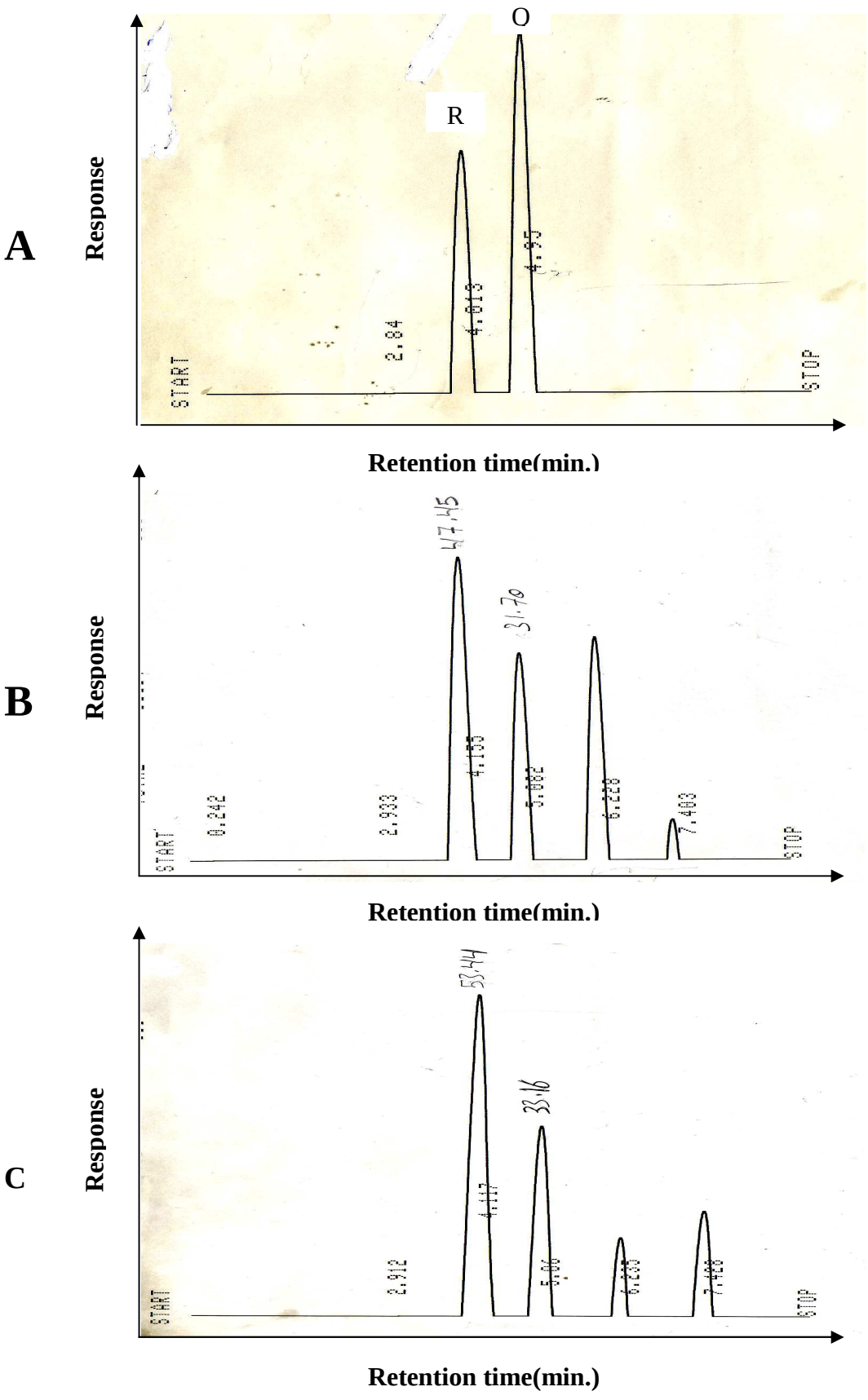
For further identification and in addition to chemical detection of active compounds in *C.spinosa* and *R. acetosella* , HPLC analysis was done for detecting the most important active compounds flavonoids (Rutin and Quercetin) in both plants using rutin and quercetin standard as a reference .

HPLC analysis was done using C18-column , 1250x , 4.6 mm I.D column , the mobile phase used was 0.1 % acetic acid in deionized water: acteto-nitrile (60:40), and the flow rate was 1ml/min .at 275 nm (Handa *et al*., 1998).

HPLC showed two major peaks have different retention times for *R. acetosella* .These two peaks are also had a retention time, semi identical to the retention time of rutin standard and quercetin standard .

And also for *C.spinosa* and by depending on retention time it was show that also two major peaks had (Rutin and quercetin).

Figure (4-1 A,B,C) shows that two plant extracts have high concentration from these two standards .



Figure(4-1) HPLC analysis of Rutin(R) and Quercetin (Q) standard(A),*C.spinosa*(B).*R.acetosella*(C).

4.4.Cytogenetic Effects of MTX Drug, *C.spinosa* and *R.acetosella* Extracts on Mouse :

4.4.1. The Effects on Mitotic Index of Bone Marrow Cells :

Under normal experimental conditions , the Balb/C mice had a mitotic of (4.5%) in their bone marrow cells, this was considered as a negative control.

Table (4-2) shows that 50µg/kg a dose of MTX causes a significant reduction ($P<0.05$) in MI (1.56%) after 7 days of treatment in comparison with the negative control .

Treatment with ethanolic extract of *C. spinosa* causes an increase in MI. The increase in MI is concentration dependent. In comparison with negative control (4.5%), there was a significant increase ($P<0.05$) in MI after seven days of treatment with extract at concentration 500mg/kg (6.90%)and also a significant increase ($P<0.05$) in MI was shown at concentration 700mg/kg to reach to (7.36%).

Treatment with methanolic extract of *R. acetosella* causes an increase in MI of mouse bone marrow cells (Table 4-2) . The increases in MI is concentration dependent , so in comparison with the negative control (4.5%) , there was a significant increase ($P<0.05$)in MI to reach to (6.90% , 9.0%) for both concentration of Rumex (100, 250) mg/kg respectively.

The results indicate that MTX with its dose resulted in the reduction of MI in mouse bone marrow cells . This may be related to the proteins required for mitosis which were not produced at the same quantities , or the code was not reached the cell to induce it to proliferate , or the drug may cause the death of bone marrow cells (Turner *et al* ., 1988)or due to defect occurred in the mitotic spindle composition during cell division (Shiraishi , 1978).

This result was in agreement with the result of Theodor *et al* ., (1998), who found that MTX at concentration 25 and 50 µg/kg caused reduction in

the MI of mouse bone marrow cells .Almiry , (1999) also pointed that MTX caused reduction in MI of mouse bone marrow cells .

There are other chemotherapeutic drugs that also cause mitotic index inhibition like cyclophosphamide (CP) (AL-Fayadh , 2000 ; Hassan , 2000) mitomycin-C (MMC)(Littlefield *et al* ., 1980) and tamoxifen (TAM) (Syhood, 2000).

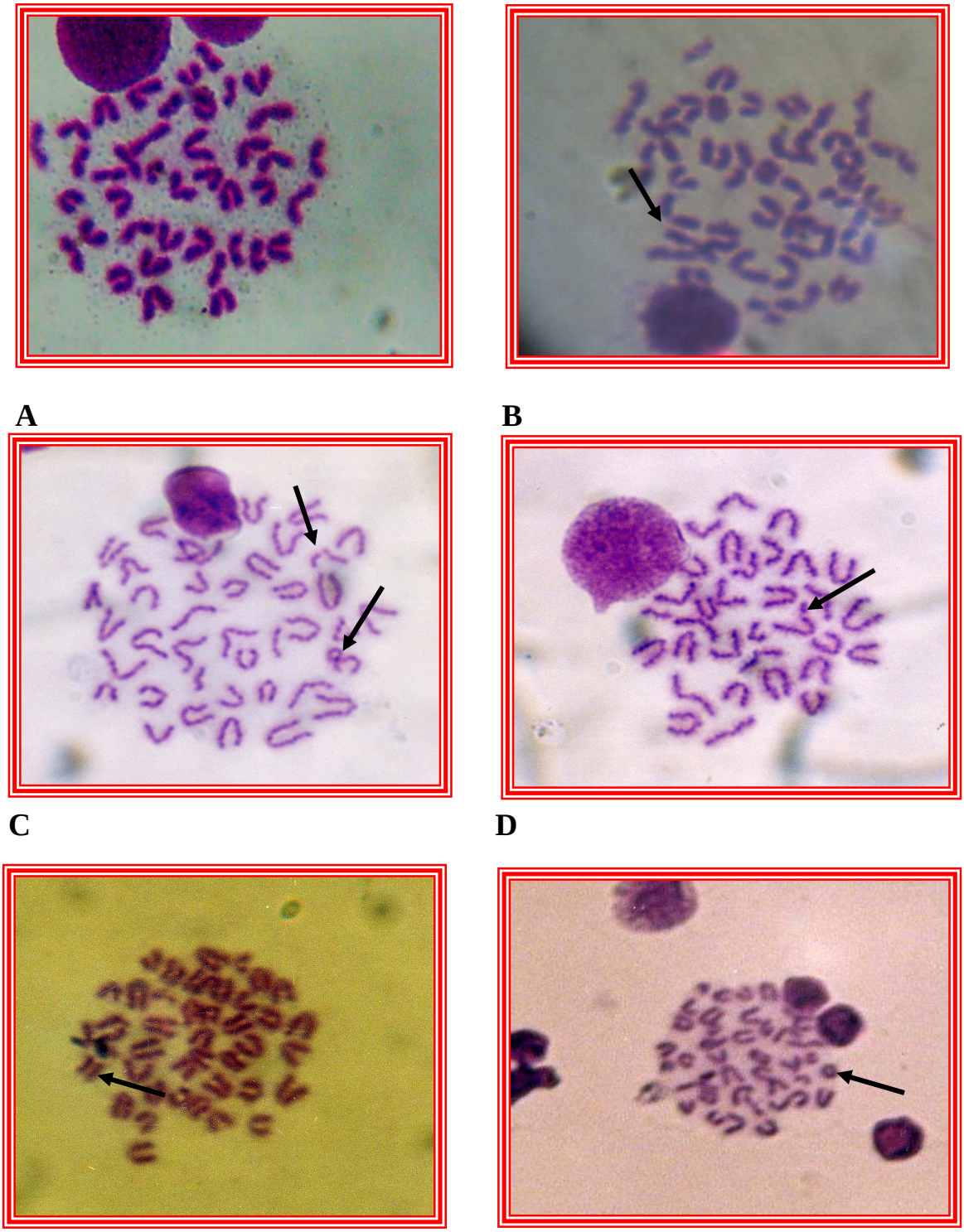
Decreased MI reflects inhibition of cell cycle progression or loss of proliferative capacity (Marucia *et al* ., 2000). And also depression of the MI is usually a consequence of a reduced rate of cell proliferation (Mitotic delay) (Galloway *et al*., 1994, Hagelstrom *et al* ., 1995).

While the increase in MI after treatment with the *C.spinosa* and *R.acetosella* extract may be returned to that these two plants extract contained mitogenic agents in its constituent because of that , MI might increase after treatment , the antioxidant represents the main constituent (rutin and vitamins) which was able to induce cell division without any other mitogen (Travis , 1975).

4.4.2. Effects on Chromosomal Aberrations (CAs):

The spontaneous frequency of chromosomal aberrations in mouse bone marrow cells was (0.8%) which represents a negative control (Table 4-2) .

The results of this experiment indicats that 50µg/kg of MTX can increase CAs frequencies (chromatid break , chromatid gap, deletion , dicentric chromosome , ring chromosome , chromosome break and chromosome gap) (Figure 4-2) which reached to (2.38%) after 7 days of MTX treatment . These results were significantly different ($P<0.05$) from the negative control .



Figure(4-2) Cell in metaphase stage taken from mice treated with methtrexate showing normal chromosome(A) ,deletion(B), dicentric, gap(C),chromatid break(D) acentric chromosome (E), Ring chromosome(F) (100X).

C.spinosa have the ability to reduce the spontaneous CAs from (0.8%) in the negative control to (0.23%) after seven days of treatment with (500mg/kg) and (0.15%) with (700mg/kg) of *C.spinosa* extract .The results were non significantly different (P>0.05) in comparison with the negative control . On the other hand, Rumex has also the ability to reduce spontaneous frequencies of CAs in mouse bone marrow cells to reach to (0.53 %, 0.19 %) for both (100, 250)mg/kg respectively and this reduction was non significant (P≥0.05) in comparison with the negative control (Table 4-2).

The result of the effects of MTX on CAs may suggest that MTX at this concentration may limits the intracellular supply of reduced folate (tetrahydrofolate) through inhibition of dihydrofolate reductase (DHER) and, therefore, is considered to induce cytotoxic effects with resultant inhibition of new DNA, thymidylate and purine synthesis (Lorico *et al*., 1988 , Sano *et al*., 1991).

Methotrexate may act as cytotoxic agents on bone marrow cells by interferes and damages the chromosomal proteins (Weiss and Gurpid , 1988). It was pointed that the percentage of chromatid break was higher than the percentage of chromosome breaks this may be related to the differences in the repair systems for each change (Loric *et al*., 1990; Holoden *et al.*,1995).

C.spinosa and *R.acetosella* extracts contain chemical compounds which may 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 ethanolic extract of different plants have more activity for decreasing the spontaneous frequency of CAs and MN which occur as a result of

metabolic changes inside the body or as a result of presence of undetected environmental and genetic mutagen (Shubber, 1981), because the ethanolic extracts contain the more active compounds which dissolved in alcohol but not in water like coumarin which have the ability to remove the cytotoxic effect of more chemical compounds (Ohta *et al.*, 1983).

Another reason for CAs decreased proposed by the *in vivo* antioxidant/radical scavenger activity which was assessed by determining the ability of *C.spinosa* to reduced UV-B induced skin erythema in healthy human volunteers (Bonina *et al*., 2002).

These results were in agreement with Jagetia and Ganapthi , (1991) who has reported that chromosomal aberrations were repaired by liv.52 ,a non toxic herbal preparation composed from *C.spinosa* and other herb .

Other considered that Rumex contains antioxidant agents (vitamin C) which act as scavengers for the free radicals in the cells and like other desmutagen vitamin C work in different way, Its act as anticlastogen for CAs for the mutagenic drug Di-iodohydroxy quinolin in mouse bone marrow (Kola *et al*., 1989 ; Ghaskadbi *et al* ., 1992) . Eylar *et al*., (1996) found that vitamin C can also protect from oxidative damage to DNA and lipid that may lead to aging (cancer) and other dysfunction.

4.4.3.The Effects on Micronucleus Induction (MN):

The mouse bone marrow micronucleus (MN) assay holds a key position in all schemes for detecting potential carcinogens and mutagens (Helen and John, 1994).

Micronucleus frequencies of polychromatic erythrocytes from negative control mice was (0.80%) (Table 4-2) .The percentage was increased to (2.33%)after treatment with 50 µg/kg methotrexate.This result is significantly different ($P<0.05$) from the negative control (Figure 4-3) .

C.spinosa causes reduction in MN frequency, and the reduction in MN was depending on the concentration of *C.spinosa* (Table 4-2) . The non significant decrease in MN was observed after seven days of treatment with extract to reach to (0.33%) at the concentration (500mg/kg) and (0.20%) at the concentration (700mg/kg) when compared with negative and positive control .

As a result of Rumex treatment , the spontaneous frequency of MN decreased from (0.80%) in the negative control to(0.33%,0.23%)for both concentration of Rumex respectively. The reduction of MN was non significant in comparison with the negative control but it was significant in comparison with positive control . This experiments indicates that Rumex has the ability to reduce the spontaneous frequency of MN, and this reduction depend on the concentration of plant extract , in which the highest reduction was seen with concentration (250mg/kg) when compared with the negative control .

Micronucleus induction by MTX may be resulted from chromosomal damage or damage to the mitotic apparatus in the erythroblasts of the mouse bone marrow (Armstrong and Galloway , 1993).Kasahara *et al* ., (1992) found that MTX has been a clastogenic agent and a micronucleus inducer in mouse bone marrow cells and human. Miller and Adler , (1990) pointed that 5-15% of the micronuclei induced by clastogens (hydroquinone and mitomycin C).

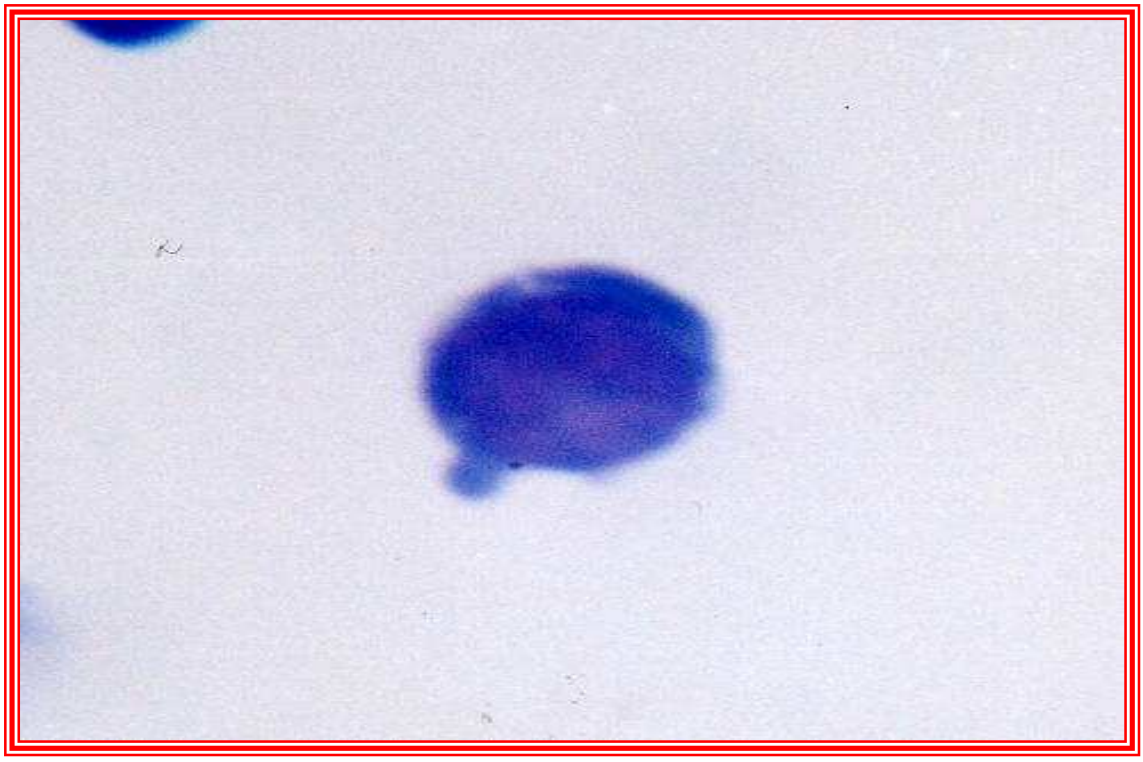


Figure (4-3) Micronucleated bone marrow cell from mouse treated with methotrexate(100 X).

The reduction in frequency of MN after *C.spinosa* treatment may be due to the presence of more active herb constituent which is rutin and qurecetin which have protective effects with highest anticancer activity and rutin protected DNA from damage (Sahelian , 2004).

The ability of Rumex to reduce MN frequency might due to antioxidant and free-radical scavenging activity of vitamins which represents the main constituent of Rumex .It is also due to its antimutagenic and detoxification activities (Weitbery , 1987).

Although various authors (Hose *et al* ., 1987 , Ayllon and Garcia – Vazquez , 2000) suggest that variations in the shape of nucleus could represent an alternative approach for detecting genotoxicity.

Table(4- 2):Cytogenetic effects of MTX drug ,*C.spinosa* and *R.acetosella* in mouse bone marrow cells *in vivo*.

Concentration µg/ml	Cell viability (Absorbance) (m±SE)				
	Ref			Ref	
	C.spinosa	*R.acetosella*	Concentration µg/ml	MTX	MMC
Negative control	a 0.235±0.003	a 0.235±0.003		a 0.235±0.003	a 0.235±0.0005
62.5	a 0.221±0.006	a 0.219±0.006	1	b 0.086±0.02	b 0.050±0.002
125	a 0.215±0.005	a 0.219±0.006	2	b 0.073±0.027	b 0.048±0.0005
250	a 0.206±0.004	a 0.210±0.003	4	b 0.071±0.028	c 0.047±0.002
500	a 0.195±0.001	a 0.195±0.013	6	b 0.07±0.025	b 0.047±0.0005
750	b 0.189±0.006	b 0.184±0.006	8	c 0.059±0.008	c 0.046±0.0005

Different letter represent significant differences (P≤0.05) between means of the same column

4.4.4. The Effects on Mitotic Index of Mouse Germ Cells and on Sperm Morphology :

Under normal experimental conditions mice had a mitotic index (10.8%) in their testis this was considered as a negative control . The MTX at concentration 50µg/kg caused significant reduction ($P<0.05$) in mitotic index to reach to (3.52%) in comparison with the negative control(Table4-3).

The results show that there was a significant increase ($P<0.05$) in the mitotic index of testis cell to reach (12.6%) after 7 days of treatment with (500mg/kg) of *C.spinosa* and (15.7%) after treatment with (700mg/kg) of *C.spinosa* in comparison with negative control.

The results after treatment with (100,250) mg/kg of Rumex extract show that there was a significant increase ($P<0.05$) in mitotic index of mouse testis to reach to (12.9%, 16.0%) for both concentrations respectively in comparison with negative and positive control .

The reduction in MI caused by MTX may be related to the testicular damage as manifested by reduced testicular volume , oligozoospermia , elevated FSH and LH protein and lower testosterone concentrations which may be required for mitosis (Chatterjee *et al*.,2000).Several chemotherapeutic such as fludarabin ,cyclophosphamid can cause these damage (Agarwal and Tamer ,2003).

This result is in agreement with Hassan , (2002) which pointed that MTX cause reduction in the MI of testis and this reduction may return towards the toxic and mutagenic effects of this drug .

The increase in MI of mouse testis may be due to the *C.spinosa* and *R.acetosella* have mitogenic effects through the presence of active compounds acts as or stimulate the division process without addition of any mitogenic agents (Haq *et al*., 1995).These plants extracts may improve animal fertility and it can cross testis barriers.

Table (4- 3): Effects of MTX drug,*C.spinosa* and *R.acetosella* extracts on mitotic index of mouse germ cells

Groups		Mitotic index % (m±SE)
Negative control		a 10.8±0.44
Positive control (MTX) (50 µg/kg)		b 3.52±0.30
Capparis spinosa	500 mg/kg	c 12.6±0.44
	700 mg/kg	d 15.7±0.14
Rumex acetosella	100 mg/kg	c 12.9±0.34
	250 mg/kg	d 16.0±0.28

Different letter represent significant differences (P≤0.05) between means of the same column

The results pointed that under normal experimental conditions , the percentage of normal sperms in negative control was (60.1, 60.5 , 57.6) after 7,21 and 35 days respectively (Table 4-4) . On the other hand, and on the effects of MTX on sperm morphology (head and tail), the results showed that MTX cause significant reduction ($P<0.05$) in number of normal sperms after 35 days of treatment to reach to (34.0%) in comparison with the negative control (57.0), while MTX causes no significant reduction in number of normal sperms after 7 and 21 days of treatment (61%, 54.5%) respectively in comparison with the negative control (Table 4-4).

MTX also causes significant increase ($P<0.05$)in number of abnormal head after 35 days of treatment to reach to (32.5%) in comparison with the negative control (22.5%) and also there is no increase in number of abnormal head caused by MTX after 7 and 21 days (19 % , 22.5%) in comparison with the negative control (19.0%, 19.5%).

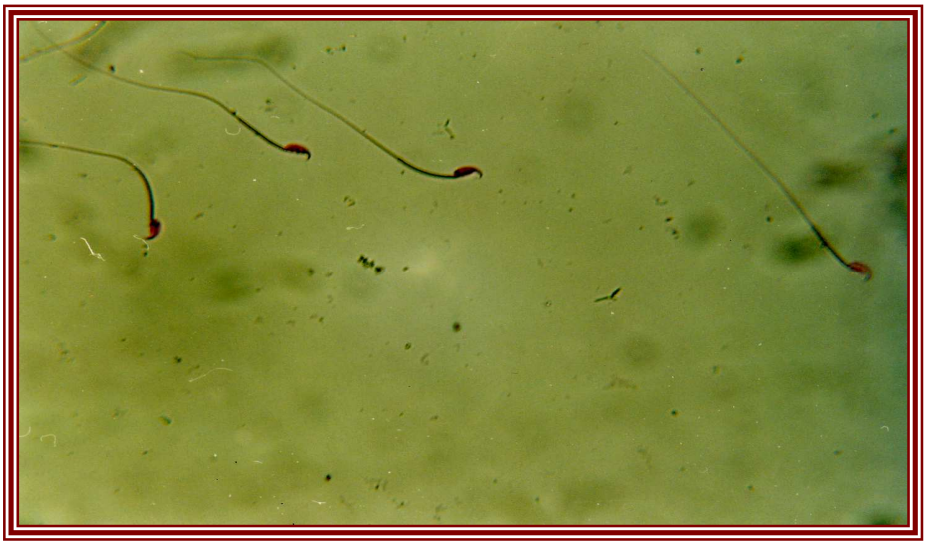
On the other hand, MTX causes significant increase ($P<0.05$) in number of abnormal tail after 35days of treatment to reach to (34.5%) in comparison with the negative control (20.2%) and no significant differences showed after 7 and 21 days of treatment with MTX (20.5%,22.5%) respectively in comparison with the negative control (20.5%, 19.5%) respectively (Figure 4-4).

Table (4- 4): Mean ± Standerd error of sperm head and tail morpholog

Treatment period		No.of normal sperm %m±SE	No.of head abnormalities%m±SE	No.of tail abnormalities%m±SE
Negative control	7days	a 60.1 ± 0.60	a 19.3 ± 0.49	a 20.5 ± 0.31
	21days	a 60.5 ± 0.49	a 19.5 ± 0.49	a 19.5 ± 0.49
	35days	a 57.6 ± 1.10	a 22.5 ± 0.49	a 20.2 ± 0.75
Positive control	7days	a 61.0 ± 0.99	a 19.0 ± 0.99	a 20.5 ± 0.31
	21days	a 54.5 ± 0.49	a 22.5 ± 0.49	a 22.5 ± 0.49
	35days	b 34.0 ± 1.99	b 32.5 ± 2.50	b 34.5 ± 0.49
Capparis spinosa	500mg/kg 7days	a 70.7 ± 1.25	a 15.2 ± 0.25	a 14.0 ±0.99
	21days	a 73.5 ± 0.49	a 13.2 ± 0.25	ab 13.5 ± 0.49
	35days	b 81.0 ± 0.99	b 9.0 ± 0.99	b 10.0 ± 0.43
	700mg/kg 7days	a 75.0 ±0.99	a 12.0 ± 0.99	a 13.0 ± 0.43
	21days	a 77.7 ± 0.25	ab 11.5 ± 0.49	a 11.5 ± 0.49
	35days	b 86.5 ± 0.99	b 8.0 ± 0.99	b 6.0 ± 0.99
Rumex acetosella	100 mg/kg 7days	a 65.0 ± 0.99	a 16.5 ± 0.49	a 17.5 ± 0.49
	21days	ab 68.2 ± 0.25	ab 15.7 ± 0.25	a 16.2 ± 0.25
	35days	b 73.5 ± 1.49	b 13.5 ± 0.49	a 14.0 ± 0.99
	250mg/kg 7days	a 70.5 ± 1.49	a 15.0 ± 0.99	a 14.5 ± 0.49
	21days	a 75.0 ± 0.99	a 13.0 ± 0.99	a 13.0 ± 0.49
	35 days	b 79.5 ± 1.49	a 10.0 ± 0.99	b 10.5 ± 0.49

Different letter represent significant differences (P≤0.05) between means of the same column

A



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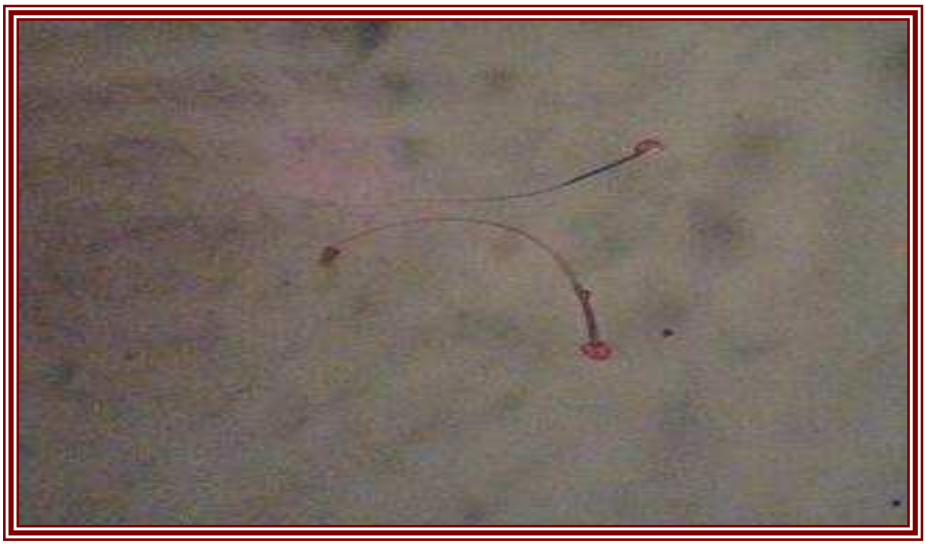


C

Figure(4-4)Sperm morphology from mouse treated with methotrexate showing normal sperm(A), abnormal head(B),abnormal tail(C)(40X).

The normal sperms percentage after 7,21 and 35 days of treatment with *C.spinosa* extract shown in (Table 4-4), the result indicated that the percentage of normal sperms after 7 days of treatment was (70.7 , 75.0%) for both concentration (500 ,700)mg/kg respectively and (73.5%, 77.7%) after 21 days of treatment and also for both concentration respectively.These results indicated there were not significant differences when compared with negative control , but significant differences ($P<0.05$) was shown after 35 days of treatment with extract and the percentage of normal sperms reach to (81.0% , 86.5%) for both concentrations respectively .

On the other hand, the percentage of head and tail abnormalities was decreased , the head abnormalities were decreased to reach to (15.2%, 12.0%) after 7 days of treatment and (13.2% , 11.5%) after 21 days of treatment and for both concentration (500,700)mg/kg respectively .These results were not significant when compared with negative control (19.3 %, 19.5%) after 7 and 21 days respectively , but the significant differences ($P<0.05$) were shown after 35 days of treatment to reach to (9.0%, 8.0%) for both concentrations respectively in comparison with negative control (22.5%).

The percentage of tail abnormalities were decreased from (20.5%, 19.5 %, 20.2%) after 7,21 and 35 days respectively in negative control to reach to (14.0% ,13.0%) after 7 days of treatment and (13.5% , 11.5%) after 21 days of treatment for both concentrations respectively which was not significant ($p>0.05$) from negative control. A significant decrease ($P<0.05$) in tail abnormalities was shown after 35 days of treatment with extract to reach to (10.0%, 6.0%) for both concentration respectively in comparison with negative control (Table 4-4).

The results of effects of *R. acetosella* on the sperm morphology (head and tail) showed that there is non significant differences ($P>0.05$)in number of normal sperm after seven days of treatment to reach to (65.0% 70.5%) for

both concentration (100,250)mg/kg respectively , and after 21 days of treatment (68.2 % , 75.0%) in comparison with the negative control (60.1% ; 60.5%) after 7 and 21 days respectively .But the significant increase ($P<0.05$) in number of normal sperms was shown after 35 days of treatment to reach to (73,5% ; 79.5%) for both concentrations respectively in comparison with the negative control (Table 4-4) .

In its effect on sperms head, it was shown that Rumex extract cause significant decrease ($P<0.05$) in number of sperms head abnormalities after (21,35) days of treatment to reach to (15.7%, 13.0 %) after 21 days and (13.5, 10.0%) after 35 days of treatment for both concentrations (100, 250)mg/kg respectively , while after 7 days there is no significant differences ($p>0.05$) in sperms head abnormalities (16.5% , 15.0%) for both concentrations respectively in comparison with the negative control (19.3%).

On the other hand, and on effect of Rumex extract on sperm tail morphology , it was shown that there were non significant differences ($P>0.05$)in number of abnormal tail for three periods (7,21,35) days of (100mg/kg) of Rumex treatment to reach to (17.5% , 16.2%, 14.0%) respectively in comparison with the negative control (20.5%, 19.5%,20%) respectively. Also there are non significant differences ($P>0.05$) was shown after (7,21) days of (250mg/kg) of Rumex extract treatment (14.5%,13.0%) respectively, but significant decrease ($P<0.05$)in tail abnormalities was shown after 35 days of treatment to reach to (10.5%) in comparison with the negative control (20.2%) (Table 4-4).

The results of effects of MTX on sperms morphology are in agreement with AL-Kadi , (1997) who noticed that the effect of most mutagenic agents on sperm morphology (head and tail) are investigated in the five week of treatment .This may reflect that the sperms exposed to drug when they occur in the spermatogonia which represent 1^{st} stage of sperm formation process.This stage is more sensitive to be effected by drug because of their

high mitotic activity which represent a source of all sperms (Tates and Natarajan , 1976) . While in the first week of treatment, the sperm in this period occur in spermatogenesis stage in which the spermatide converted to sperms (Wyroberk and Bruce , 1978 ; Zdzienicke *et al* ,1982) and because the sperms have a full form in this stage ,so the drug cannot interfere with sperms formation .On the other hand, Clemont , (1970) demonstrates that spermatogenesis regulated by the accurately coordinated expression of many genes ,disruption of this process can be brought about mutations of many genes, so any mutation of these genes which resulted from drug effect may effect sperms head and tail and produce abnormal sperms .

Another reason for which MTX treatment causes abnormal sperms morphology is that ,the male reproductive function is under hormonal control ,spermatognic process is under control of follicular stimulating hormone (FSH) and testosterone (Seeley *et al* ., 1996),while the formation of type A spermatogonia and conversion of primary spermatocyte into secondary spermatocyte (Meiosis I) are dependent on testosterone and the final step of maturation of spermatids are dependent on FSH (Ganong, 1991). So the abnormal sperm morphology may reflect on abnormal intratesticular maturation as a result of drug treatment (Acosta *et al* , 1988), also the drug induces an alteration in androgen secretion that usually produce changes in the reproductive system ,such changes might include the production of abnormal sperms (Tesarik *et al*.,1992),also any effect on spermatogenesis lead to production of abnormal sperms (Arab *et al* ., 1989).

The decreasing in the abnormalities of sperms (head and tail)especially after 35days of treatment with both plants extracts occur when the sperms occur in the spermatogonia stage and before mitotic division which represents a source of sperms , and this will be in agreement with AL-Rubia , (2000) in which this extract does not contain any mutagenic agents . Wyrobek and Bruce , (1978) point that these mutagenic agent can induce the

abnormalities in sperms head and tail while non-mutagenic agent does not induce these abnormalities.In addition to these compounds which were detected in Capparis and Rumex extract may play a role in antioxidant protection against sperm damage (Sierene *et al.,* 2002). This test represents a more sensitive test for detection of the mutagenic compounds, or the decreasing in the sperms head and tail abnormalities may be due to that these extracts have protective effects in germ stem cells (spermatogonia) which act as a source of all sperms (Tates and Natarajan , 1976).

4.4.5.Effects on liver enzymes (GOT,GPT) :

The effect of MTX ,*C.spinosa* and *R.acetosella* on GOT and GPT level was shown in (Table 4-5) .

It was shown that MTX causes significant increase ($P<0.05$) in the GOT level to reach to (294.3 U/L) in comparison with the negative control (228.0 U/L).

Also a significant increase ($P<0.05$) on GPT was shown after MTX treatment to reach to (95.6 U/L) in comparison with the negative control (68.3 U/L).

The *C.spinosa* extract shows no significant differences ($P>0.05$) on the level of GOT , the level of GOT reach to (191.3 , 189.3)U/L for both (500, 700) mg/kg respectively in comparison with the negative control (228.0 U/L) .On the other hand ,and on the effect of *C.spinosa* extract on GPT, the results also indicated that there were no significant differences in level of GPT. The level of GPT reach to (47.3 , 55.3) U/L for both concentrations respectively in comparison with negative control (68.3 U/L).

On the other hand Rumex extract causes a non significant increase ($P<0.05$) in GOT level to reach to (230.0, 243.6)U/L for both (100,250)mg/kg respectively in comparison with the negative control (228.0 U/L). Also in GPT level the Rumex extract causes a non significant decrease

(P>0.05) in GPT level for both concentration to reach to (57.6,62.0)U/L respectively in comparison with the negative control (68.3 U/L)(Table 4-5).

The increase of GOT and GPT serum levels may be due to that MTX has cytotoxic effect on liver cells and this leads to increase the permeability of liver cell membrane which causes the movement of high quantity of these enzyme to blood serum and this explains the increases of the enzyme level in blood serum and decreases in liver after using of these toxic agents (Bonnefoi *et al*., 1989).

Rawat *et al*., (1997) point that the agents that have cytotoxic effects cause lysosomes lyses which are damaged in all the organelles inside the cell and lead to death of paranchymatous cell which causes an increases in the serum levels for GOT and GPT. At the same time most of chemical compounds and MTX have one of these compounds lead to inhibition the activity of the detoxification enzymes such as superoxide dismutase (SOD) and glutathione -S- transferase (GST) that scavenging free radicals from the cell (Banerjee *et al* 1994).

Also, there are chemotherapeutic drugs like tamoxifen that cause an increase in serum levels for GOT and GPT in animal treated with it , this may be returned to cytotoxic effects on the liver cells that cause damages of the liver tissue and increase GOT and GPT level (D'Mello *et al*., 1999).

The no cytotoxic effect of *C.spinosa* extract on the enzymes level may be due to presence of flavonoids which are known for their excellent antioxidative capacity in various model system (Sahelian , 2004).In addition to *R.acetosella*, they have in their content active compound such as vitamin A,B,C,E, and K, with high minerals content such as sodium iron , and calcium. All these compounds have a strong antioxidant activity against reactive oxygen species (ROS), and the hepatoprotective activity of Rumex was possibly due to its antioxidant properties (Qiusheng *et al* ., 2004).

These results are in agreement with Tennekoon *et al*.,(1991) and Hassan , (2002) who found that black seed extracts have no cytotoxic effect on the enzymes level and this is in agreement with results of Khan *et al*., (1995) who found that carry and mustard plants have no cytotoxic effect on GPT and GPT level of rats serum .

4.4.6. Effects on the Immune Enzyme ADA:

Under normal experimental conditions , mice had ADA enzyme activity level (0.359 U/gm), this was considered as a negative control (Table 4-5).

The result of this experiment indicated that MTX causes significant inhibition ($P<0.05$) of ADA activity to reach to (0.159U/gm) in comparison with the negative control .

Treatment with *C.spinosa* causes a significant increase ($P<0.05$) in the ADA activity to reach to (4.45,5.01)U/gm for both concentrations (500,700)mg/kg respectively in comparison with negative control (0.359 U/gm). Results presented in this study demonstrates that *R.acetosella* causes significant increase ($P<0.05$) in the activity of ADA to reach to (0.702,1.473)U/gm for both (100,250)mg/kg respectively in comparison with the ADA activity in the negative control (0.359 U/gm) .

Adenosine deaminase (ADA) is important for the development and function of the immune system. ADA deficiency results in a combined immunodeficiency brought a bout by the immunotoxic properties of elevated ADA substrates (Michael *et al* ., 2000).

The mechanism by which MTX affects the kinetics and dynamics of adenosine has partially been elucidated by previous animals studies .Most probably MTX interferes with the de novo purine synthesis pathway in cells and leads to modulate in the adenosine metabolism and later leads to accumulation of toxic compounds which cause an inhibition in the ADA activity and later ADA deficiency (Huang *et al*., 1997; Cutolo *et al*., 2001).

Any stimulator's that can raise WBCs can also increase the ADA activity *in vivo* and *in vitro* (Hag *et al*., 1998). Capparis extract has active constituent that can enhance immune system such as rutin and quercetin by maintaining the functional and structural integrity of important immune cells (Schoenherr and Jewell , 1997).

So, the increase in ADA activity may be due to the stimulation of T-lymphocyte for producing 1L-3 which is important for activating CD4 for division and differentiation (Hag *et al*., 1995), or may be due to presence of flavonol glycosides which is one of the active constituent that might contribute to the antioxidant defenses of blood (Hollman and Katan , 1999).

R.acetosella extract is rich with many vitamins. Vitamin C is also a powerful antioxidant. It enhances the immune system and it is known as the most versatile supplement in the helping the immune system and increased T-cell activity. So the increases in the ADA activity may be due to this causes in addition to vitamins A and E which are known to enhance immune function (Gey , 1998). The enhances in the immune function by the vitamins may be due to its activity to eliminate toxic activity substances from the body and to enhance cellular oxygen and active enzymatic reactions (Jialal , 1993).

Table (4-5):Effects of MTX ,*C.spinosa and R.acetosella* on liver enzymes (GOT,GPT and ADA enzyme activity)

Groups		GOT (m±SE)U/L	GPT (m±SE)U/L	ADA activity (m±SE)U/mg
Negative control		a 228.0±23.24	a 68.3±14.74	a 0.359±0.44
Positive control (MTX) (50 µg/kg)		b 294.3±29.19	b 95.6±6.96	b 0.159±0.06
Capparis spinosa	500 mg/kg	a 191.3±12.57	a 47.3±4.17	c 4.45±0.37
	700 mg/kg	a 189.3±8.66	a 55.3±3.33	c 5.01±0.68
Rumex acetosella	100 mg/kg	a 230.0±10.96	a 57.6±3.18	c 0.702±0.07
	250 mg/kg	a 243.6±22.15	a 62.0±2.88	c 1.473±0.30

Different letter represent significant differences(P≤0.05) between means of the same column

4.4.7.The Effects on the Total and Differential Counts of Leukocyte on Mouse :

MTX (50µg/kg) treatment causes significant decrease (P<0.05) in the total counts of leukocytes to reach to (48.83%) in comparison with the negative control (100.83%)(Table 4-6).

A significant decrease (P<0.05) in the number of lymphocyte and neutrophil were shown after MTX treatment to reach to (1390,1808)cell/cu.mm.blood respectively in comparison with the negative control (5742,2512) cell /cu.mm.blood respectively.

Also, MTX causes a significant decreases (P<0.05) in number of monocyte to reach to (666.3)cell/cu.mm.blood in comparison with the negative control (1769.2)cell/cu.mm.blood, while in it's effect on eosinophil and basophil ,MTX causes significant increases (P<0.05) in both number to reach to (381.0,524.0)cell/cu.mm.blood respectively in comparison with the negative control.

Table (4-6) showes that the two different concentrations of *C.spinosa* extract causes an increase in the total number of leukocyte which reached to (155.50%) for (500mg/kg) and (172.83%) for the (700mg/kg) of Capparis extract. The increase in total counts was significant (P<0.05) for both concentration in comparison with total counts of negative control (100.83%).

Also there is a significant (P<0.05) increase in number of both lymphocyte and neutrophil for both concentration of Capparis , the number of lymphocyte reach to (10113, 11063)cells/cu.mm.blood, for both (500 and 700)mg/kg respectively in comparison with negative control (5742) cell/ cu.mm.blood, and the number of neutrophil reach to (3791.7 , 3973.7) cell/cu.mm.blood respectively for both concentrations in comparison with negative control (2512)cell /cu.mm.blood.

While the number of monocyte increases was not significant (P>0.05), the number of monocyte reach to (1644.3, 2246.8) cell/cu.mm.blood for both concentrations respectively in comparison with negative control (1769.2)cell/cu.mm.blood. No significant differences (P>0.05) in the number of eosinophil and basophil was shown after treatment with both concentration of Capparis extract in comparison with negative control .

On the other hand, the two different concentrations (100,250)mg/kg of Rumex extract cause significant increase (P<0.05) in the totals number of WBC to reach to (162.17% , 174.67%) for both concentrations respectively in comparison with the totals counts of the negative control (100.38%).

Also there is a significant increase (P<0.05) in number of lymphocyte and neutrophil for both concentration of Rumex extract, the number of lymphocyte reachs to (10322,11411)cell/cu.mm.blood respectively in comparison with negative control (5742)cell/cu.mm.blood and the number of neutrophil reachs to (3838.8, 4076.3) cell/cu.mm.blood respectively in comparison with the negative control(2512)cell/cu.mm.blood.The increases in number of monocytes were non significant for both concentrations of Rumex extract in comparison with the negative control (Table 4-6) .

Cytotoxic effect of MTX which causes damage to the cell in addition to it's effects on bone marrow (Decreasing in cellular division).This damage expected to have effects on the quantity of WBCs .A decreasing in total counts of leukocyte may be due to cytotoxic effects of MTX (Braunwald *et al* ., 1987). One possible explanation of the methotrexate effects is the diminution of the size and reactivity of the T-lymphocyte population.There are suggestions that this may be accomplished by the induction of apoptosis in activated T-cells (Genestir *et al*., 1998). This suggestion is consistent with the observations of reduction in peripheral blood T and B-lymphocyte populations after short term methotrexate treatment (Wastcher *et al* ., 1994).

Table (4- 6): Effects of MTX drug ,*C.spinosa* and *R.acetosella* extracts on differential and total counts of blood *in vivo*

Groups		Total WBCs (m±SE) %	Differential count (m±SE)				
			No.of Lymphocyte cells /cu. mm. blood	No.of Neotrophil cells /cu. mm. blood	No.of Monocyte cells /cu. mm. blood	No.of Eosinophil cells /cu. mm. blood	No.of Basophil cells /cu. mm. blood
Negative control		a 100.83±12.14	a 5742±936.4	a 2512±132.7	a 1769.2±220.5	a 60±30.4	a 0.00±0.00
Positive control (MTX) (50 µg/kg)		b 48.83±1.16	b 1390±137.4	b 1808.5±91.0	b 666.3±54.8	b 381.0±52.0	b 524.17±82.9
Capparis spinosa	500 mg/kg	c 155.50±3.27	c 10113±366.0	c 3791.7±263.0	a 1644.3±298.3	a 0.00±0.00	a 0.00±0.00
	700 mg/kg	cd 172.83±1.92	c 11063±204.9	c 3973.7±77.5	a 2246.8±24.9	a 0.00±0.00	a 0.00±0.00
Rumex acetocella	100 mg/kg	cd 162.17±0.72	c 10322±189.9	c 3838.8±124.5	a 2055.3±151.6	a 0.00±0.00	a 0.00±0.00
	250 mg/kg	d 174.67±1.42	c 11411±86.6	c 4076.3±133.3	a 1979.2±53.3	a 0.00±0.00	a 0.00±0.00

Different letter represent significant differences (P≤0.05) between means of the same column

The decreasing in number of monocyte is thought to be that MTX causes reduction in monocytic IL-1 production.Some have suggested that alterations in IL-1 responses were related to diminution in the ability of cells to respond to IL-1 rather than to direct inhibition of it's production (Segal *et al* ., 1989; Brody *et al*., 1993). Another reason of monocyte reduction may be resulted from effect of MTX on suppression of TNF-α synthesis in T-cell and causes macrophages suppression (Becker *et al* ., 1998). The increase in basophil and eosinophil number may be due to that the drug interferes or effects the hypersensitivity type 1 reaction and make some T-lymphocyte and mast cells to release some cellular kinetics that stimulate production of these cells in the affected tissue and peripheral blood (Roitt *et al* ., 1998), or the increase in number of these cell may be returned to ADA deficiency mice caused by MTX. So, the decreasing of ADA level leads to accumulation of these cell in ADA deficient mice (Michael *et al*.,2000). These result are in agreement with Murad ,(2005) who found that these cells will be increased in breast cancer patients and in albino female rats after treatment with tamoxifen drug.

All these effects on total and differential counts of leukocyte by Capparis extract have one explanation in which Capparis has no cytotoxic effect. This is due to most active compounds which have medicinal activity (Evans, 1999), like glycosides , flavonoids especially rutin and quercetin which have an important roles in immune system as an immune stimulators (Beresford , 1999), and these increases were cleared in WBCs especially lymphocyte which acts as a regular of immune response through it's secretion to cytokines which act as activator for immune system through induces to phagocytes and the induction of B-cell to produce antibody (Roitt and Rabson ,2000). In addition to the increases in number of monocytes they have an important role in phagocytosis (Rosenbery and Gallin , 1999).

On the other hand, Capparis contains compounds that may increase the activity of phagocytes and natural killer cells, it stimulates the cell to initiate the immune response and accelerating the immune system's ability to produce T- cell aggregation (Chu *et al* ., 1970).Or these may be active compounds act as a regulator for proliferation and differentiation of bone marrow progenitor cell population (Clark and Kamen , 1987), and also stimulates the mobilization of performed mature neutrophils from the bone marrow cavity or other pooling organs such as blood vessels (Okabe *et al* ., 1990) .

The above results showed that *R.acetosella* can cause an enhancement of immune response due to it's cause an increase in total and differential counts of leukocytes. This may be due to it's active constituents in which vitamin C,E and carotenoids are naturally occurring antioxidant nutrients that play important roles in animals health by inactivating harmful free radicals produced through normal cellular activity and from various stressors . Both *in vitro* and *in vivo* studies showed that these antioxidant vitamins generally enhance different aspects of cellular and non cellular immunity (Chew , 1995).

4.5.Cytogenetic Effects of MTX , *C.spinosa* and *R.acetosella* on Human Blood Lymphocyte:

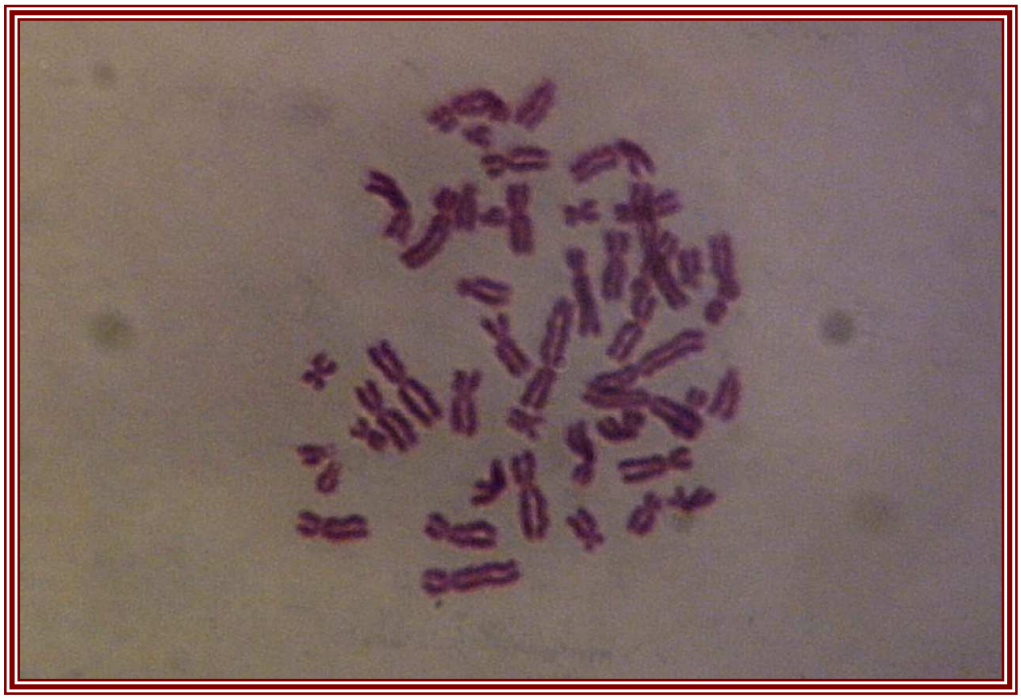
The blast index of human blood cells from healthy individual was (34.1%).

Methotrexate treatment also causes a significant decrease ($P<0.05$) in BI for all concentrations of methotrexate used in this experiment (0.4,2,4)µg/ml to reach to (25.24%,16.24%.11.92%) respectively in
comparison with the negative control.

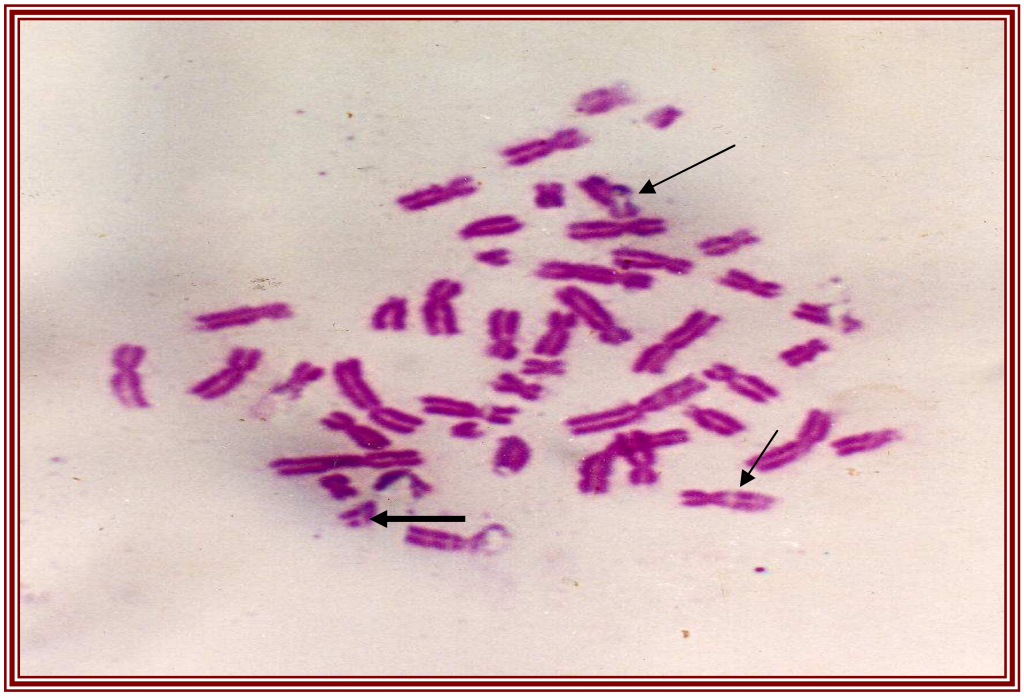
Also, MTX causes a significant inhibition (P<0.05) in MI of human blood lymphocyte for all concentrations to reach to (0.86%,0.35%, 0.19%) respectively in comparison with the negative control (2.92%) and a high significant inhibition of MI which was noticed with the (4µg/ml)(Table 4-7). Other cytogenetic effects of MTX in human blood cells *in vitro* were the increases in the spontaneous frequencies of micronuclei (MN) and chromosomal aberrations (CAs). The increase in MN were significant (P<0.05) for all concentration of MTX (0.4,2,4) µg/ml to reach to (1.38%,1.57%,1.83%) respectively in comparison with the negative control (1.06%) (Table 4-7) . While increases in CAs were significant (P<0.05) only at the concentration (4 µg/ml)to reach to (2.01%) in comparison with the negative control (1.57%) (Table 4-8) .

Spontaneous frequencies of chromatid break , chromatid gap, deletion and dicentric chromosomes were increased depending on the concentration of MTX in human blood culture (Figure 4-5)(4-6)(4-7) .

Another effect of MTX occurs on the replicative index in human blood culture .MTX also causes a significant decrease (P<0.05) in RI at the concentration (4 µg/ml) to reach to (1.69%) while in other two concentrations (0.4,2) µg/ml the decreasing in RI was non significant in comparison with the negative control (Table 4-9).



Figure(4-5)Metaphase of normal human blood lymphocyte(100 X).



Figure(4-6)Metaphase of human blood lymphocyte treated with MTX showing chromosome break(100 X).

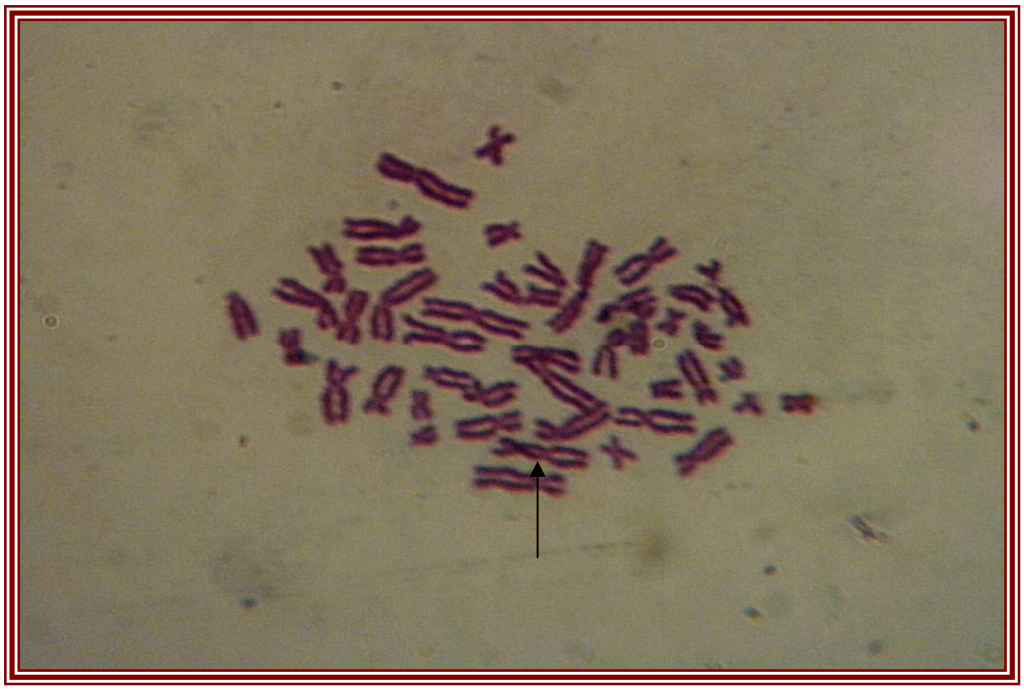
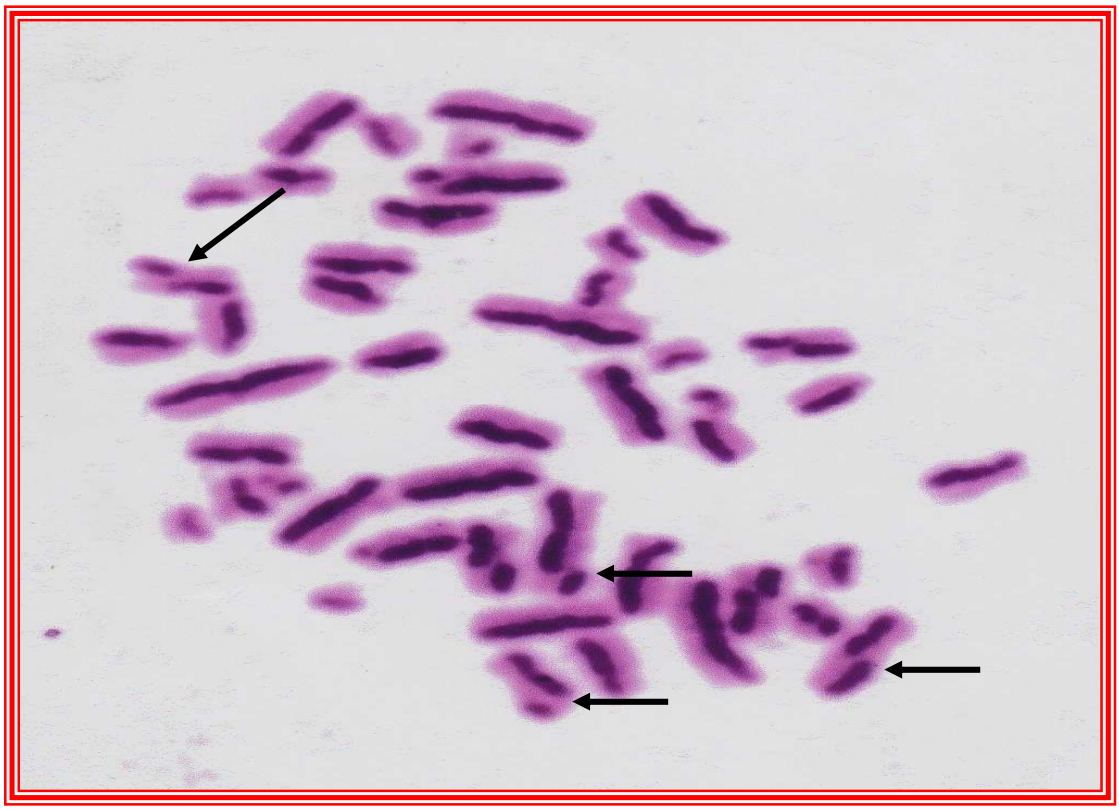


Figure (4-7)Metaphase of human blood lymphocyte treated with MTX showing dicentric chromosome(100 X).

The percentage of cells in M3 was decreased after treatment with MTX concentrations (0.4,2,4)µg/ml to reach to (30%,27%,22.8%) respectively in comparison with the negative control (31%).Cells in M2 was also decreased to reach to (31%,29%,24%) respectively in comparison with the negative control (33%), while cells percentage in M1 was also increased to reach to (39%,44%,53.2%) respectively in comparison with the negative control (36%) (Table 4-9). MTX also causes a significant increase ($P<0.05$) in SCEs and for all MTX concentrations to reach to (5.32%,6.55%,8.10%)respectively in comparison with the negative control (4.41%) (Figure 4-8).



Figure(4-8)Metaphase of human blood lymphocyte stained with hoechest stain, showing the sister chromatid exchange(1000 X).

The blast index appears to increase gradually in correlation with concentration of *C.spinosa* extract when compared with that of normal human blood lymphocyte culture (34.1%) , the value for (200,400)µg/ml of extract was (51.90 , 55.1%) respectively. There is no significant differences ($P>0.05$) between these concentrations, but significant increase ($P<0.05$) was shown when compared with negative control, a significant increase ($P<0.05$) in blast index was also shown with the concentration (800µg/ml) to reach to (61.18%) in comparison with other two concentrations and with the negative control (Table 4-7).

The mitotic index of human blood cells from healthy individual was (2.92%) , *C.spinosa* causes a significant increase (P<0.05) in MI and in different concentrations in comparison with the negative control , so the increases in MI was concentrations dependent. In the concentration (200µg/ml) of Capparis extract, the MI reach to (4.37%), while at the (400µg/ml) the MI reachs to (4.93%), so there is no significant differences between these two concentrations. A significant increase (P<0.05) was observed at the concentration (800µg/ml) the MI reachs to (6.12%)in comparison with other two concentrations and with negative control (Table 4-7).

Other cytogenetic parameters used in this study was chromosomal aberrations and micronucleus. For CAs , *C.spinosa* cause a significant decrease (P<0.05) in totals of chromosome aberrations for three different concentrations (200, 400,800)µg/ml to reach to (0.58%,0.37%, 0.24%) respectively in comparison with the negative control (1.57%). A high significant reduction in spontaneous CAs was shown with the concentration (800µg/ml) in comparison with other concentrations . So the reduction was also concentration dependent , and this indicated that *C.spinosa* has no cytotoxic activity on human blood lymphocytes *in vitro*.

Spontaneous frequencies of chromatid break , chromosome break , chromatid gap, chromosome gap, deletion, and dicentric were decreased depending on concentration of Capparis extract in human blood culture , while ring chromosomes were not observed in the concentration (800µg/ml) in comparison with negative control (Table 4-8).

For the MN , *C.spinosa* extract shows that there was a significant decrease (P<0.05) in micronucleus , the MN was decreased from (1.06%) in the negative control to (0.33%, 0.20%, 0.14%) for the concentrations (200,400,800) mg/ml respectively , a high significant decrease was clear at the concentration (800µg/ml)(Table 4-7).

The percentage of cell in M3 after treatment with *C. spinosa* reach to (33%) for the concentration (400µg/ml). A significant increase ($p<0.05$) was shown in the concentration(400,800)µg/ml to reach to (36%,37.6%) in comparison with negative control (Table 4-9) .In M2, it was also shown that *C. spinosa* causes a significant increase in cells to reach to (34%, 35%, 36.4%) for three different concentration respectively (200,400,800)µg/ml in comparison with cells in M2 of negative control (33%) .On the other hand , cells in M1 were decreased to reach to (33%, 29%, 26%) respectively in comparison with negative control (36%).

So, the RI was increased significantly ($P<0.05$) with the increase of the concentration of Capparis extract to reach to (2%,2.08%,2.13) for all concentrations of Capparis extract respectively in comparison with RI of negative control (1.95%).

C.spinosa causes non significant decrease in the percentage of SCEs with the all concentrations of plant extract to reach to (4.04%, 3.97%,3.68%)µg/ml for the concentrations (200,400,800)µg/ml respectively in comparison with the negative control (4.41%) (Table 4- 9) .

Treatment with Rumex extract causes a significant increase ($P<0.05$) in blastogenic index (BI) in human blood lymphocytes *in vitro* to reach to (49.48%, 54.62% , 62.46%) for (200,400,800)µg/ml respectively , the increase in BI was significant ($P<0.05$) for all concentrations in comparison with the negative control ,and a high increases was clear at the concentration (800µg/ml). A gradual increase in MI was noticed after using different concentrations of Rumex extract in human blood culture . The Rumex causes a significant increase ($P<0.05$) in MI to reach to (4.76%,5.50%,6.36%)for (200,400,800)µg/ml respectively in comparison with the negative control (2.92%). Also a high significant increase was shown at concentration (800µg/ml) in comparison with other two concentrations and with the negative control (Table 4-7) .

Other cytogenetic effects of Rumex extract in human blood cells *in vitro* were the decrease in spontaneous frequencies of micronuclei (MN) and chromosomal aberrations (CAs). The Rumex extract causes a significant decrease ($P<0.05$) in MN for all concentrations of Rumex to reach to (0.36% ,0.24% , 0.22%)for (200, 400, 800)µg/ml respectively in comparison with the negative control (1.06%)(Table 4-7).Rumex extract cause a significant decrease ($P<0.05$) in totals CAs. Spontaneous frequencies of chromatid break , chromosome break , chromatid gap , chromosome gap , deletion and dicentric were decreased depending on the concentration of Rumex extract in human blood culture. Ring was not observed in the (800µg/ml) of Rumex extract in comparison with the negative control (Table 4 - 8).

The percentage of cells in M3 vary with different concentrations of Rumex extract.In the negative control the cells in M3 was (31%), while cells in M3 was increased to (33%, 35%,37.2%) for (200,400,800) µg/ml of Rumex extract respectively . Cells in M2 was also increased to reach to (34.8% , 35.8%, 36%) for three different concentrations respectively in comparison with the negative control (33%) , while cells in M1 was also decreased to reach to (32.2%, 29.2%, 26.8%) in comparison with the negative control (36%).So, the RI was increased significantly ($P<0.05$)with the increase of the concentrations of plant extract to reach to (2%,2.05%,2.10%)for three different concentrations respectively and in comparison with the negative control (1.95%)(Table 4-9).

On the other hand, SCEs percentage was decreased non significantly ($P>0.05$) with all the concentrations of Rumex extract to reach to (4.31%,3.63%,3.3%) respectively in comparison with the negative control.

The reduction in BI and MI by MTX indicates that MTX has a cytotoxic activity on human blood lymphocyte *in vitro* . This reduction in BI and MI caused by MTX are considered as indicators of cytogenetic damage (Morris and Helfich ,1984). Puck *et al*., (2002) demonstrates that repair of mutated

G2 cells causes a lag in their reaching mitosis and, therefore, a drop in mitotic index. Another reason for decreasing the MI by MTX suggestes that an indicator of changes in lymphocytes and their ability to respond to culture conditions and may be an early marker of cytotoxicity and genotoxicity in humans (Gonsedatt *et al* ., 1994).

The increase of MN in peripheral blood indicates that MTX may be mutagenic agent (Liu Weijun , 1992). Schmid, (1982) suggestes that micronuclei may originate from a centric chromosome fragments , either resulting from double strand DNA damage before cell division , or after the breakage of anaphase bridges that thought to be caused by MTX .

The MTX causes an inhibition in the (DHFR), so the resultant inhibition of new DNA , thymidylate and purin synthesis (Li and Kaminskas , 1984, Loric *et al* ., 1988), hence , DNA strand breaks arise from spontaneous and normally repaired DNA lesions that are not repaired due to a depletion of dTTP, dATP and dGTP. There is also evidence that MTX influences the cellular topoisomerase II content and causes, therefore, an increase in CAs and MN (Loeico *et al*., 1990 , Holden *et al*., 1995).

Some investigations have indicated that nuclear irregularities may be also associated with chromosomal aberration (Atkin and Baker, 1979).The results indicate that the formation of different type of CAs and MN are closely related to the breakage fusion bridge type of mitotic disturbances , and abnormalities in nuclear shape may thus primarily be regarded as an indicator of genetic instability (David *et al* ., 2001).

The increases on the frequency of SCEs and the reduction of RI caused by genotoxic agents are considered as indicators of cytogenetic damage (Morris and Heflich ,1984) and, therefore, SCEs appear to have an application in the clinical prediction of tumor sensitivity to potential chemotherapeutics (Deen *et al*.,1986; Lialiaris *et al* ., 1992) .It is found that MTX leads to increase the SCE , reduction of new DNA synthesis , causes

DNA fragmentations through inhibition of different enzyme mechanisms and induced cell growth inhibition and cell killing (Li and Kamiuskas , 1984 ; Sano *et al*., 1991) . Another explanation for which the MTX causes an increases in CAs and SCEs were that MTX is a folate anti metabolite and enter the cell through folate carrier or by passive diffusion. When enter the cell methotrexate bind to DHFR strongly and ignore any folic acid, they might see and inhibit dihydrofolate reductase thus the nucleotide precursors for DNA and RNA synthesis will inhibited. The inhibition of thymidylate synthesis apears to the most important effect of methotrexte and results in a greater inhibition of DNA synthesis (Kasahara *et al* .,1992).

Our results are in agreement with the Hassan , (2002) and AL-Janabi , (2004) in which the MTX has a genotoxic effect on the human lymphocyte cells *in vitro* through the decreasing of the MI, RI and CCP in cultured lymphocyte treated with it .

The increases in MI and BI indicat that plants extracts contain active constituents that might stimulates cell mediated immunity by stimulating blastogenesis or blast transformation of lymphocyte and their cell division (Mowery , 1986).

The reduction in the percentage of CAs and MN after treatment with *C.spinosa* gives an indication that Capparis extract has no genotoxic effects on human blood cells *in vitro* this may be due to presence of rutin and quercetin in the Capparis extract , it was found that quercetin reduced the DNA strand breakage which induced by mitomycin C and rutin were protected from DNA damage.Thus, in human lymphocytes quercetin and rutin displayed protective effects on DNA damage in concentration dependent manner (Sahelian , 2004).

Flavonoids have a variety of biological effects in numerous mammalian cell systems , *in vitro* as well *in vivo* . Recently , much attention has been paid to their antioxidant properties and to their inhibitory role in various

stages of tumor development (Hollman and Katan , 1997) . Flavonoids have also acted as desmutagen and Bio-antimutagen that can reduc or decrease the percentage of SCEs , and can interfere with DNA repair process, so, its act as Bio - antimutagen (Francies *et al* ., 1989).

Quercetin, the major representative of the flavonol subclass , and one of Capparis active constituent, is a strong antioxidant and prevents oxidation of low density lipoproteins *in vitro* (Hollman and Katan , 1999), in addition to the ability of flavonoids quercetin and rutin to protect living cell against DNA strand breaks by way of their metal ion chelating mechanism (Miski *et al* ., 1983).

The reduction in the percentage of CAs and MN after *R.acetosella* treatment may be due to presence of vitamin C,E and vitamin K which acts as anticlastogenic compounds (Ghaskdbi and Vaidya, 1989). Gebhart, (1984) pointes that vitamin C has the ability to reduce the CAs which induced by CP and Trenimon .

The increases in percentage of M3 and the decrease in the percentage of M1 after treatment with both plants extracts point that plants extracts stimulate cell to replicate and also lead to increase CCP especially M3 , and this may be due to that the plant extract contains active compounds acts as stimulator for cell cycle , and the decrease in SCEs mean by Rumex extract may be due to the presence of vitamins (Gebhart , 1992), which may be acted as an inhibitor for the mutagenic agents which may be found in human lymphocyte culture. So vitamins were included in the natural antimutagenic compound (Deflora and Ramel, 1988), which was able to reduce SCE in human lymphocyte culture in addition to antigenotoxic activity, which may be returned to antioxidant ability or to the competitive possibility with the molecules that interfere with the DNA (Madrigal –Bujaidar and Diaz – Barriga , 1995)

Table (4-7): Cytogenetic effects of methotrexate ,*C.spinosa* and *R.acetosella* on human blood lymphocytes *in vitro*.

Groups		Mitotic index m±SE%	Blast index m±SE%	Micronucleus m±SE%
Negative control		a 2.92 ± 0.20	a 34.1± 0.72	a 1.06 ± 0.05
Positive control (MTX)	0.4 µg/ml	b 0.86 ± 0.07	b 25.24 ± 0.85	b 1.38 ± 0.02
	2.0 µg/ml	b 0.35 ± 0.03	c 16.24 ± 0.34	c 1.57 ± 0.02
	4.0 µg/ml	b 0.19 ± 0.03	c 11.92 ± 0.54	d 1.83 ± 0.03
C.spinosa	200 µg/ml	c 4.37 ± 0.10	d 51.90 ± 1.95	f 0.33 ± 0.05
	400 µg/ml	c 4.93 ± 0.19	d 55.1 ± 1.67	f 0.20 ± 0.04
	800 µg/ml	d 6.12 ± 0.17	e 61.18 ± 1.94	f 0.14 ± 0.04
R.acetosella	200 µg/ml	c 4.76 ± 0.29	d 49.48 ± 1.68	f 0.36 ± 0.05
	400 µg/ml	cd 5.50 ± 0.32	d 54.62 ± 1.56	f 0.24 ± 0.06

Different letter represent significant differences (P≤0.05) between means of the same column

	800 µg/ml	**d** **6.36 ± 0.34**	**e** **62.46 ± 2.29**	**f** **0.22 ± 0.04**

Table (4-8): Chromosomal aberration of methotrexate ,*C.spinosa* and *R.acetosella* on human blood lymphocytes *in vitro*.

Treatment groups		Chromosomal Aberration % m±SE								
		Chromatid break	Chromatid gap	Deletion	Dicentric	Acentric	Ring	Chromosome break	Chromosome gap	Total
Negative control		a 0.18±0.005	a 0.51±0.01	a 0.41±0.006	a 0.17±0.01	a 0.12±0.008	a 0.01±0.001	a 0.08±0.008	a 0.09±0.006	a 1.57±0.02
Positive control (MTX)	0.4 µg/ml	b 0.07±0.01	b 0.08±0.01	b 0.18±0.01	b 0.13±0.02	a 0.08±0.01	a 0.01±0.009	ab 0.04±0.01	b 0.07±0.01	b 0.66±0.02
	2 µg/ml	c 0.13±0.02	c 0.12±0.01	b 0.21±0.02	b 0.17±0.02	b 0.19±0.02	b 0.05±0.009	b 0.08±0.01	c 0.12±0.01	a 1.07±0.002
	4 µg/ml	d 0.23±0.02	d 0.21±0.01	d 0.49±0.02	c 0.27±0.01	c 0.31±0.03	c 0.12±0.01	c 0.18±0.02	d 0.20±0.01	c 2.01±0.03
Capparis spinosa	200 µg/ml	c 0.07±0.008	b 0.05±0.008	b 0.21±0.008	ad 0.06±0.007	a 0.09±0.006	ab 0.01±0.005	ab 0.06±0.009	b 0.03±0.006	b 0.58±0.03
	400 µg/ml	b 0.03±0.007	b 0.03±0.008	b 0.17±0.01	a 0.05±0.007	a 0.06±0.008	a 0.006±0.03	a 0.02±0.003	a 0.01±0.008	d 0.37±0.01
	800 µg/ml	b 0.02±0.006	b 0.03±0.008	b 0.13±0.01	a 0.02±0.004	a 0.03±0.006	a 0.0±0.00	a 0.01±0.004	a 0.004±0.002	e 0.24±0.01
Rumex acetocella	200 µg/ml	b 0.1±0.005	b 0.07±0.005	b 0.26±0.01	b 0.18±0.007	b 0.16±0.007	a 0.01±0.005	b 0.08±0.008	b 0.09±0.006	f 0.95±0.02
	400 µg/ml	b 0.07±0.01	b 0.04±0.007	b 0.21±0.007	d 0.1±0.01	ab 0.12±0.008	a 0.004±0.002	b 0.05±0.004	a 0.02±0.006	b 0.61±0.02
	800 µg/ml	b 0.03±0.005	b 0.02±0.006	b 0.11±0.007	a 0.05±0.003	a 0.04±0.007	a 0.0±0.00	a 0.01±0.004	a 0.01±0.005	e 0.27±0.01

Different letter represent significant differences (P≤0.05) between means of the same column

Table (4-9): Effects of methotrexate drug ,*C.spinosa* and *R.acetosella* extracts on cell cycle progression on human blood lymphocytes from healthy individual *in vitro*

Groups	Replicative Index % m±SE	Cell Cycle Progression % m			Sister chromatid exchange % m±SE
		M1	M2	M3	
Negative control	a 1.95±0.02	36	33	31	a 4.41±0.07
Positive control 0.4 µg/ml MTX	a 1.91±0.03	39	31	30	b 5.32±0.02
Positive control 2 µg/ml MTX	ab 1.83±0.04	44	29	27	c 6.55±0.85
Positive control 4 µg/ml MTX	b 1.69±0.02	53.2	24	22.8	d 8.10±0.19
200 µg/ml *C.spinosa*	c 2±0.02	33	34	33	a 4.04±0.17
400 µg/ml *C.spinosa*	c 2.08±0.05	29	35	36	a 3.97±0.14
800 µg/ml *C.spinosa*	c 2.13±0.03	26	36.4	37.6	a 3.68±0.13
200 µg/ml *R.acetosella*	c 2 ±0.04	32.2	34.8	33	a 4.31±0.22
400 µg/ml *R.acetosella*	c 2.05±0.05	29.2	35.8	35	a 3.63±0.25
800 µg/ml *R.acetosella*	c 2.10±0.02	26.8	36	37.2	a 3.3±0.13

4.6. Cytotoxicity of Plants Extracts on Tumor Cells:

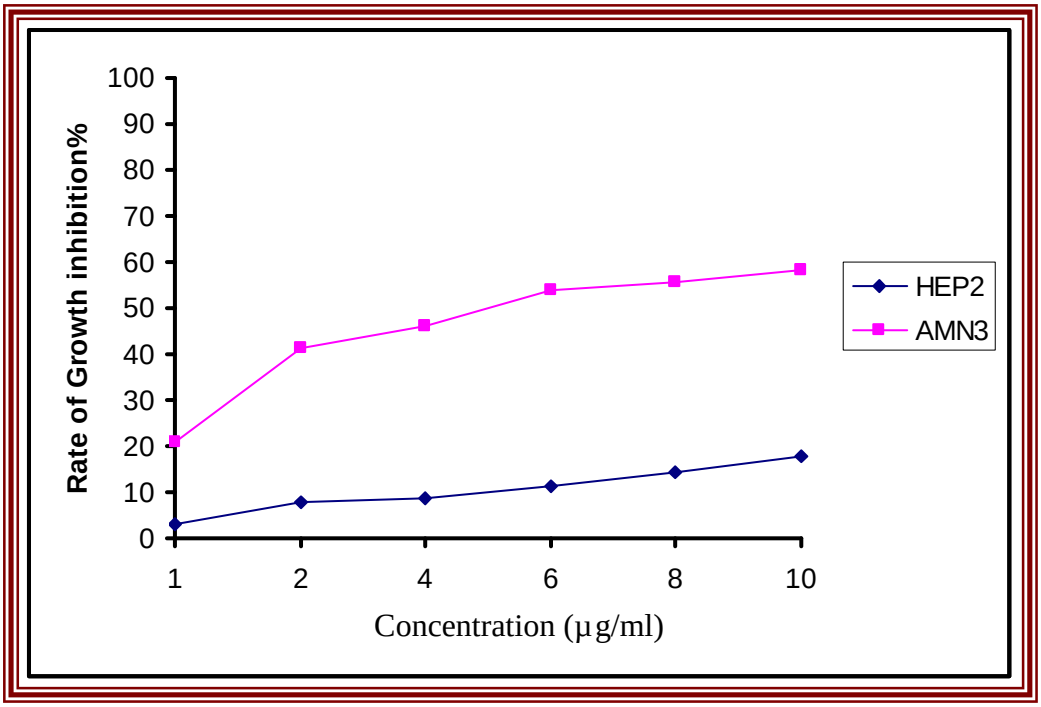
Statistical analysis reveales significant differences between means of cell viability of tumor cells AMN-3 and HEP-2 cultures treated with methotrexate and mitomycin-C drugs.

In the cytotoxic effect of methotrexate on AMN-3 cell culture the results show that MTX causes a significant reduction ($P<0.05$) in cell viability especially at the concentration 10µg/ml in comparison with the negative control (Table 4-10). The growth inhibition percentage GI reach to (58.1%). On the other hand, the result of the effect of MTX on HEP-2 cell line shows there is a significant reduction ($P<0.05$) in cell viability and also at the concentration 10 µg/ml of MTX in comparison with the negative control, the GI percentage reachs to (17.8%)(Figure4-9).

Table(4- 10):Cytotoxic effect of MTX and MMC drug on the growth of cancer cell line (AMN-3,HEP-2).

Concentration µg/ml	Cell viability (Absorbance)(m±SE)			
	AMN -3		HEP-2	
	MTX	MMC	MTX	MMC
Negative control	a 0.659±0.0005	a 0.659±0.0005	a 0.359±0.0005	a 0.359±0.0005
1	c 0.52±0.0005	c 0.494±0.05	ab 0.348±0.02	b 0.295±0.0005
2	d 0.386±0.01	d 0.385±0.0005	a 0.331±0.005	b 0.233±0.0005
4	d 0.355±0.0005	e 0.294±0.01	a 0.328±0.0005	bc 0.199±0.0005
6	c 0.302±0.07	b 0.142±0.006	d 0.318±0.002	bc 0.142±0.006
8	c 0.292±0.0005	b 0.116±0.03	e 0.308±0.0005	d 0.086±0.004
10	c 0.276±0.0005	b 0.086±0.008	c 0.295±0.01	b 0.073±0.0005

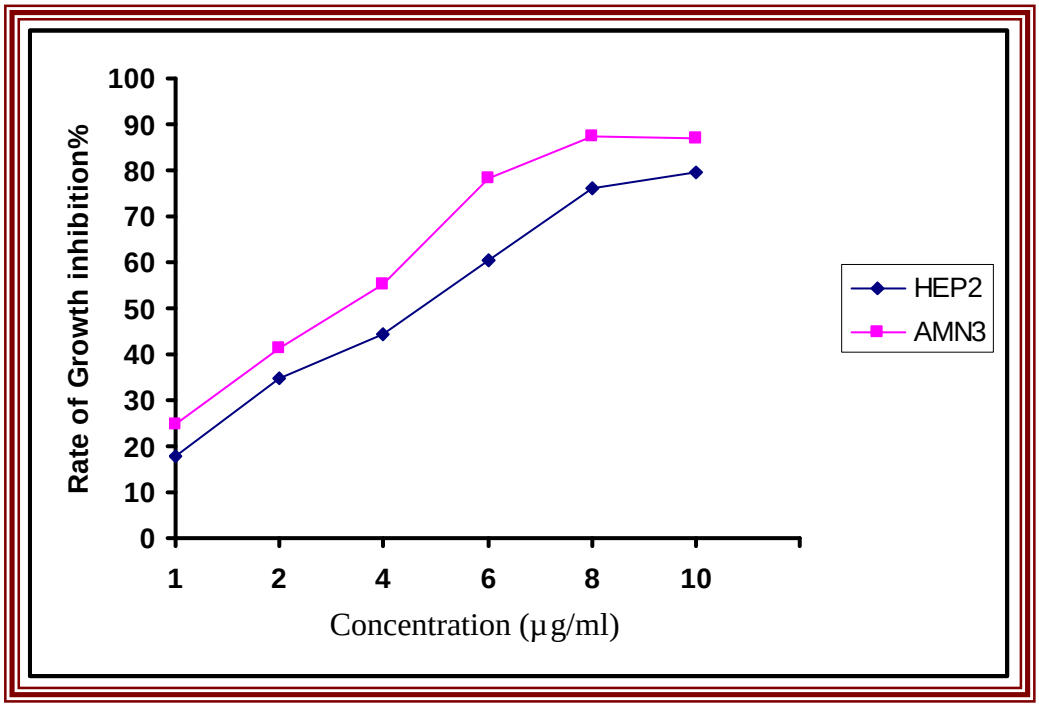
Different letter represent significant differences (P≤0.05) between means of the same column



Figure(4-9) Growth inhibition percentage of MTX drug on HEP-2 and AMN-3 cell line after 72hr of incubation.

The cytotoxic effect of mitomycin-C drug on both AMN-3 and HEP-2 show that there are significant differences ($P<0.05$) between means of cell viability of AMN-3 and HEP-2 cell lines (Table 4-10). MMC causes significant reduction ($P<0.05$) in AMN-3 cell growth in comparison with the negative control . The growth inhibition percentage reachs to (86.9%) for (10µg/ml). No significant reduction ($P>0.05$) was shown on HEP-2 cell line treated with the (1,2,4) µg/ml of MMC , while significant reduction ($P<0.05$) was shown at other concentrations (10 µg/ml) of MMC in comparison with the negative control (Table 4-10), and the GI percentage reachs to (79.6%) for (10µg/ml).

From the above results , it is noticed that MMC has greater effect on HEP-2 and AMN-3 cell line than MTX, while the more decrease in cell viability of MMC is shown on the AMN-3 than HEP-2cell line with high growth inhibition percentage (Figure 4-10).



Figure(4 -10) Growth inhibition percentage of MMC drug on HEP-2 and AMN-3cell line after 72hr of incubation.

The results showes that cell survival in both MTX and MMC treated cultures were progressively decreased with increasing the concentration . Statistical analysis shows significant decrease in cell viability with the increase in concentration of both drugs in both cell lines (Table 4-10) .

The results of plants extract on their effect on both cell lines showes there are also a significant differences ($P<0.05$) between means of cell viability of each HEP-2 and AMN-3 cultures treated with *C.spinosa* extract (Table 4-11).*C.spinosa* cause a significant decrease ($P<0.05$) in cell viability of AMN-3 cell line for all concentrations to reach maximum significant decrease at concentration 1000µg/ml in comparison with the negative control and the growth inhibition percentage reachs to (89.3%) .

The results of HEP-2 cell viability show that there is also significant decrease ($P<0.05$) in cell viability when treated with *C. spinosa* and for all concentrations to reach maximum reduction at the concentration 1000µg/ml

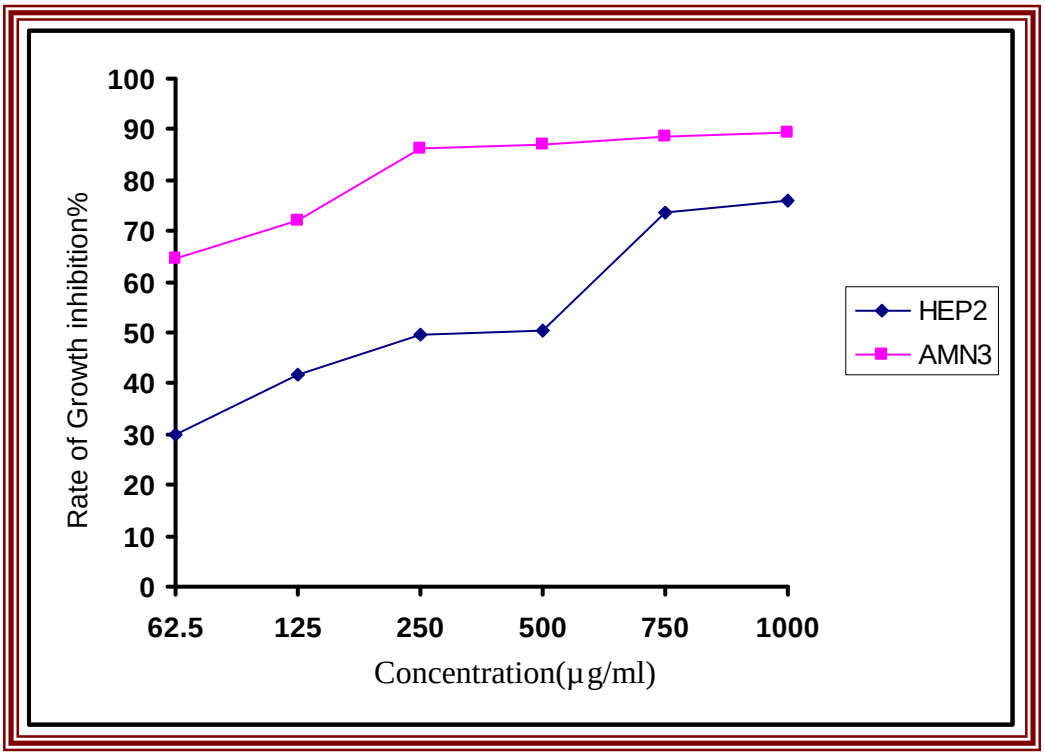
in comparison with the negative control , and the growth inhibition percentage reach to (76%) .

By comparison between the cytotoxic effect exhibited by Capparis extract on both cell lines, the results show that the extract has greater effect (GI. 89.3%) on AMN-3 cell line than the HEP-2 cell line (GI.76%) (Figure 4-11).

Table(4-11)Cytotoxic effect of *Capparis spinosa* and *Rumex acetosella* extracts on the growth of cancer cell line(AMN-3,HEP-2).

Concentration µg/ml	Cell viability (Absorbance)(m+SE)			
	AMN -3		HEP-2	
	C.spinosa	*R.acetosella*	*C.spinosa*	*R.acetosella*
Negative control	a 0.659±0.0005	a 0.659±0.0005	a 0.359±0.0005	a 0.359±0.0005
62.5	b 0.234±0.001	b 0.278±0.01	b 0.252±0.02	b 0.282±0.002
125	b 0.183±0.008	c 0.266±0.01	b 0.209±0.02	b 0.267±0.02
250	b 0.090±0.004	c 0.231±0.02	b 0.181±0.01	c 0.215±0.014
500	b 0.087±0.004	b 0.096±0.006	b 0.178±0.002	c 0.095±0.006
750	b 0.076±0.004	b 0.080±0.005	b 0.094±0.0005	c 0.086±0.004
1000	b 0.070±0.004	b 0.076±0.0005	b 0.086±0.004	b 0.078±0.0005

Different letter represent significant differences (P≤0.05) between means of the same column



Figure(4-11)Growth inhibition percentage of *C.spinosa* on AMN-3 and HEP-2 after 72hr

On the other hand, and on the effect of *R.acetosella* on cell viability of both AMN-3 and HEP-2 cell line culture , the results show that Rumex extract causes significant decrease ($P<0.05$) of AMN -3 cell viability , and this decrease in cell viability is increased with the increasing the Rumex concentrations in comparison with the negative control and the growth inhibition percentage reachs to (88.4%).

There is also a significant decrease ($P<0.05$) of HEP-2 cell viability treated with Rumex extract and for all concentrations in comparison with the negative control (Table 4-11) and the growth inhibition percentage reachs to (78.2%) (Figure 4-12) .

And by comparison between the cytotoxic effect exhibited by Rumex extract on both cell lines , the general trend of these cell lines study showed that Rumex extract had greater effect on AMN-3 which the growth inhibition

percentage reach to (88.4%) than the HEP-2 cell line (GI .78.2%)(Figure 4-12).

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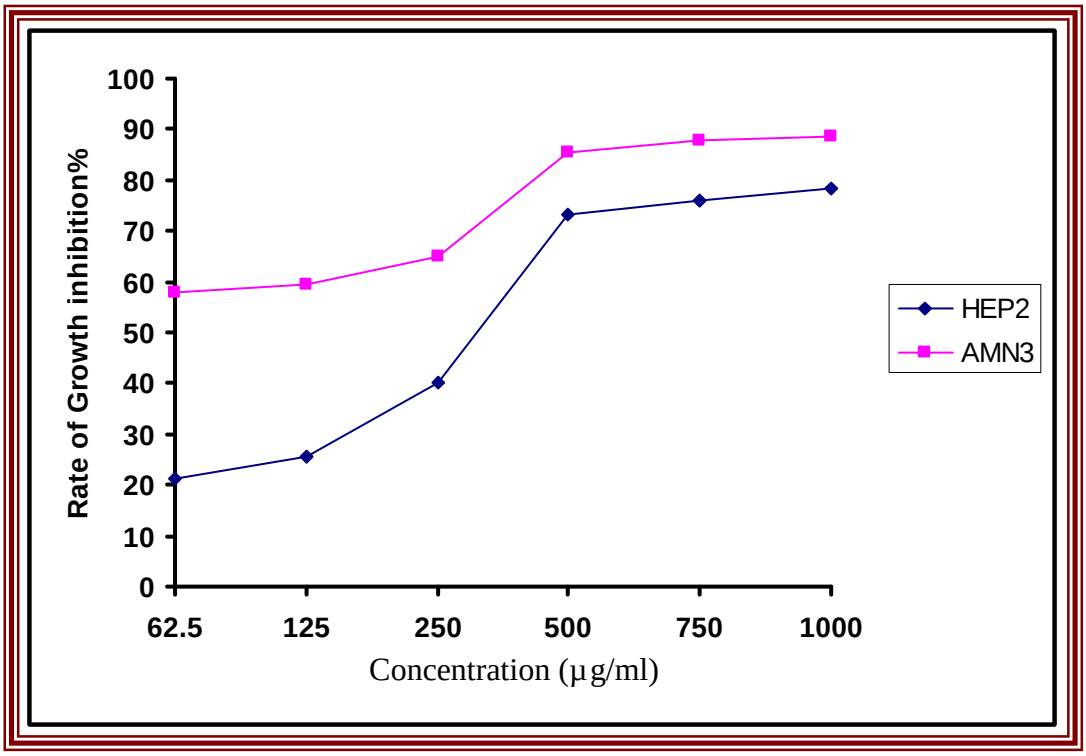
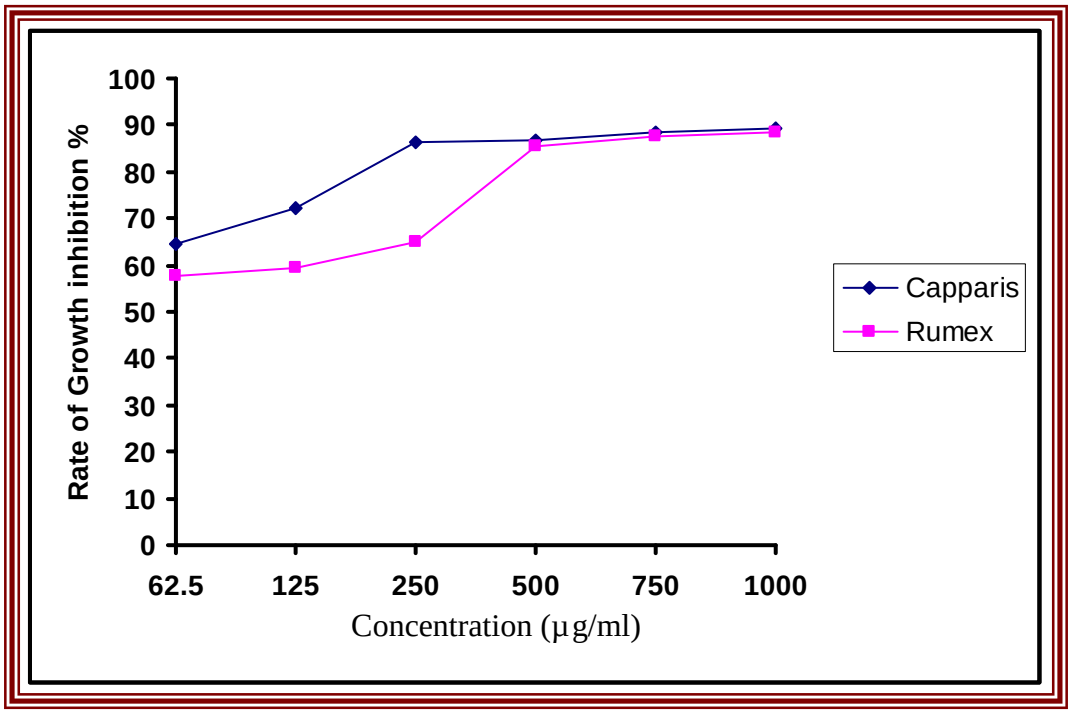
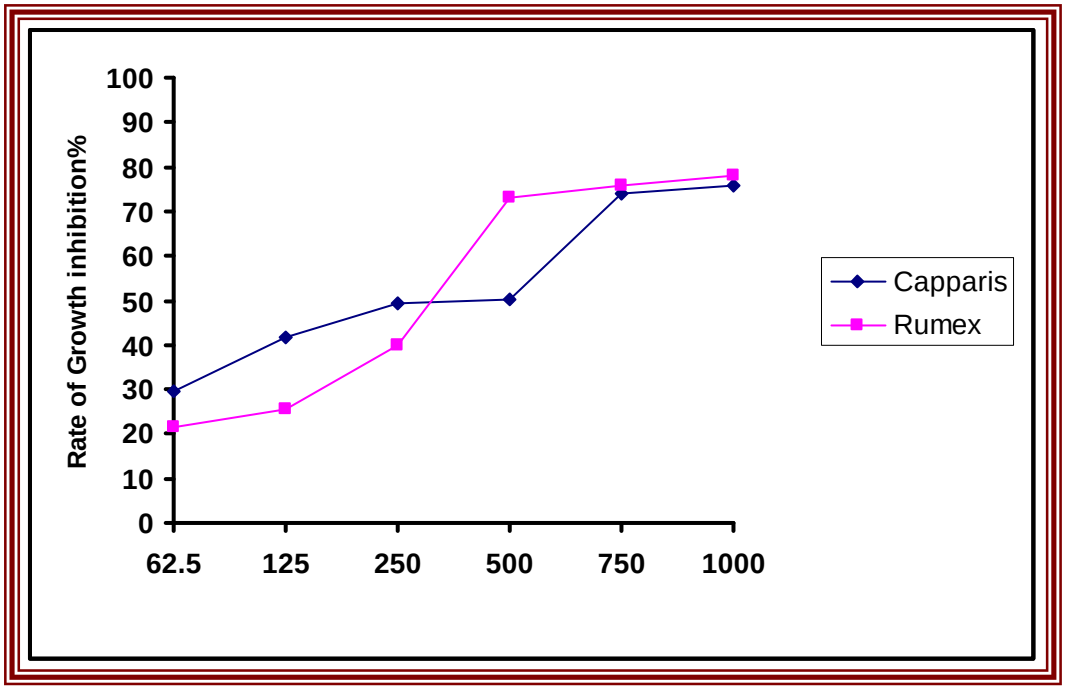


Figure(4-12)Growth inhibition percentage of *R.acetosella* on AMN-3 and HEP-2 after 72hr

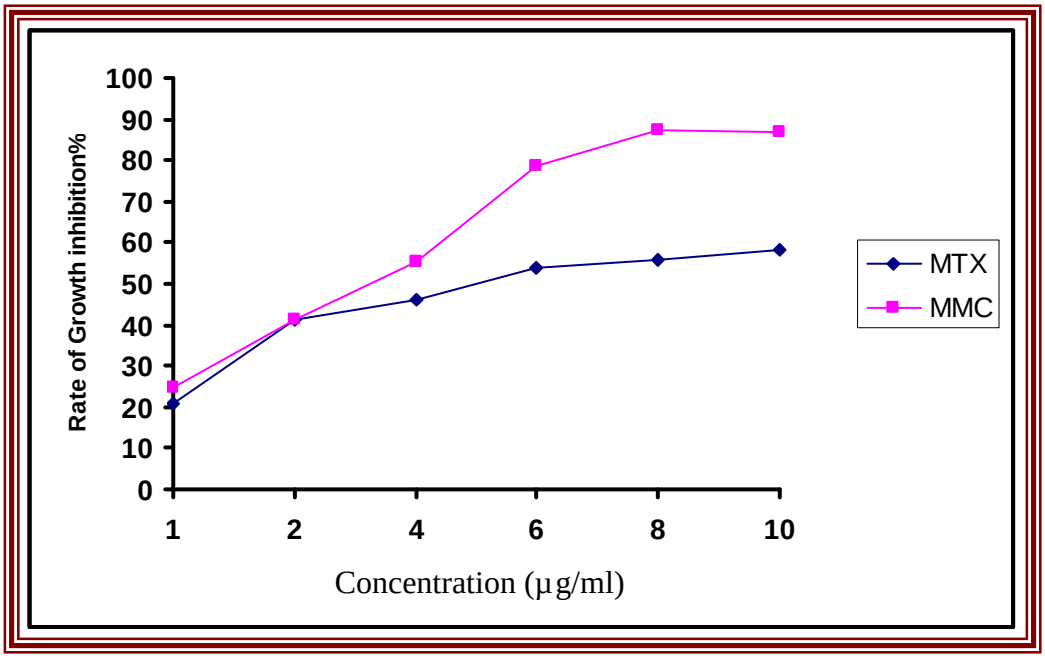
By comparing between the two cell lines from its sensitivity to plants extract show that AMN-3 cell line is more sensitive to both plants extract than the HEP-2 cell line Figure (4-13) and (4-14), while in comparing between the cytotoxic effect exhibited by both plants extract on cell lines , and the cytotoxic effect exhibited by both drugs , the results show that both cell lines are more sensitive to both Capparis and Rumux extract than the both drugs (MMC and MTX)Figure (4-15) and (4-16) .



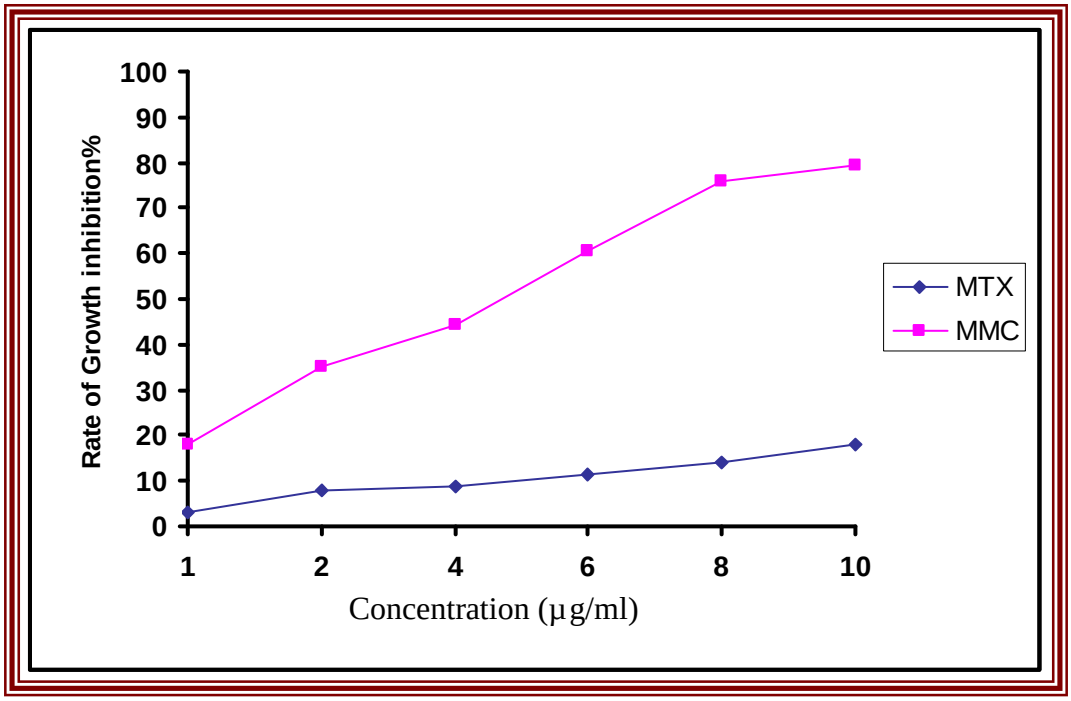
Figure(4 -13) Growth inhibition percentage of *C.spinosa* and *R.acetosella* extracts on AMN-3 cell line after 72hr of incubation.



Figure(4-14) Growth inhibition percentage of *C.spinosa* and *R.acetosella* extracts on HEP-2 cell line after 72hr of incubation.



Figure(4 -15)Growth inhibition percentage of MTX and MMC drug on AMN -3 cell line after 72hr of incubation.



Figure(4-16) Growth inhibition percentage of MTX and MMC drug on HEP-2 cell line after 72hr of incubation.

Cell cycle progression is an important biological events having controlled regulation in normal cells , which almost universally becomes aberrant or deregulated transformed and neoplastic cells. In this regard, targeting deregulated cell cycle progression and its modulation by various natural and synthetic agents are gaining wide spread attention in recent years to control the unchecked growth and proliferation in cancer cells . It was show that many phytochemicals halt are uncontrolled cell cycle progression in cancer cells. Among these phytochemicals , natural flavonoids have been identified in *C.spinosa* as a one of the major classes of natural anticancer agents exerting antineoplastic activity via cell cycle arrest as a major mechanism in various types of cancer cells (Singh and Agarwal ,2006).

The inhibition effect of plants extract in both cell lines may be due to the active constituents of these two plants .Quercetin is a flavonoid molecule ubiquitous in nature , it was detected in *C.spinosa* , a number of its actions make it a potential anti cancer agent , including cell cycle regulation , interaction with type II estrogen binding sites , and tyrosin kinase inhibition (Davis *et al* .,2000).Hollman and Katan,(1997) pointes that quercetin has an inhibitory role in various stage of development in animal studies .

Shen and Weber , (1997) suggestes that quercetin is an anti cancer agent arrests the cell cycle at G1 and S phase boundary. In addition, it has been found that the increase of the concentration of chemotherapeutic agents in some cell resistant cell lines and *in vitro* research demonstrates that quercetin can increase the anti tumor activity of Cisplatin and Busulfan and can be used in conjunction with doxorubicin and etoposide without interfering with their therapeutic action (Bracke *et al* ., 1999).

In addition, *R.acetosella* contains chlorophyll and carotenoids compounds. Chlorophyll raises the amount of oxygen in the body ,some believe that cancer cannot live or thrive when oxygen levels are increased in the body .Carotenoids is known as antioxidant and is very high oxalic acid

which is a contributing factor to the antitumor and anticancer aspects of the herb and it is though that this herb *R.acetosella* work in formula to stop cancer cells from metastasizing (Melanie Nathan , 2002).

Another cause for decreasing the cell viability of both cell lines by *R.acetosella* may be returned to the vitamin K. The anticancer activity of vitamin K has also been demonstrated in a number of *in vitro* studies using both rodent (Chlebowski *et al*.,1985)and human cancer cell lines (Juan and Wu,1993). Patrick , (2002) suggeste that vitamin C is able to inhibit the growth of melanoma in culture ,yet when combine their anticancer activity was much stronger .

Some of secondary metabolites have a selective toxicity on its effect on cancer cells, the cytotoxic effect of plants extract on both tumor cell lines (AMN-3 and HEP-2) and normal cell line (Ref) were compared and this represents a control for comparison ,and by depending on the result, it was found that plant extract has greater cytotoxic effect on two cancer cell line, while it has no identical or significant effect on the REF or normal cell line in comparison with the negative control (Table 4-12). From this, we conclude that selective toxicity may be due to the metabolic factor found in the cancer cell lines but not found in normal cells , like the angiogenic promoters and inhibitors , and associated signaling in both tumor as endothelial cells (Folkman,2002 ,Moteki *et al*.,2002). In addition to DNA of tumor cell found in relaxant shape ,and the DNA molecule was found in a unstable figure because the far away between the H-bond which connect the both strand of DNA and this make easy for compound to interfere or associated to both strands of DNA, while DNA of normal cell has a strong H-bond connect the both strands to each other and make it more stable, so the compounds cannot interfere or associated with DNA strand (Belijanski, 2000).

Table(4-12):Cytotoxic effect of *Capparis spinosa* , *Rumex acetosella* extracts, MTX,MMC drug on the growth of normal cell line(Ref).

Concentration µg/ml	Cell viability (Absorbance) (m±SE)				
	Ref			Ref	
	C.spinosa	*R.acetosella*	Concentration µg/ml	MTX	MMC
Negative control	a 0.235±0.003	a 0.235±0.003		a 0.235±0.003	a 0.235±0.0005
62.5	a 0.221±0.006	a 0.219±0.006	1	b 0.086±0.02	b 0.050±0.002
125	a 0.215±0.005	a 0.219±0.006	2	b 0.073±0.027	b 0.048±0.0005
250	a 0.206±0.004	a 0.210±0.003	4	b 0.071±0.028	c 0.047±0.002
500	a 0.195±0.001	a 0.195±0.013	6	b 0.07±0.025	b 0.047±0.0005
750	b 0.189±0.006	b 0.184±0.006	8	c 0.059±0.008	c 0.046±0.0005
1000	b 0.181±0.002	b 0.057±0.004	10	c 0.057±0.012	c 0.046±0.0005

Different letter represent significant differences (P≤0.05) between means of the same column

Summary

The present study was designed to detect the active constituent of different plants including *Capparis spinosa ,Rumex acetosella*, *Trifolium pratensa* ,and *Solanium nigrum*. The cytogenetic effects of ethanolic extract of *C.spinosa*, methanolic extract of *R.acetosella* and methotrexate on laboratory mice (*in vivo*) and on human blood lymphocyte (*in vitro*), with the comparison between the cytotoxic effects of these two plants extracts with those of MTX and MMC drugs on the tumor and normal cell lines were studied.These investigations were performed under four successive profiles:

A-profile One:

Chemical investigation of *C.spinosa ,R.acetosella,T.pratense and S.nigrum* for different active class compounds are evaluated .The HPLC analysis was carried out for ethanolic extract of *C.spinosa* and methanolic extract of *R.acetosella* using the flavonoids standards (Rutin and Qurecetin) . The following results were obtained:

1- Chemical investigation shows that *C.spinosa* and *R.acetosella* contain different active compounds such as flavonoids and glycosides.

2- HPLC analysis showes that both *C.spinosa* and *R.acetosella* contain high level of rutin and qurecetin flavonoids.

B-Profile Two :

The effects of ethanolic extract of *C.spinosa* ,methanolic extract of *R.acetosella* and methotrexate on albino mice (*in vivo*) were evaluated by employing the following parameters: Mitotic index, chromosome aberration, micronucleus in bone marrow, the total and differential count of leukocyte, mitotic index in mouse testis, sperm head and tail morphology, in addition to serum level of liver function enzyme Glutamic Oxaloacetic Transaminase (GOT),Glutamic Pyruvic

Transaminase(GPT) and specific activity of serum adenosine deaminase (ADA). The following results were obtained:

1- Methotrexate reveals clear effects in reducing mitotic index and increased chromosomal aberration in mouse bone marrow cells(*in vivo*) and caused an increase in the percentage of micronucleus .

2- Methotrexate caused a significant reduction in mitotic index of mouse testis and cause an induction in the abnormalities of sperm head and tail morphology.

3- Methotrexate reveals clear reduction in the total and differential counts of white blood cells by the reduction in lymphocytes, and neutophils.

4-The specific activity of ADA was significantly decreased while GOT and GPT was significantly increased in animals treated with MTX.

5- Ethanolic extract of *C.spinosa* and methanolic extract of *R.acetosella* cause increased in mitotic index, and decrease the spontaneous frequency of chromosome aberration, and micronucleus in mouse bone marrow.

6- Both plants extracts caused a significant increase in mitotic index of mouse testis and decrease the abnormalities in sperms morphology.

7- Both plants extract increase the total and differential counts of white blood cells, through the increasing in the number of lymphocyte , monocyte and neutrophil.

8-The specific activity of ADA was significantly increased while the GOT and GPT were decreased in experimental animals treated with ethanolic extract of *C.spinosa* and methanolic extract of *R.acetosella* .

C-Profile Three:

The cytogenetic effects of ethanolic extract of *C.spinosa*, methanolic extract of *R.acetosella* and MTX drug on human blood lymphocytes (*in vitro*) were evaluated with further parameter: Blast index ,mitotic index , replicative index, chromosomal aberration, micronucleus and sister chromatid exchange. The following results were obtained:

1- Methotrexate reveals clear effects in reducing mitotic index in human blood lymphocytes(*in vitro*) ,and also caused reduction in Blast index, replicative index but an increases was observed in chromosomal aberration and sister chromatid exchange, and an increase in micronucleus *in vitro.*

2- *C.spinosa* and *R.acetosella* extract causes a significant increase in MI,BI,RI and both cause reduction in CA, MN, and SCEs in human blood lymphocyte *in vitro.*

D-Profile Four:

The cytotoxic activity of *C.spinosa , R.acetosella* extract, MTX and MMC drug on tumor cell lines were evaluated by using two tumor cell lines Human Epidermoid Larygocarcinoma(HEP-2) ,and Ahmed – Mohammed, Nahi mammary adenocarcinoma(AMN-3) ,and on normal cell line Rat embryo fibroblast (Ref). The following results were obtained:

1- Both MTX and MMC cause decrease in the number of cell viability of both tumor cell lines(HEP-2 and AMN-3) at high concentration of these drugs and both drugs have cytotoxic activity on normal cell line(Ref).

2- Both plants extract *C.spinosa* and *R.acetosella* indicated a clear cytotoxic activity with high significant in two tumor cell lines (HEP-2,AMN-3) and for all concentration .

3- These two plants *C.spinosa* and *R.acetosella* carried a compounds have less genotoxic effects than used anti cancer drugs MTX or MMC but they were very effective against tumor cells *in vitro*.

Dedication

TO MEMORY OF MY MOTHER

TO MY BROTHER

AND

MY SISTERS

EBTEHAL

قَالُواْ سُبْحَانَكَ لاَ عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ ۝]

صدق الله العظيم

سورة البقرة "٣٢"

الخلاصة

أجريت هذه الدراسة بهدف الكشف عن بعض المركبات الفعالة في نباتات مختلفة تتضمن نبات الكبروالحميض و البرسيم ،وعنب الذيب ، ودراسة تأثيرمستخلصات نبات الكبر ونبات الحميض وعقار الميثوتركسيت في الصفات الو راثية الخلوية لخلايا نقي العظم في الفئران المختبرية (داخل الجسم) وخلايا الدم المحيطي للإنسان (خارج الجسم)،بالاضافة إلى دراسة تأثير الفعالية السمية لهذه النباتات مع عقار الميثوتركسيت وعقارالمايتومايسين في خطوط خلايا ورمية وخط خلايا طبيعي ومن خلال المعايير التالية :

أ-الجانب الأول

الفحوصات الكيميائية لنباتات الكبر والحميض ونبات البرسيم وعنب الذيب للكشف عن مختلف مجاميع المكونات الفعالة .استخدمت تقنية كروموتوغرافيا الأداء العالي السائلة على نبات الكبر والحميض للكشف عن الفلافينويدات باستخدام مركب Quercetin,Rutin القياسية. وقد تم الحصول على النتائج آلاتية:

١- بينت نتائج الكشف الكيميائي احتواء نباتات الكبر والحميض على المركبات الفعالة مثل الكلايكوسيدات والفلافينويدات .

٢- اظهرتحليل كروموتوغرافيا الأداء العالي السائلة احتواء كل من نبات الكبر ونبات الحميض على كمية عالية من مادتي Quercetin، Rutin مقارنة بالمركبات القياسية.

ب- الجانب الثاني

تضمن الجانب الثاني دراسة التأثيرات الو راثية الخلوية للمستخلص الايثانولي لنبات الكبر والمستخلص الميثانولي لنبات الحميض وعقار الميثوتركسيت وباستخدام الفئران المختبرية(داخل الجسم)ومن خلال المعايير الاتية : معامل الانقسام الخيطي والتغيرات الكروموسومية وتكوين النوى الصغيرة في خلايا نقي العظم ،ودراسة العدد الكلي والتمايزي في خلايا دم الفئران ومعامل الانقسام الخيطي في الخلايا الجنسية للفأر ودراسة شكل الرأس والذيل لنطف الفئران بالاضافة إلى الفعالية النوعية لأنزيم ادينوسين دي امينيز وقياس فعاليةانزيمات وظائف الكبد(GOT,GPT) في المصل.

وقد تم الحصول على النتائج آلاتية:

١- أظهر الميثوتركسيت تأثيرات سلبية تمثلت بانخفاض معامل الانقسام وزيادة في التغييرات الكروموسومية في خلايا نقي العظم(داخل الجسم) وزيادة نسبة تكوين النوى الصغيرة .

٢- سبب الميثوتركسيت انخفاضا في معامل الانقسام الخيطي للخلايا الجنسية في الفار وحفز على زيادة التشوهات لرأس وذيل النطف.

٣- لقد أظهر الميثوتركسيت تأثيرات سلبية تمثلت بانخفاض العدد الكلي والتفريقي لكريات الدم البيض من خلال الانخفاض لاعدادالخلايا اللمفية والوحيدة والعدلة.

4- انخفاض مستوى أنزيم ADA وأرتفاع مستوىانزيمي**GOT,GPT** في حيوانات التجربة المعاملة بالميثو تركسيت.

٥- ادى كل من المستخلص الايثانولي لنبات الكبروالمستخلص الميثانولي لنبات الحميض إلى زيادة في معامل الانقسام الخيطي ونقصان في التغيرات الكروموسومية وتكوين النوى الصغيرة التلقائي.

٦- سبب كل من النباتيين زيادة معنوية في معامل الانقسام للخلايا الجنسية للفأر ونقص في نسبة التشوهات في رأس وذيل نطف الفأر .

٧- ظهورزيادة في العدد الكلي والتفريقي لكريات الدم البيض من خلال الزيادة بأعداد الخلايا اللمفية والوحيدةوالعدلة بعد المعاملة بمستخلص نبات الكبر ونبات الحميض.

٨- لوحظ الزيادة في مستوى **ADA** وانخفاض في مستوى **GOT,GPT** في حيوانات التجربة المعاملة بالمستخلص الايثانولي لنبات الكبروالمستخلص الميثانولي لنبات الحميض.

ت-الجانب الثالث

تضمن هذا الجانب دراسة التأثيرات الو راثية الخلوية للمستخلص الايثانولي لنبات الكبروالمستخلص الميثانولي لنبات الحميض وعقار الميثوتركسيت في خلايا الدم المحيطي للإنسان(خارج الجسم)اعتمادا على المعايير الاتية :معامل الارومي والانقسام الخيطي ومعامل التضاعف والتغيرات الكروموسومية وتكوين النوى الصغيرة والتبادل الكروماتيدي الشقيق خارج الجسم . وقد تم الحصول على النتائج آلاتية:

١- سبب الميثوتركسيت في خلايا الدم المحيطي(خارج الجسم) انخفاضا واضحا في معامل الانقسام الخيطي وادى أيضا الى انخفاض في المعامل الارومي ومعامل التضاعف ،و سبب زيادة في نسبة التغيرات الكروموسومية والتبادل الكروماتيدي الشقيق ، وزيادة في نسبة تكوين النوى الصغيرة خارج الجسم.

٢- ادت المعاملة بمستخلص نبات الكبر ونبات الحميض الى زيادة في معامل الانقسام الخيطي والمعامل الارومي ومعامل التضاعف، وكلا المستخلصين سببا انخفاضا في التغيرات الكروموسومية ومعدل تكوين النوى الصغيرة والتبادل الكروماتيدي الشقيق في خلايا الدم المحيطي خارج الجسم.

ث-الجانب الرابع

درس الفعالية السمية لمستخلص الكبر والحميض وعقار الميثوتركسيت والمايتومايسين على نوعين من خطوط الخلايا الورمية هما سرطان الحنجرة وسرطان غدد الثدي بالإضافة إلى خلايا الخط الطبيعي لجنين الجرذ. وقد تم الحصول على النتائج آلاتية:

١- ادى كلا العقارين MTX,MMC الى انخفاض في نمو الخلايا الورمية HEP-2,AMN-3 وعند التراكيز العالية لهاذين العقارين ، وكلا العقارين اظهرا تاثيرا سميا واضحا في خط الخلايا الطبيعي لجنين الجرذ (Ref).

٢- اظهرت مستخلصات كل من النباتين فعالية سمية واضحة في خطي خلايا سرطان الحنجرة وسرطان غدد الثدي (HEP-2,AMN-3) ولجميع التراكيز.

٣- تحتوي مستخلصات كلا النباتين على مركبات ذات سمية اقل من استخدام الادوية المضادة للسرطان (MTX,MMC) وذات فعالية كبيرة ضد الخلايا السرطانية خارج الجسم .

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

التأثيرات البايولوجية لمستخلص نبات الكبر و الحميض في الخلايا الطبيعية والورمية للانسان والحيوان

رسالة

مقدمة الى كلية العلوم جامعة النهرين

وهي جزء من متطلبات نيل درجة دكتوراه فلسفة في التقانة الاحيائية

من قبل

إبتهال حسين محمود النعيمي

بكالوريوس تقانة احيائية / جامعة النهرين/ ٢٠٠٠

ماجستير تقانة احيائية / جامعة النهرين/ 2002

صفر ١٤٢٨هـ

اذار ٢٠٠٧م