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

Antimicrobial and Cytogenetic studies on the effect of *Ocimum sanctum* on albino male mice

A Thesis

Submitted to the College of Science of Al-Nahrain University as a Partial Fulfillment of the Requirements for the Degree of Master of Science in Biotechnology

By

Haider Jabbar Ibrahim

B.Sc. Biotechnology (2003)

Al-Nahrain University

Thul-huja **December**

1429 **2008**

Supervisors Certification

We certify that this thesis was prepared under our supervision in the College of Science, Al-Nahrain University as a partial requirement for the degree of Master of Science in Biotechnology.

Signature:
Supervisor: **Dr. Khulood W. Al-Samarae**
Scientific Degree: Professor

Signature:
Supervisor: **Dr. Nabeel K. Al-Ani**
Scientific Degree: Assistant Professor

In view of the available recommendations, I forward this thesis for debate by the examining committee.

Signature:
Name: **Dr. Kadhim Mohammad Ibrahim**
Scientific degree: Professor
Title: Head of Biotechnology Department
Date:

Committee Certification

We, the examining committee, certify that we have read this thesis and examined the student in its contents and that, according to our opinion, is accepted as a thesis for the degree of Master of Science in Biotechnology.

Signature:
Name:
Scientific Degree:
Date:

Signature:
Name:
Scientific Degree:
Date:

(Member\ supervisors)

Signature:
Name:
Scientific Degree:
Date:
(Member)

Signature:
Name:
Scientific Degree:
Date:
(Member)

Signature:
Name:
Scientific Degree:
Date:
(Chairman)

I hereby certify upon the decision of the examining committee

Signature:
Name: **Dr. Laith Abdul Aziz Al-Ani**
Scientific Degree: Assistant Professor
Title: Dean of College of Science
Date:

Acknowledgments

At the beginning praise to great **Allah** the lord of the Universe and creation, the merciful and kindness, and blessing upon **Mohammed** prophet of Allah and upon his family and companions.

First I thanks and appreciate my supervisors **Dr. Khulood Al-Samaraie** and **Dr. Nabeel Al-Ani** for great support throughout my studies and special thanks to the head of Biotechnology Department Dr**. Kadhim Mohammad Ibrahim** and all staff of Biotechnology Department College of science.

Special thanks to **Dr. Ali Ad'hiah** *and* **Dr. Ikhlas.**

It's my pleasure to thank my collogues **Azad, Omar, Anas, Hussain, Hussam Ali, Dhilal, Worood, Marriam, Rojwan, Noor, Mena, Haneen** and **Asmaa** for their assistance.

Haidar

Summery

The present study was carried out to investigate the chemical analysis of *Ocimum sanctum* for different active compounds, and prepare methanolic and hexane extracts to determine their antimicrobial activity (*in vitro*). The cytogenetic effects of (methanolic and hexane extracts) as well as methotrexate (MTX) was evaluated in albino male mice (*in vivo*). The cytogenetic analyses were extended to include mitotic index and chromosomal aberrations.

The study also tried to investigate the role of *O. sanctum* methanolic and hexane extracts in reducing the activity of albino mice sperms morphology.

The antimicrobial effects of plant extracts were investigated on different types of microorganisms (*Staphylococcus aureas, Pseudomonas aeruginosa* and *Candida albicans*), the tested concentrations of methanolic and hexane extracts were 75, 37.5 and 18.75 mg/ml. Results showed that methanolic extract had a good effect on *Candida albicans* and slight effect on *Staphylococcus aureas* at three concentrations used, while hexane extract showed there was no effect on tested microorganisms.

The cytogenetic effects of plant extract were investigated after seven days of treatment mice with three doses of MTX, the doses were 0.6, 0.8 and 1 mg/kg, while doses of *O. sanctum* were 200, 400, and 600 mg/kg.

The following results were obtained:

1. Chemical analyses showed that *O. sanctum* methanolic leaves extract contain flavonoids.
2. Chemical analyses showed that *O. sanctum* hexane leaves extract contain steroids.

3. Methotrexate revealed clear effects in reducing mitotic index and increased chromosomal aberrations in mouse bone marrow cells.
4. The inductions in abnormalities of sperm morphology were observed in MTX treatment especially after 35 days of treatment.
5. Methanolic extract of *O. sanctum* exhibited an increase in mitotic index and decrease in chromosomal abbrations, while hexane extract has no effects.
6. Treatment with *O. sanctum* methanolic extract results in increasing of malformation of sperm morphology like the formation of amorphous head and coiled tail especially after 35 days of treatment, while hexane extract has no effects.

List of Content

List of abbreviations

Abbreviations	key
CA	Chromosomal Aberrations
DHFR	Dihydrofolate reductase
DNA	Deoxyribonucleic acid
D.W	Distilled water
FSH	Follicle stimulating Hormone
g	gram
I.P	Intrapritonial
Kg	Kilo gram
LD50	Lethal Dose 50
M	Molarities
mg	Milligram
MI	Mitotic index
min	minute
Ml	millileter
µg	microgram
MTX	Methotrexate
O.sanctum	*Ocimum sanctum*
PBS	Phosphate Buffer Saline
PCE	Polychromatic erythrocytes
RNA	Ribonucleic acid
RPM	Round per minute
WHO	World Health Organization

List of Tables

List of Figures

Chapter One

Introduction and Literatures Review

Introduction and Literature Review

1.1 Introduction

Herbal and traditional medicines had been used for thousands of years to improve human health, and proved to have both medicinal and nutritional value (Eisenberg *et al*., 1993).

A variety of herbs and herbal extracts contain different phytochemicals, including flavonoids, terpenoids, lignans, sulfides, polyphenolics, cartenoids, coumarins, saponins, and plant sterols with biological activity that can provide therapeutic effects like reducing high blood cholesterol concentrations, some protection against cancer, and stimulate the immune system (Farrell, 1985). Today herbal preparations are widely used for a host of common ailments and conditions, such as anxiety, arthritis, cold, coughs, constipation, fever, headaches, infections, intestinal disorders, stress, ulcers, and weakness (Tyler, 1994). Additionally the plants had served humans well as valuable components of seasonings, beverages, cosmetics, and dyes. The World Health Organization (WHO) estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts or their active components like salicylic acid (a precursor of aspirin) obtained from willow tree bark (*Salix alba*) to help relieve fevers (Bruneton, 1995).

Several commonly used herbs had been identified by the National Cancer Institute (NCI) as possessing cancer – preventive properties, these herbs include members of the Alliums family; members of the Labiatae family; members of the Zingiberaceae family; licorice root; green tea and others (Caragay, 1992).

Leukaemia is a progressive, malignant disease of the blood forming organs, characterized by distorted proliferation and development of leukocytes and their precursors in the blood and bone marrow, which can be classified clinically on the basis of duration and character of disease into (a) acute leukaemia and (b) chronic leukaemia (Goldmen and Tarig, 1999).

Cytotoxic chemotherapeutic agents are the standard therapy for acute lymphoblastic leukaemia and known to be mutagenic and carcinogenic (Andersone *et al.,* 1972), so the *O. sanctum* was one of the plants that selected to study its biological activity including cytotoxicity due to the presence of certain active compounds like flavonoids, terpenoids, lignans, sulfides, polyphenolics, cartenoids, coumarins, saponins, (Kanazawa *et al*., 2004).

Aims of study:

1. Detection of some classes of active compounds in methanolic and hexane extracts from *O. sanctum* leaves.
2. Studying the antimicrobial activity of methanolic and hexane extracts from *O. sanctum* leaves against different types of microorganisms.
3. Studying the cytogenetic effects of methotrexate by using (mitotic index and chromosomal aberration) in mouse bone marrow cells (*in vivo*).
4. Studying the cytogenetic effects of methanolic and hexane extracts by using (mitotic index and chromosomal aberration) in mouse bone marrow cells (*in vivo*).
5. Studying the effect of *O. sanctum* methanolic and hexane extracts and methotrexate on sperm head and tail morphology in albino male mice.

1.2 Literature Review

1.2.1 Medicinal Plants

Plants are the oldest friends of mankind. They do not only provided food and shelter but also served the humanity to cure different ailments, and according to the world health organization (WHO), about three-quarters of the world population relies upon traditional remedies (mainly herbs) for the health care of its people (Calixto, 2005). Herbal medicine is increasingly gaining greater acceptance from the public and medical profession due to greater advances in the understanding of the mechanisms by which herbs positively influence health and quality of life (Berman, 2000). Historians from all around the world have produced evidence to show that apparently all primitive peoples used herbs, and often in a sophisticated way. Quinine from Cinchona bark was used to treat the symptoms of malaria long before the disease was identified, and the raw ingredients of a common or garden aspirin tablets were a popular painkiller for far longer than we had and access to tablet-making machinery (Pieters and Vlietinick, 2005). By the middle of the nineteenth century at least 80% of all medicines were derived from herbs. Even today, many pharmacological classes of drugs include a natural product prototype. Aspirin, atropine, artimesinin, colchicines, digoxin, ephedrine, morphine, physostigmine, pilocarpine, quinidine, reserpine, taxol, tubocurarine, vincristine, and vinblastine are a few examples of what medicinal plants have given us in the past (Gubri-Fakim, 2005). More of these plants-derived drugs were originally discovered through the study of traditional cures and folkloric knowledge of indigenous people and

some of these could not be substituted despite the enormous advancement in synthetic chemistry (Gilani and Atta-ur-Rahman, 2005). In this regards, basil (*Ocimum Sanctum*) is a further subject of medical plants, and the literatures are rich in overwhelming us with its medicinal uses (Kasinathan *et al*., 1972; Vrinda and Uma Devi, 2001; Singh *et al*., 2005)

1.2.2 Common Names and Classification

In English literature, several common names are used to describe *Ocimum sanactum*. However, from taxonomists point view, the plant classification is (Internet, 2004):

1. Kingdom: Plantae
2. Division: Magnoliophyta
3. Class: Mangoliopsida
4. Order: Lamiales
5. Family: Lamiaceae
6. Genus: Ocimum
7. Species: *O. sanctum* or *O. tenuiflorum*

Figure (1-1): *Ocimum sanctum* plant.

1.2.3 Plant Description and Distribution

***Ocimum tenuifolium or sanctum* L.** (Lamiaceae) (known as **Holy basil** in English, and **Tulasi** in Sanskrit), commonly known, as Tulsi in India is a local herb containing potent antioxidants flavanoids (orientin, vicenin) and phenolic compounds (eugenol, cirsilineol, apigenin) (Sethi *et al*., 2003). It is well known as an aromatic plant in the family Lamiaceae. Apart from its culinary uses, for which it is known across the world, it is also used as a medicinal plant, and has an important role within many traditions of Hinduism, wherein devotees perform worship involving Tulasi plants or leaves. Native to India, it is a short lived perennial herb or small shrub, often grown as an annual. The foliage is green or purple, strongly scented. Leaves have petioles, and are ovate, up to 5cm long, usually somewhat toothed. Flowers are white, tinged purple, borne in racemes.

Its aroma (Eugenol and caryophyllene) is distinctively different from its

close cousin, the Thai Basil which is sometimes wrongly called Holy Basil, in shops and on the internet, but they can be distinguished by their aroma and flavour. Holy Basil is slightly hairy, whereas Thai Basil is smooth and hairless; Holy Basil does not have the strong aniseed or licorice smell of Thai Basil; and Holy Basil has a spicy flavor sometimes compared to cloves (Gupta, *et al*., 2002).

1.2.4 Chemical constituents

Chemical analyses of basil revealed that the plant is rich in the following constituents:

1.2.4.1 Essential oil

The principle components are monoterpenoids, such as eugenol, thymol, estragole and 1,8-cineol, together with sesquiterpenes such as α-humulene, β-caryophyllen and viridiflorol (Pitatevic *et al*., 1984; Lawrence, 1992). Ocimum sanctum oil was found to possess significant antiulcer activity, anti-bacterial and insecticidal (Singh and Majumdar, 1999; Mahajan and Wasule, 2008).

1.2.4.2 Hydroxycinnamic acid derivatives

Principally they are presented by caffeic acid dimmer, rosmarinic acid. Caffeic acid trimers have also been isolated. Collectively, these and similar compounds are sometimes described as "tannins". Other hydroxycinnamic compounds are also present, including 6-feruloyl-glucose and a polyalcohol derivative of it, three hydroxycinnamic esters of disaccharides and free caffeic acid (Lu *et al*., 1999).

1.2.4.3 Phenolic diterpenes

Carnosic acid, a tricyclic diterpene, occurs in the fresh leaves and to some extent in the dried leaves and certain types of extracts. However, carnosic acid is fairly unstable and readily auto-oxidiseto form lactones,

especially the bitter-tasting lactone carnosol. In turn, carnosolcan degrade further to produce other phenolic diterpenes with lacton structures, such as rosmanol, epirosmanol, 7-methoxyrosmanol and galdosol, which have been identified in *Ocimum sanctum* leaves (Kavvadiase *et al*., 2003).

1.2.4.4 Triterpenes

They occur as pentacyclic triterpenes acids, which are mainly ursolic and oleanolic acid, and the triterpene alcoholic α-and β-amyrin (Wang *et al*., 2000).

1.2.4.5 Flavonoids

Such as orientin, vicenin, apigenin, polyphenols, and anthocyanins (Juliani and Simon 2002; Nayak and Uma Devi, 2005).

1.2.4.6 Phenolic glycosides

They represent a diverse ring including, picin (4-hydroxyacetophenone glucoside), 4-hydroxyacetophenone 4-(6'-apiosyl)-glucoside**,** *cis*- and *trans*-*p*-coumaric acid 4-(2'-apiosyl)- glucoside isolariciresinol 3-glucoside and 1-hydroxypinoresinol 1- glucoside (Wang *et al*., 2000).

1.2.4.7 Polysacchrides

Crude fractions rich in water-soluble arabinogalactans, high molecular weight pectin and glucuronoxylan-related polysaccharides have been isolated from the aerial parts of basil (Cepek *et al*., 2003).

1.2.4.8 Other constituents

Include small amount of benzoic acid derivatives (*p*-hydroxybenzoic, gentisic, syringic and other acids) and phytosterols (β-sitosterol and stigmasterol) (Kennedy *et al*., 2006).

1.2.5 Biological potentials

Several biological potentials had been described for different extracts of *Ocimum sanctum* or its natural products. They could be summarized in the following:

1.2.5.1 Antioxidant activity

The leaf extracts exhibit strong antioxidant activity, largely attributable to various phenolic constituents including phenolic diterpenes such as carnosol and hydroxycinnamic acid derivatives, notably rosmarinic acid (Lamaison *et al*., 1991; Wang *et al*., 2000). In a carotene bleaching test, the antioxidative activity of a dry acetone extract from leaves was found to be 101-116% of that of the synthetic antioxidant butylated hydroxytoluene (Dapkevicius *et al*., 1998). Lipid peroxidation in both enzyme-dependent and enzyme- independent test system were inhibited more effectively by a dry 50%-methanolic extract from aerial parts of leaves than by α-tocopheryl acid succinate (as a positive control). The antioxidant activity was attributed mainly to phenolic compounds, rosmarinic acid being the main contributor due to its high concentration in the extract (Hohmann *et al*., 1999; Zupko *et al*., 2001). Recently, it had been demonstrated that the leaf methanolic extract of basil can improve the antioxidant status of livers in mice and rats (Lima *et al.,* 2005).

1.2.5.2 Antimicrobial activity

Antiviral, antibacterial and antifungal activites of basil have been demonstrated. With respect to antiviral potentials, extracts of the plant were effective in the treatment of hepatitis B virus (Xiong, 1993), vesicular stomatitis viral infection (Sivropoulou *et al*., 1997) and *Herpes labialis* (Saller *et al*., 2001). As antibacterial, different extracts of basil were effective against Gram-negative, as well as, Gram-positive bacteria (*Bacillus subtilits, Echerichia coli, Proteus mirabilis, and Staphylococcus*

aureus) (Hammer *et al*., 1999; Bariceviv *et al*., 2001; Pereira *et al*., 2004; Rios and Recio, 2005).

Similarly, different fungus species (*Botrytis cinerea, Ehrysanthemum morifolium, Trichophyton mentagrophytes and Epidermophyton floccosum*) showed a sensitive response to the fixed oils extracted from basil (Catra *et al*., 1996).

1.2.5.3 Antimutagenic and antitumor activities

Ocimum is considered as plant that has chemical constituents with antimutagenic and antitumor potentials. In this regard, 18 compounds were isolated from the species *Ocimum sanctum* and tested for their cytotoxicity *in vitro* against cancer cells from colon, lung, ovary and skin. Results suggested that such compounds were effective in inhibiting the proliferation of these cells (Sato *et al*., 1992; Ryue *et al*., 1997). Such findings were further confirmed on mice with induced skin tumer, and the conclusion favored that the plant compounds are cytostatic rather than cytotoxic (Gali-Muhtasib and Affra, 2000). However, cytotoxic and DNA-damaging effects of diterpenoid quinines from the roots of *Ocimum sanctum* on cultures of colonic and hepatic human cells have been demonstrated *in vitro* (Santos-Gomes *et al*., 2002). Furthermore, *Ocimum sanctum* extracts with *in* vivo antimutagenic effects have also been reported (Vujosevic and Blagojevic, 2005).

1.2.6 Medicinal uses of *Ocimum sanctum*

1.2.6.1 Anthelmintic activity

The essential oil of *Ocimum sanctum* and eugenol, tested in vitro, showed potent anthelmintic activity in the *Caenorhabditis elegans* model. Eugenol exhibited an ED_{50} of 62.1 μg/ml. Eugenol being the predominant

component of the essential oil, is suggested as the putative anthelmintic principle (Fitoterapia, 2001).

1.2.6.2 Effect on carbohydrate metabolism

Ocimum sanctum (OS) has been mentioned in Indian system of traditional medicine to be of value in the treatment of diabetes mellitus. We have previously shown that OS shows a dose-dependent hypoglycemic effect and prevented rise in plasma glucose in normal rats. It also showed significant antihyperglycemic effect in STZ-induced diabetes. The present study was undertaken to assess the effect of OS on three important enzymes of carbohydrate metabolism [glucokinase (GK) (EC 2.7.1.2), hexokinase (HK) (EC 2.7.1.1) and phosphofructokinase (PFK) (EC 2.7.1.11)] along with glycogen content of insulin-dependent (skeletal muscle and liver) and insulin-independent tissues (kidneys and brain) in STZ (65 mg/kg) induced model of diabetes for 30 days. Administration of OS extract 200 mg/kg for 30 days led to decrease in plasma glucose levels by approximately 9.06 and 26.4% on 15th and 30th day of the experiment. Liver and two-kidney weight expressed as percentage of body weight significantly increased in diabetics ($P<0.0005$) versus normal controls. OS significantly decreased renal ($P<0.0005$) but not liver weight. Renal glycogen content increased by over 10 folds while hepatic and skeletal muscle glycogen content decreased by 75 and 68% in diabetic controls versus controls. OS did not affect glycogen content in any tissue. Activity of HK, GK and PFK in diabetic controls was 35, 50 and 60% of the controls and OS partially corrected this alteration (Vats, *et al*.; 2004).

1.2.6.3 Antiulcer activity

Ocimum sanctum (OS) is known to possess various therapeutic properties. We evaluated its anti-ulcerogenic activity in cold restraint (CRU), aspirin (ASP), alcohol (AL); pyloric ligation (PL) induced gastric ulcer models in Sprague–Dawley rats, histamine-induced duodenal (HST) ulcer in guinea pigs, and ulcer-healing activity, in acetic acid-induced (AC) chronic ulcer model. We found that OS decreased the incidence of ulcers and also enhanced the healing of ulcers. OS at a dose of 100 mg/kg was found to be effective in CRU (65.07%), ASP (63.49%), AL (53.87%), PL (62.06%), and HST (61.76%) induced ulcer models and significantly reduced free, total acidity and peptic activity by 72.58, 58.63, 57.6%, respectively, and increased mucin secretion by 34.61%. Additionally, OS completely healed the ulcers within 20 days of treatment in AC. We observed that anti-ulcer effect of OS may be due to its cytoprotective effect rather than antisecretory activity. Conclusively, OS was found to possess potent anti-ulcerogenic as well as ulcer-healing properties and could act as a potent therapeutic agent against peptic ulcer disease (Dharmani, *et al*.; 2004).

1.2.6.4 Effect on thyroid function

The effects of*Ocimum sanctum*leaf extract on the changes in the concentrations of serum triiodothyronine (T_3), thyroxine (T_4) and serum cholesterol; in the activities of hepatic glucose-6-phosphatase (G-6-P), superoxide dismutase (SOD) and catalase (CAT); hepatic lipid peroxidation (LPO) and on the changes in the weight of the sex organs were investigated. While the plant extract at the dose of 0.5 g kg^{-1}body wt. for 15 days significantly decreased serum T_4concentrations, hepatic LPO and G-6-P activity, the activities of endogenous antioxidant enzymes, SOD and CAT were increased by the drug. However, no marked changes were observed in serum T_3level, T_3/T_4ratio and in the

concentration of serum cholesterol. It appears that *Ocimum sanctum* leaf extract is antithyroidic as well as antioxidative in nature (Panda and Kar, 1998).

1.2.6.5 Antiasthma activity

The ethanol extract of *ocimum sanctum* was evaluated for antiasthmatic activity by using various *in vitro* and *in vivo* animal models. *In vitro* models like isolated goat tracheal chain preparation and isolated guinea pig ileum preparation were studied to know basic mechanism by which extract shows relaxant activity. The study showed that extract is effective against histamine-induced contraction. In isolated goat tracheal chain preparation and isolated guinea pig ileum preparation extract exhibits maximum relaxant effect ($p<0.01$) against histamine at concentrations 100mg/ml and 25mg/ml respectively. Animal studies involved use of histamine induced bronchoconstriction in guinea pigs, egg albumin induced passive paw anaphylaxis in rats and haloperidol-induced catalepsy in mice. These studies showed significant ($p<0.01$) protection at lower doses while further increase in the dose level showed reduced activity. Biochemical estimations in milk-induced total leukocytes count and milk-induced differential leukocyte count were also studied. In this study there was maximum increase in leucocytes and lymphocytes (99%) and maximum decrease in eosinophils up to 0% at dose 375mg/kg p.o. body weight was observed. The results of these studies indicated usefulness of ethanol extract of *ocimum sanctum* in asthma (Pandit, *et al*.; 2008).

1.2.7 Methotrexate

Methotrexate is an antimetabolite drug that acts by inhibiting the metabolism of folic acid. MTX empirical formula is $C_{20}H_{22}N_8O_5$ and it is structural name N-[4-(2, 4-diamino-6-pteridinyl) methyl] methyl glutamic acid with a molecular weight 454.4 (Klareskog *et al*., 2004).

Methotrexate is a yellow fluid may be given as and injection intravenously, intramuscularly or intrathecally, also it is available as a yellow tablets of 2.5 mg and 10 mg (Martindale, 2002).

The most widely used and best understood antifolate in cancer therapy is MTX which differs from the essential vitamin, folic acid, by having an amino substituted with a hydroxyl at the 4- position on the petridine ring (figure 1-2), and this change transforms the enzyme substrate into a tight-binding inhibitor of Dihydrofolate reductase (DHFR), a key enzyme required to maintain adequate intracellular levels of reduced folates (Allegra and Collins, 1990).

1.2.7.1 Clinical uses of methotrexate

Almost 50 years ago, MTX was developed as a chemotherapeutic agent for the treatment of cancer especially leukemia (Cronstein, 1997).

Methotrexate has since been used in the treatment of various malignancies including osteosacroma, non Hodgkin's lymphoma, Hodgkin's disease, cutanous T cell lymphoma, head and neck cancer, lung cancer, and breast cancer (Jolivet *et al*., 1983). Subsequently MTX was found to play a major therapeutic role in non- neoplastic diseases, acting as anti-inflammatory and immunosuppressive drug (Seitz, 1999).

Currently, MTX is commonly used to treat rheumatoid arthritis (Nash *et al*., 1996; Weinblatt *et al*., 1998), graft-vs-host diseases (Feagen

et al., 2000) psoriasis, primary biliary cirrhosis, Crohn disease, and intrinsic asthma (Genestier *et al*., 2000).

1.2.7.2 Mechanism of action

Methotrexate inhibits dihydrofolate reductase (DHFR) an enzyme that is a part of the folate synthesis metabolic pathway and this enzyme (DHFR) catalyses the conversion of folic acid to the reduced folates (i.e., tetrahydrofolate) (Klareskog *et al*., 2004).

The tetrahydrofolate is essential cofactor that donates one carbon group in the enzymatic biosynthesis of thymidylate and purine nucleotide that is a precursor for DNA synthesis (Allegra *et al*., 1986; Allegra *et al*., 1987).

Therefore, MTX inhibits the synthesis of DNA, RNA, thymidylates, and proteins so, MTX is cell cycle S-phase selective, and has a greater negative effect on rapidly dividing cells (such as malignant cells), and thus inhibits the growth and proliferation of these cells (Klareskog *et al*., 2004).

1.2.8 Investigated Parameters

Cytogenic analyses have been employed widely to detect the effect of mutagens and carcinogens on the genetic make-up. This is carried out using in vivo and in vitro system. Chick embryos were the first to be utilized in vivo analysis, (Bloom and Hsu, 1975), but later mice became the animals of choice in such experiments due to their fast reproduction, small size and easy handling, moreover, they represent a typical mammalian system (Haung *et al*., 1990). In vitro studies generally employ live cells of a mammalian origin, and in this regard the

lymphocytes are the cells of choice in investigation the mutagenic effects of some agents (Nath *et al*., 1988).

In the present study the in vivo cytogenetic effects of two extracts of basil was investigated in the laboratory animal mouse through two parameters; micronucleus formation and sperm-head abnormality assay. The micronucleus formation was further explored in vitro using blood cultures of lymphocytes obtained from ALL patients.

1.2.8.1 Mitotic activity

The mitotic activity is expressed by the MI which the number of dividing cells in 100 cells.

Many studies that determine the activity of immune system and the effect of different agents on it depend on the ability of lymphocytes to proliferate in lymphoid organs and/or depend on dividing cells of bone marrow because it is the source of all blood cells (Hughes, 2001). Mitotic index assay is defined as the ratio of the numbers of cells in a population undergoing mitosis to the total numbers of cells (Gosh *et al*., 1991). Therefore, by the employment of this assay the effect of different physical and chemical agents on the mitotic response can be detected, and studies have reveled that the mitotic index can be affected negatively or positively by chemicals, radiation, drugs and medicinal plants (Ad'hiah *et al*., 2001a; Ad'hiah *et al*., 2002; Ad'hiah *et al*., 2004; Ad'hiah *et al*., 2006). For bone marrow cell to study the effect of the different doses of the examined drugs on the mitotic activity of the cells. 2000 cells per animal were counted and the number dividing cells including prophases and metaphases was determined.

1.2.8.2 Chromosomal aberrations (CAs)

The increasing variety of chemicals, radiations and other physical agents we are exposed to nowadays have stimulated the development of many rapid, reliable assay for the detection of the mutagenicity or carcinogenicity of such agents, one of these methods is the chromosome aberration (CA) assay (Lambert *et al*., 1978; Ardito *et al*., 1980). Paul and Buul, (1977) confirmed that physical and chemical agents have shown the ability to induce CA. The other purpose of the CA test is to identify agents that cause structural CAs in cultured mammalian cells. Structural aberrations may be of two types, chromosome or chromatid and the majority of chemical mutagens induced aberrations of chromatid type but chromosome type aberration occur ,while an increase in polyploidy may indicate that the chemical has the potential to induce numerical aberration (Evans, 1976; Ishidate and Sofuni, 1985; Galloway *et al*., 1987).

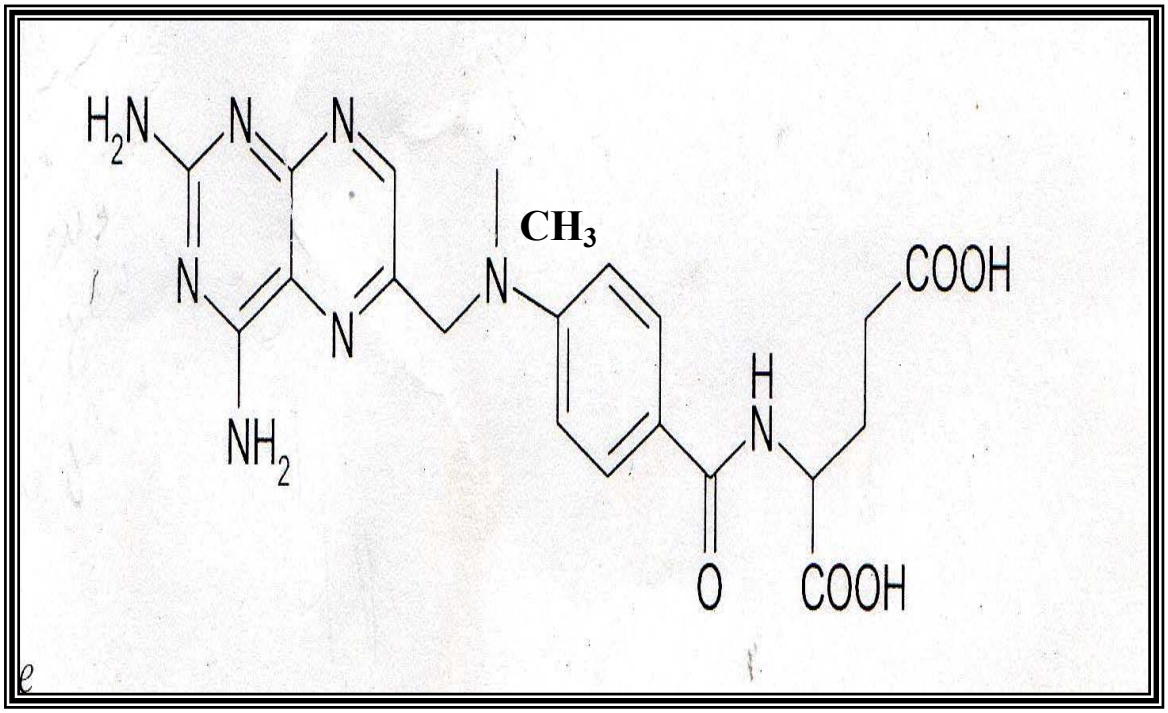
Chromosomal aberrations occur spontaneously (Goodenough, 1978), but chromosomes can be broken by ionizing radiation, physical stress, or chemical compounds, if broken ends are not brought together they can remain broken but, if broken chromatid ends are brought into apposition there are several alternative ways in which they can be rejoined, first, the two broken ends of a single chromatid can be reunited, second, the broken end of one chromatid can be fused with the broken end of another chromatid, resulting in an exchange of chromosomal material (Robert, 1996).

Jensen and Nyfros ,(1979) confirmed that the cytogenetic examination of the bone marrow cells must be included in the study of the possible chromosome damaging effect of chemical agents *in vivo* because the bone marrow cells are very sensitive to chemicals, so they

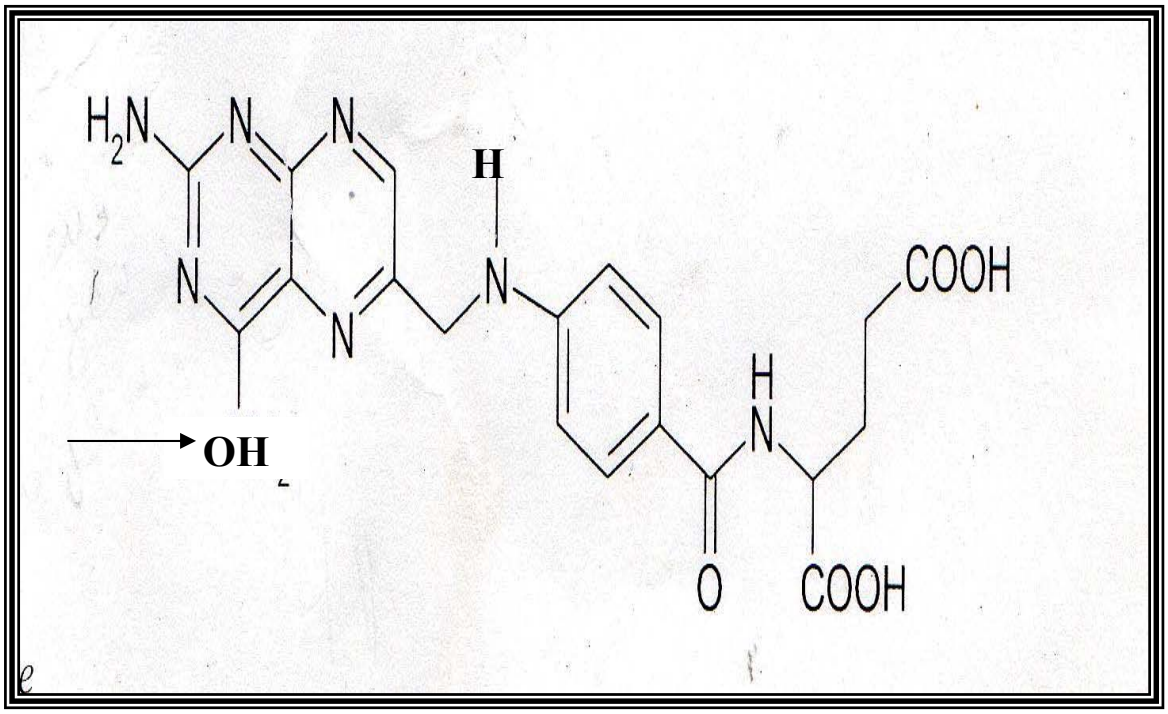
represent a good indicator for the effect of chemical oncogens and mutagens.

1.2.8.3 Sperm Abnormality Assay

The entire process of sperm formation, from spermatogonia to spermatozoa is referred to as spermatogenesis, which occurs in the seminiferous tubules of the testis (Seeley *et al*., 1996). The stem cell (spermatogonia) is divided mitotically to give primary spermatocytes that undergo meiosis I to give rise to haploid secondary spermatocytes that undergo meiosis II to produce spermatids. The spermatids are then transformed to spermatozoa by a series of morphological changes (Hafez and Hafes, 2000). These events are under a genetic control, especially the morphological changes involved in sperm head formation. Therefore, an assay was developed to detect genotoxic effects induced by physical and chemical agents. The assay scores the percentage of sperms with abnormal head morphology. Such morphological changes are dependent on the stage spermatogenesis. If the abnormality appeared at the end of first week post-treatment, it means that the genetic abnormality occurred in the stage spermatid formation, while if the abnormality appeared after 3-5 weeks post-treatment, it means that the abnormality occurred in the stages of spermatocytes or spermatogonia. Therefore, this test has been qualified to assess the mutagenic effects of chemical and physical mutagens in vivo (Topham, 1980; Al-Rubaiey, 2000).



Methotrexate



Folic acid (Vitamin M)

(Figure 1-2): The formula of methotrexate and folic acid (Gescher, 2004)**.**

Chapter Two

Materials and Methods

Materials and Methods

2.1 Materials

2.1.1 Equipments and apparatus

The following equipments and apparatus were employed throughout this study:

No.	Apparatus	Company / Country
1.	Cold incubator	Memmert – Germany
2.	Centrifuge	Beckman – England
3.	Autoclave	Dixone – UK
4.	Microscope	Olympus – Japan
5.	Micropipette	Gelson – France
6.	Electric balance	Sartorius – Germany
7.	Water bath	Gallenkamp – England
8.	pH- Meter	Radiometer – Denmark
9.	Vortex mixer	Griffin – England
10.	Rotary evaporator	BUCHI - Switzerland

2.1.2 Chemicals

The following chemicals were used in this study:

No.	Materials	Company / Country
1.	Methanol	Fluka – Switzerland
2.	Glacial acetic acid	Fluka – Switzerland
3.	Methotrexate (MTX)	Hexal – German
4.	Colchicine	Ibn Hayan – Syria
5.	Giemsa stain	Fluka – Switzerland
6.	Glycerin	Fluka – Switzerland

7.	Ethanol	Ferak – German
8.	Eosin	Reidal – DE Haen AG seelze – Hanno - ven
9.	KCl KH_2PO_4	Fluka – Switzerland
10.	HCl NaCl	Fluka – Switzerland
11.	KOH Na_2HPO_4	Fluka – Switzerland
12.	Sodium bicarbonate	BDH – England
13.	NaOH $FeCl_2$	Fluka – Switzerland
14.	Roshail salt	Fluka – Switzerland
15.	Sulfuric acid	Ferak – German
16.	Chloroform	BDH – England
17.	Nigrosin	LA – Chema - Russia

2.2 Plant Collection

Ocimum sanctum plants were collected from Al-Nahrain University garden during November and December (2006), and identified by the director of Baghdad University Hurberum, Prof. Dr. Ali Al-mosewy.

Leaves of this plant were air dried in shade at room temperature and grinded to powder using electric grinder.

2.3 Chemical Preparations

a. Colchicine

Colchicine was prepared by dissolving one tablet (0.5 mg) of

colchicine in (0.5 ml) of phosphate buffer saline (PBS) to be used for mouse injection. Each animal was injected with 0.25 ml of this solution in the intraperitoneal membrane (I.P) (Allen *et al*., 1977).

b. Phosphate buffer saline (PBS)

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

Sodium chloride (NaCl) 8 g

Potassium chloride (KCl) 0.2 g

Sodium phosphate hydrate (Na_2HPO_4) 1.15 g

Potassium phosphate dihydrate (KH_2PO_4) 0.2 g

The pH was an adjusted to 7.2 and the solution was sterilized by autoclaving at 121ºC for 15 minute, and then stored at 4°C (Hudson and Hay, 1989).

c. Potassium chloride (KCl) (hypotonic Solution)

A concentration of 0.075 M, of this solution was prepared by dissolving 5.75 g of KCl salt in 1000 ml of D.W. This solution was sterilized by autoclaving and stored at (4°C) (Allen *et al.*, 1977).

d. Fixative Solution

This solution was freshly prepared by mixing 3 parts of absolute methanol with 1 part of glacial acetic acid (Patton, 1967).

e. Sodium bicarbonate ($NaHCO_3$)

Sodium bicarbonate (4.4 g) was dissolved in 100 ml sterile D.W. and stored at 4°C until used (Allen *et al*., 1977).

f. Sodium hydroxide solution

This solution was prepared by dissolving 40 mg of NaOH in 1000 ml of D.W.

g. Potassium hydroxide solution

It was prepared by dissolving 50g of potassium hydroxide in 100 ml of D.W. This solution was used to detect the flavonoids.

h. Giemsa stain (Allen *et al*., 1977)

Giemsa stock solution was prepared by dissolving 1 g of Giemsa powder in 33 ml glycerin in a water bath 60°C for two hours with continuous shaking, and then left for 30 min at room temperature, and then 66 ml of absolute methanol was added with continuous shaking. The solution was kept in dark bottle at room temperature.

For slide staining, Giemsa solution was prepared as follow:

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

i. Eosin stain

This stain was prepared by dissolving 1 g of yellowish eosin in 100 ml distilled water (Wyrobek and Bruce, 1975).

j. Fehling reagent (Sarkas *et al*., 1980)

Solution A: 35 gm of copper sulfate were dissolved in 100 ml of D.W., and then diluted by D.W. until volume 500 ml.

Solution B: 7 gm of sodium hydroxide and 175 gm of roshail salt were dissolved in 100 ml of D.W.; the volume was completed until 500 ml by adding distilled water.

A and B solutions were mixed in equal volume. This reagent used for glycosides detection.

k. Ferric chloride solution

The solution was prepared by dissolving 1g of ferric chloride in 100 ml distilled water. It was used for saponins detection.

2.4 Extraction of plant extracts

2.4.1 Extraction of flavonoids (methanolic extract)

The leaves powder of *O. sanctum* was put in soxhlet device and used for extract 250 ml of methanol at temperature 60°C for 6 hours to obtain the active compound as crude compound, and then the extract was dried by using rotary evaporator. Several dilutions were made for injection in mice (intrapretonial) to detect the cytogenetic effect of the plant extract (Sabahi *et al*., 1987).

2.4.2 Extraction of steroidal compounds (Hexane extract)

The leaves powder of *O. sanctum* was put in soxhlet device and used for extract 250 ml of hexane at temperature 60°C for 6 hours to obtain the active compound as crude compound, and then the extract dried by using rotary evaporator to obtain drying powder. Several dilutions were made for injection in mice (intrapretonial) to detect about the cytogenetic effect of the plant (Al-Jeboory, 1994).

2.5 Detection of some active compounds in *Ocimum sanctum* leaf extract

2.5.1 Detection of flavinoids

Ten milliliters of 50% ethyl alcohol were mixed with 10 ml of 50%

potassium hydroxide solution, and then added to equal volume of the plant extract. The appearance of yellow color indicated the presence of flavinoids (Harborne, 1973).

2.5.2 Detection of steroids

1 g of hexane extract was participating in a few drops of chloroform, and then a drop of acetic anhydride and drop of concentrated sulfuric acid were added. Dark blue color appeared represented the presence of steroids in the extracts (Al-Abid, 1995).

2.5.3 Detection of saponins

A: Plant extract was shacked vigorously in a test tube; the formation of foam remaining for few minutes indicated the presence of saponins.

B: Five milliliters of the plant extract were added to 3 ml of ferric chloride solution, and the appearance of white precipitate indicated the presence of saponins (Stahl, 1969).

2.5.4 Detection of glycosides

a. Non-hydrolyzed extract:

Equal volumes of the plant extract and Fehling reagent were mixed, and then left in a boiling water bath for 10 minutes; the appearance of red precipitate indicated the presence of free sugars.

b. Hydrolyzed extract:

Few drops of 10% hydrochloric acid were added to 5 ml of the plant extract, and then left in a boiling water bath for 20 minutes. The acidity was neutralized by adding few drops of sodium hydroxide solution, finally equal volume of Fehling reagent was added and the

development of red precipitate was an indication for the aglycon part of the glycoside (Harborne, 1973).

2.5.5 Detection of terpene

One gram of plant extract was precipitated in a few drops of chloroform, and then a drop of acetic anhydride and concentrated sulfuric acid were added. The appearance of dark brown color indicated the presence of terpenes (Al-Abid, 1985).

2.6 Laboratory animals

Forty four Swiss male, obtained from Biotechnology Research Center, were used. Their age ranged between (8-12) weeks and weighting 23-27 gm. They were divided into 11 groups; each group was put in a separate plastic cage. The cages were placed in a room at a temperature (23°C-25°C). The animals were fed with a suitable quantity of water and standard pellets.

2.7 Preparation of methotrexate

Methotrexate was obtained from Hexal Company at a concentration of 25 mg/ml, and from this stock solution the concentrations 0.6, 0.8, and 1 mg/ml were prepared to be used in mice studies.

2.8 Preparation of *O.sanctum* extract

Two grams of dry extract was dissolved in (10 ml) of D.W. for methanolic extract and (10 ml) of olive oil for hexane extract to make a stock solution, and from this stock the concentrations 200, 400, 600 mg/kg were prepared to be used in mice studies.

2.9 Antimicrobial activities

The microorganisms used for anti-microbial activity include:

No.	Microorganisms	Origin
1	*Staphylococcus aureus*	Biotechnology department Al-Nahrain university
2	*Pseudomonas aeroginosa*	Biotechnology department Al-Nahrain university
3	*Candida albicans*	Biotechnology department Al-Nahrain university

2.9.1 Sterilization methods

A-Culture media were sterilized by autoclaving at 121 °C, 15 1b/ in² for 15 minutes.

B- Glassware was sterilized using electric oven at 180-200 °C for 2 hours.

2.9.2 Preparation of culture media

The following culture media were used routinely in this study:

A-Nutrient agar-

This medium was prepared as recommended by the manufacturer, autoclaved at 121º C for 15 minutes.

B-Modified sabouraud Dextrose Agar:

Fungi were cultured on modified sabouraud dextrose agar according to Finegold *et al*. (1982), by mixing the following ingredients:

Peptone 10 g

Glucose 20 g

Agar	20 g
Cycloheximide	500 mg
Cephalaxin	500 mg
D. W.	1000 ml

2.10 Preparation of antibacterial samples

For hexane extract that insoluble in water, 5ml of olive oil were added to the dried hexane extract and mixed well to obtain a dissolved hexane extract.

On the other hand, 5ml of distilled water were added to the dried methanolic extract and mixed well to obtain a dissolved methanolic extract.

Nutrient agar media were prepared as controlling plate. The plant extracts were prepared at different concentrations starting with 75µg/ml, 37.5µg/ml, and 18.75 µg /ml.

These concentrations were calculated according to the equation:

$C1\ V1 = C2\ V2$

(C value: means the concentration, V value: means the volume).

The medium was mixed well, poured in Petri-dishes and left to solidify, 100 µl overnight cultures (O.D. about 0.2-0.4) was spotted on the agar medium and distributed with spreader. The inoculated plates were placed at room temperature for 30 minutes to allow absorption of excess moisture. The plates were incubated for 24 hours at 37°C (NCCL, 1993).

2.11 Preparation of antifungal samples

The same concentration of plant extracts were prepared as mentioned later, but plant extracts were added to modified sabouraud

dextrose agar, all Petri-dishes were inoculated with fungal spore and incubated at 30°C for 7-14 days (Al-samarae *et al*., 2001).

The diameter of fungal colonies was determined after the period of incubation, and then the inhibition percent was calculated according to the equation:

Inhibition average of clonal diameter in control plate-average of clonal dia.
In treated

$$= \frac{\text{plate}}{\text{Percent average of clonal diameter (In control plate)}} \times 100$$

2.12 Administration of drug on laboratory animals

2.12.1 Methotrexate

2.12.1.1 Cytogenetic experiment

The animals in this experiment were treated with cumulative doses of methotrexate in a short time. The main aim of this experiment was to evaluate the acute treatment effect of MTX by applying cytogenetic analysis and examining the inhibition in mitotic activity and the induction of chromosomal aberration in normal bone marrow cells. It also aimed to select the most suitable concentration of MTX treatment with the highest side effects to be used in the next experiment.

The mice were divided into 4 experimental groups. Each groups consisted of 4 mice.

Group I: Negative control (4 mice)

Treated with (0.1 ml) PBS.

Group II: MTX treatment (4 mice)

Treated with (0.1 ml) of MTX (0.6 mg/kg)

Group III: MTX treatment (4 mice)

Treated with (0.1 ml) of MTX (0.8 mg/kg)

Group IIII: MTX treatment (4 mice)

Treated with (0.1 ml) of MTX (1mg/kg).

Methotrexate was given orally for 7 successive days, and then the mice were scarified after seven days along with the first group of control mice. Bone marrow samples were taken and cytogenetic analyses were carried out as described in 2.12.2.1.

2.12.1.2 Sperm abnormality

Animals in this experiment were treated with MTX to evaluate the abnormalities of sperm in mice.

Two groups of mice were taken and treated as follows:

Group I: control (4 mice)

Treated with (0.1 ml) PBS.

Group II: MTX treatment (12 mice)

Treated with (0.1 ml) of MTX (1 mg/kg).

Methotrexate was given by oral gavage for 7, 21, 35 days, then mice were scarified along with the first group of control mice. Sperms samples were taken from vas deferens and the experiments were carried out as described in 2.12.2.2.

2.12.2 *Ocimum sanctum*

2.12.2.1 Cytogenetic experiment of methanolic extract

This experiment was carried out to detect the genotoxic effects of *O. sanctum* methanolic extract in mice by applying cytogenetic analyses (MI, CA) in normal bone marrow cells. It also aimed to select the most suitable concentration of *O. sanctum* methanolic extract to be used in next steps.

Four groups of mice were used and treated as follows:

Group I: Negative control (4 mice)

Treated with (0.1 ml) PBS.

Group II: *O.sanctum* methanolic extract treatment (4 mice)

Treated with (0.1 ml) of extract 200 mg/kg.

Group III: *O. sanctum* methanolic extract treatment (4 mice)

Treated with (0.1 ml) of extract 400 mg/kg.

Group IIII: *O. sanctum* methanolic extract treatment (4 mice)

Treated with (0.1 ml) of extract 600 mg/kg.

Doses were given intrapretonially for 7 successive days, and then the mice were scarified along with the first group of control mice. Bone marrow samples were taken and cytogenetic analyses were carried out.

2.12.2.2 Sperm abnormality of methanolic extract

The purpose of this experiment was to evaluate the ability of the methanolic extract for *O. sanctum* to reduce the abnormalities in sperm morphology.

Two groups of mice were used and treated as follows:

Group I: Negative control (4 mice)

Treated with (0.1 ml) PBS.

Group II: *Ocimun sanctum* methanolic extract treatment (4 mice)

Treated with (0.1 ml) of extract 600 mg/kg.

The doses were given intrapretonially for 7, 21, 35 days, then mice were scarified along with the first group of control mice. Sperms samples were taken from vas deferens and the experiment were carried out as described later.

2.12.2.3 Cytogenetic experiment of hexane extract

This experiment was carried out to detect the genotoxic effects of

O. sanctum hexane extract in mice by applying cytogenetic analyses (MI, CA) in normal bone marrow cells. It also aimed to select the most suitable concentration of *O. sanctum* hexane extract to be used in next steps.

Four groups of mice were used and treated as follows:

Group I: Negative control (4 mice)

Treated with (0.1 ml) olive oil.

Group II: *O.sanctum* hexanes extract treatment (4 mice)

Treated with (0.1 ml) of extract 200 mg/kg.

Group III: *O. sanctum* hexanes extract treatment (4 mice)

Treated with (0.1 ml) of extract 400 mg/kg.

Group IIII: *O. sanctum* hexanes extract treatment (4 mice)

Treated with (0.1 ml) of extract 600 mg/kg.

Doses were given intrapretonially for 7 successive days, and then the mice were scarified along with the first group of control mice. Bone marrow samples were taken and cytogenetic analyses were carried out.

2.12.2.4 Sperm abnormality of hexane extract

The purpose of this experiment was to evaluate the ability of the hexane extract for *O. sanctum* to reduce the abnormalities in sperm morphology.

Two groups of mice were used and treated as follows:

Group I: Negative control (4 mice)

Treated with (0.1 ml) olive oil.

Group II: *Ocimun sanctum* hexanes extract treatment (4 mice)

Treated with (0.1 ml) of extract 600 mg/kg.

The doses were given intrapretonially for 7, 21, 35 days, then mice were scarified along with the first group of control mice. Sperms samples

were taken from vas deferens and the experiment were carried out as described later.

2.13 Cytogenetic experiments

2.13.1 Chromosomes preparation from somatic cell of the mouse bone marrow

The experiment was done according to (Allen *et al*., 1977) as follows

1. Animals were injected with 0.25 ml of colchicines which was at a concentration 1 mg/ml intraperitonially (I.P.) two hours before scarifying the animals.
2. The animals were scarified by cervical dislocation.
3. The animals were fixed on their tergal side on the anatomy plate and at the abdominal side of the animals and their thighs regions were swabbed with 70% ethanol.
4. The femus 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, and then 5 ml of PBS was injected so as to wash and drop the bone marrow in the test tube.
5. The test tubes were taken and put in a centrifuge at a speed of 2000 rpm for (10 min.).
6. The supernatant was removed and (5ml) of 0.075 M potassium chloride (KCl) was added as a hypotonic solution, then the tubes were placed in incubator at (37°C) with shaking from time to time.
7. The tubes were centrifuged at 2000 rpm for (10 min.).
8. The supernatant was removed and the fixative solution was added (as drops) on the inside wall of test tube with continuous shaking, the volume was fixed to (5 ml) and the contents were shaken well.
9. The tubes were kept at 4°C for (30 min.) to fix cells.
10. The tubes were centrifuged at 2000 rpm for 10 min. The process was

repeated four times and the cells were suspended in (2 ml) of the fixative.

11. By a Pasture pipette, a few drops from tube were dropped vertically on 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.

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

13. Three slides for each animal were prepared for cytogenetic assays.

2.14 Cytogenetic analyses test

2.14.1 Mitotic index (MI) assay

The slides were examined under (40X) of light microscope, and (1000) of dividing and non dividing cells were counted and the MI was concluded according to the following equation:

$$MI = \frac{\text{No. of the dividing cells}}{\text{Total no. of the cells}} \times 100$$

(Shubber *et al*., 1987)

2.14.2 Chromosomal aberration (CA) assay

The prepared slides were examined under oil immersion lens for 100 dividing cells per each animal. Cells were at the metaphase stage of the mitotic division where the chromosomal aberrations were clear and the percentage of these aberrations estimated.

2.15 Statistical analysis

One way analysis of variance was performed to test whether group variance was significant or not, the comparison between groups was demonstrated by analysis of variance test (ANOVA) (Al- Mohammed *et al*., 1986).

Chapter Three

Results and Discussion

3.1 Detection of some active compounds in *Ocimum sanctum* methanolic and hexane extract

By using different chemical reagents and solutions, detection of various active compounds in *Ocimum sanctum* was achieved; those were represented in (Table 3-1). Results indicated that *O. sanctum* contains flavionids and steroids.

Table (3-1): Detection of some active methanolic and hexane compounds.

Chemicals compounds	Reagent	Indication	Reaction
Saponins	1.Shaking for water extract 2.Ferric chloride	1.Foaming form 2.White precipitate	-ve -ve
Glycosides (Before)	Fehling	Red Precipitate	-ve
Glycosides (After)	Fehling	Red Precipitate	-ve
Flavonoids	Ethyl alcohol + potassium Hydroxide +plant extract	Yellow Color	+ve
Terpenes	Plant extract +chloroform +acetic anhydride +sulfuric acid	Dark brown Color	+ve
Steroids	Sulfuric acid concentrated	Blue color to green	+ve

+ve indicates the presence of the active compound.

3.2 Effect of hexane and methanolic extracts on the growth of microorganisms

Different extracts of *Ocimum sanctum* were prepared at different concentrations to find the active dose. Generally, the antimicrobial activity of each extract depends on the type of the extract, concentration and the type of microorganism.

Rani and Khullar 2004 showed in their study that *Ocimum sanctum* has a moderate antimicrobial activity.

3.2.1 Hexane extract

Hexane extract possessed no anti-bacterial activity against tested microorganisms. The growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Candida albicans* was not affected by the extract as shown in table (3-2) and figure (3-1).

This result disagrees with (Wolters, 1976). This may be due to the concentration of active compound in hexane extract was insufficient to inhibit the bacterial growth or may be due to the high concentration of bacteria.

Another reasons related to the cultured bacteria are lack or poor permeability to the extract by bacterial cell wall leading to decrease in extract accumulation and production of resistance enzymes (Chambers, 2001).

Figure (3-1): Different concentrations of hexane extract of *O. sanctum* against some microorganisms.

Table (3-2): Effect of hexane extract on the growth of microorganisms.

Concentration of the extract	*Staphylococcus aureus*	*Pseudomonas aeroginosa*	*Candida albicans*
18.75 mg/ml	- ve	- ve	- ve
37.5 mg/ml	- ve	- ve	- ve
75 mg/ml	- ve	- ve	- ve

Note: -ve = no effect on the growth.

3.2.2 Methanolic extract

The methanolic extract of *O. sanctum* possessed an inhibitory effect against the growth of *C. albicans* and *S. aureus* at all concentrations (18.75mg/ml, 37.5mg/ml, and 75mg/ml) as shown in table (3-3) and figure (3-2).

This inhibition may be attributed to the inhibition of bacterial DNA gyrase, which prevents the relaxation of positively supercoiled DNA that is required for normal transcription and replication (Chambers, 2001).

The result agrees with Rauba *et al.* (2000) who showed in their study that methanolic extract of *O. sanctum* has inhibitory effect on the growth of *S. aureus.*

Also, Geeta *et al*. (2001) showed in their study that methanolic extract of *O. sanctum* have slightly inhibition against *S. aureus.*

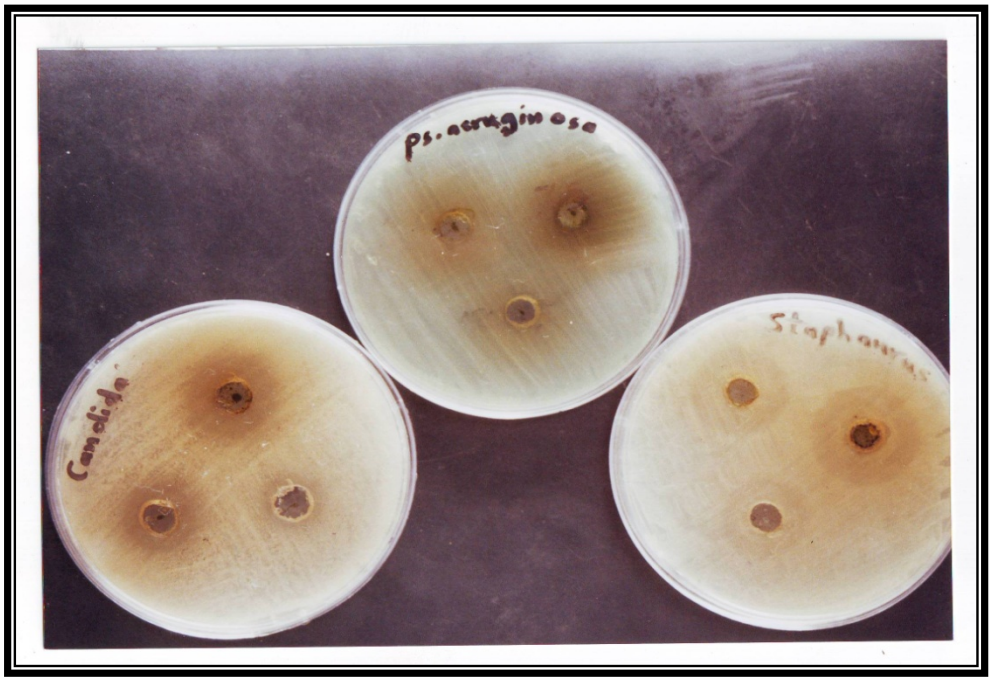


Figure (3-2): Effect of different concentrations of methanolic extract of *O.sanctum* against some microorganisms.

Table (3.3): Effect of methanolic extract on the growth of some microorganisms.

Concentration of the extract	*Staphylococcus aureus*	*Pseudomonas aeruginosa*	*Candida albicans*
18.75mg/ml	+ve	- ve	+ ve
37.5 mg/ml	+ ve	- ve	+ ve
75 mg/ml	+ ve	- ve	+ ve

Note: -ve = no effect on the growth.
+ve = inhibition of the growth.

3.3 Cytogenetic effect of methanolic and hexane extracts of *Ocimum sanctum*

3.3.1 Cytogenetic effect of *O. sanctum* extracts on mouse bone marrow cells.

3.3.1.1 Effect on mitotic index (MI)

Three doses of methanolic extract from this plant were used (200,400,600) mg/kg. The best concentration was chosen depending on the induction of MI and reduction in CAs. For methanolic extract, results showed that all these three doses led to an increase in the mitotic index (5.7, 6.8, and 7.5%), respectively as compared with negative control (5.2%). This was not significantly increased in the first concentration while the increase was significant ($P \leq 0.05$) in the second and third concentrations (Table 3-4).

The increase in mitotic index may be due to the presence of flavinoids that have an important role as inducers for immunity through the increase of the activity for lymphocytes by the induction of stem cells in mouse bone marrow.

Gebhart, (1981) showd that the flavinoids in *O. Sanctum* leaves has an important role as inducers for mitogenic agents in its constituents that stimulated cell division after treatment with this extract. Additionally, they play a role in decreasing the chromosomal aberrations in the treated mice.

These results were in agreement with Ekachai-Khumphan and Darunee-Buripakdi-Lawso (2002) who suggested that *O. sanctum* methanolic extract had a mitogenic activity so could induce cell division.

On the other hand, results of hexane extract (steroids) showed that all these three doses had no effects on mitotic index in the treated mice, because the extract did not release free radicals, plant steroids may provide a protection to the plasma lipids as a results of antioxidant activity (Moller and Loft, 2002).

These results disagree with Li *et al.* (2004) who suggested that flavinoids have the ability to generate free radicals lead to covalent damage of cellular macromolecules including DNA.

3.3.1.2 Effect on chromosomal aberrations

Methanolic extract showed the ability to reduce the spontaneous CAs from (0.79%) in the negative control to (0.57%, 0.41%, and 0.31%) for the three doses (200, 400, and 600) mg/kg, respectively (Table 3-4). These results were significantly different ($P \leq 0.05$) in comparison with control. All these reductions in CAs may be related to flavinoids.

Further evidences supporting such results may be observed in mouse bone marrow. Shymala and Devaki, (1996) and Ferreira *et al*, (2003) mentioned that the rate of CAs reduced after methanolic extract treatment. Methanolic extract is rich in flavinoids which have been reported to be efficient antioxidants by scavenging oxygen radicals, leading to decrease CAs.

While using hexane extract (table 3-5), no significant results were observed using the three doses, since the extract did not release free radicals.

These results disagree with Liehr, (2001) who suggested that flavinoids have the ability to generate free radicals leading to covalent damage of cellular macromolecules including DNA.

Table (3- 4): Cytogenetic effects of *O.sanctum* methanolic extract on mouse bone marrow *in vivo*.

Treatments	Mitotic Index % (M±SE)	Chromosomal aberration % (M±SE)						
		Chromatid Break	Chromosome Break	Acentric	Dicentric	Ring	Deletion	*Total of CA*
Negative control (PBS)	A 5.2±0.11	A 0.1±0.01	A 0.02±0.005	A 0.130±.01	AB 0.17±0.02	A 0.060±.01	AB 0.31±0.01	A 0.79±0.01
Positive control MTX	B 1.8±0.11	B 0.17±0.01	B 0.17±0.02	B 0.27±0.01	B 0.22±0.02	B 0.12±0.005	B 0.36±0.01	B 1.31±0.02
200 mg/kg *O. sanctum* extract	AC 5.7±0.12	C 0.05±0.005	C 0.02±0.005	C 0.12±0.01	A 0.13±0.01	AC 0.03±0.005	AC 0.22±0.01	C 0.57±0.01
400mg/kg *O. sanctum* extract	CD 6.8±0.54	C 0.04±0.01	C 0.01±0.00	C 0.1±0.01	B 0.04±0.005	CD 0.02±0.01	C 0.2±0.05	D 0.41±0.02
600 mg/kg *O. sanctum* extract	D 7.5±0.34	C 0.03±0.005	C 0.00±0.00	C 0.09±0.01	B 0.05±0.005	D 0.00±0.00	C 0.14±0.01	E 0.31±0.005

. **Numbers with different letters are significantly different (≤ 0.05) within the same columns.**

Table (3- 5): Cytogenetic effects of *O.sanctum* hexane extract on mouse bone marrow *in vivo*.

Treatments	Mitotic Index % M±SE)(	Chromosomal aberration % (M±SE)						
		Chromatid Break	Chromosome Break	Acentric	Dicentric	Ring	Deletion	Total of CA
Negative control (Olive oil)	A 4.9±0.11	A 0.09±0.01	A 0.015±0.005	A 0.130±.01	A 0.165±0.02	A 0.060±.01	A 0.30±0.01	A 0.76±0.01
Positive control MTX	B 1.8±0.11	B 0.17±0.01	B 0.17±0.02	B 0.27±0.01	B 0.22±0.02	B 0.12±0.005	B 0.36±0.01	B 1.31±0.02
200 mg/kg *O. sanctum* extract	A 5.1±0.12	A 0.08±0.005	A 0.02±0.005	A 0.12±0.01	A 0.16±0.01	A 0.060±0.005	A 0.30±0.01	A 0.74±0.01
400mg/kg *O. sanctum* extract	A 5.3±0.54	A 0.084±0.01	A 0.021±0.00	A 0.125±0.01	A 0.155±0.005	A 0.050±0.01	A 0.29±0.05	A 0.725±0.02
600 mg/kg *O. sanctum* extract	A 5.4±0.34	A 0.087±0.005	A 0.023±0.00	A 0.130±0.01	A 0.15±0.005	A 0.055±0.00	A 0.28±0.01	A 0.725±0.005

A, B mean there is no significant P≤ 0.05.

3.3.2 The effects of *O. sanctum* methanolic and hexane extracts on sperm morphology

In methanolic extract, the results of this experiment are displayed in Table 3-6, which indicated that methanolic extract of this plant has the ability to decreases the number of normal sperms from (64.5%) in negative control to (63.57, 59, and 56.4%) after 7, 21, and 35 days of treatment, respectively, and this was significant ($P \leq 0.05$) at 21, and 35 days of treatment but non- significant at 7 days of treatment as compared with negative control.

As a result of extract treatment, the abnormalities of head and tail increased, in head the abnormalities increased from (19.83%) in the negative control to (20.73, 25, and 26.2%) after 7, 21 and 35 days of treatment, respectively.

This increase, however, was not significant after 7 days of treatment, but it was significant after 21, and 35 days of treatment, while in tail the abnormalities were increased from (15.67%) in negative control to (15.7, 16, and 17.4%) after 7, 21, and 35 days of treatment, respectively.

This increase was not- significant in all periods of treatment as compared with negative control, as shown in (Table 3-6). (Because of the body rapier system can manage the alterations occurred in the sperms during the first 7 days while can not manage the alterations in 21, 35 days because the body saturated with the toxic components of plant extracts which affect the spermatogenesis).

These results are comparable with Kantak and Gogate (1992) and Ahmed *et al*., (2002).

Abnormalities of head and tail increased especially coiled tail, headless and broken tail, and this increase was observed especially after 35 days of treatment in which the sperms were not completed the

spermatogenesis and became mature, and this increase in abnormalities may be related to some active compounds of *O. sanctum* like flavinoids which act as anti-oxidants or scavengers for free radicals so may cause an increasing in abnormalities of sperms. It was found that was better than Eugenol, luteolin, ursolic acid, and oleanolic acid as anti-oxidant.

In contrast, hexane extract, (Table 3-7), indicated that extract had no ability to decrease or increase the normal number of sperms from (64.5%) in negative control, so there was no change, may be due to the inability of sperm cells to consume steroids as a nutrient, so steroids acts as non valuable chemical compound.

Table (3-6): Effects of *O.sanctum* methanolic extract on sperm head and tail morphology *in vivo.*

Treatment Period	Normal sperms% MS±E	Head abnormality% MS±E	Tail abnormality% M±SE
Negative Control	A 64.5±1.27	A 19.83±2.07	A 15.67±1.67
7 days *O. sanctum* extract	A 63.57±2.36	A 20.73±1.44	A 15.7±3.75
21 days *O. sanctum* extract	B 59.0±0.57	B 27.0±0.57	A 16.0±0.57
35 days *O. sanctum* extract	B 56.4±0.28	B 26.2±0.11	A 17.4±0.17

Numbers with different letters are significantly different (≤ 0.05) within the same columns

Table (3-7): Effects of *O.sanctum* hexane extract on sperm head and tail morphology *in vivo.*

Treatment Period	Normal sperms% MS±E	Head abnormality% MS±E	Tail abnormality% M±SE
Negative Control	A 64.5±1.27	A 19.83±2.07	A 15.67±1.67
7 day *O. sanctum* extract	A 64.6±2.36	A 19.5±1.44	A 15.9±3.75
21 days *O. sanctum* extract	A 64.3±0.57	A 19.6±0.57	A 16.1±0.57
35 days *O. sanctum* extract	A 64.0±0.28	A 19.8±0.11	A 16.2±0.17

A: mean there is no significant P≤ 0.05.

3.4 Cytogenetic effect of methotrexate

3.4.1 Cytogenetic effect of methotrexate on mouse bone marrow cells

3.4.1.1 Effect on mitotic index (MI)

Under normal experimental conditions, white mice have a mitotic index of (5.2%) in their bone marrow cells (Table 3-4). This considered as a negative control.

Three concentrations of MTX were used as positive control (0.6, 0.8, 1)mg/kg, and all these concentrations led to significant reduction ($p \leq 0.05$) in MI of mouse bone marrow cells as compared with negative control reaching (2.2%, 2%, and 1.8%), respectively, so the concentrations (0.6, 0.8)mg/kg were not taken and according to this the

selected concentration of MTX was (1mg/kg) because of its high cytotoxicity effects on bone marrow cells.

These results indicated that MTX resulted in a reduction of MI in mouse bone marrow cells, and this reduction was dose dependant. This may be related to MTX that limit the intracellular supply of reduced folates (Tetrahydrofolate) through inhibition of dihydrofolate reductase (DHFR) and, therefore, is considered inducing cytotoxic effect with resultant inhibition of new DNA, thymidylate and purine synthesis so, the MTX is a cell cycle S- phase selective (Li and kaminskas, 1984).

These results agree with the results of Al-Amiry (1999), who found that the MTX caused reduction in MI in mouse bone marrow cells.

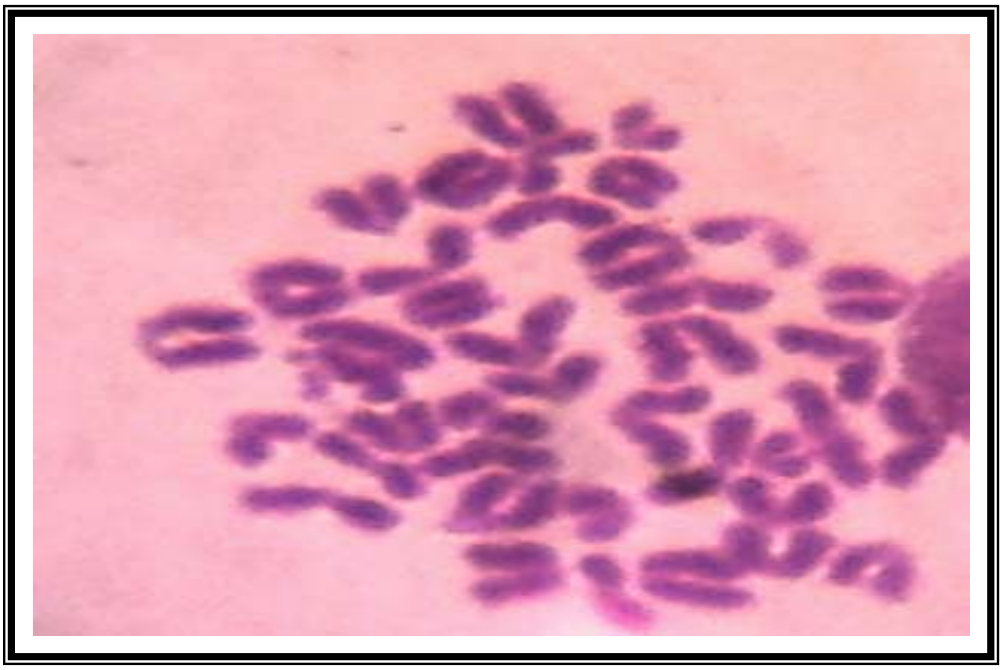
3.4.1.2 Effect on chromosomal aberrations

The spontaneous frequency of chromosomal aberration in mouse bone marrow cell was (0.79%) which represented as negative control (Table 3-4).

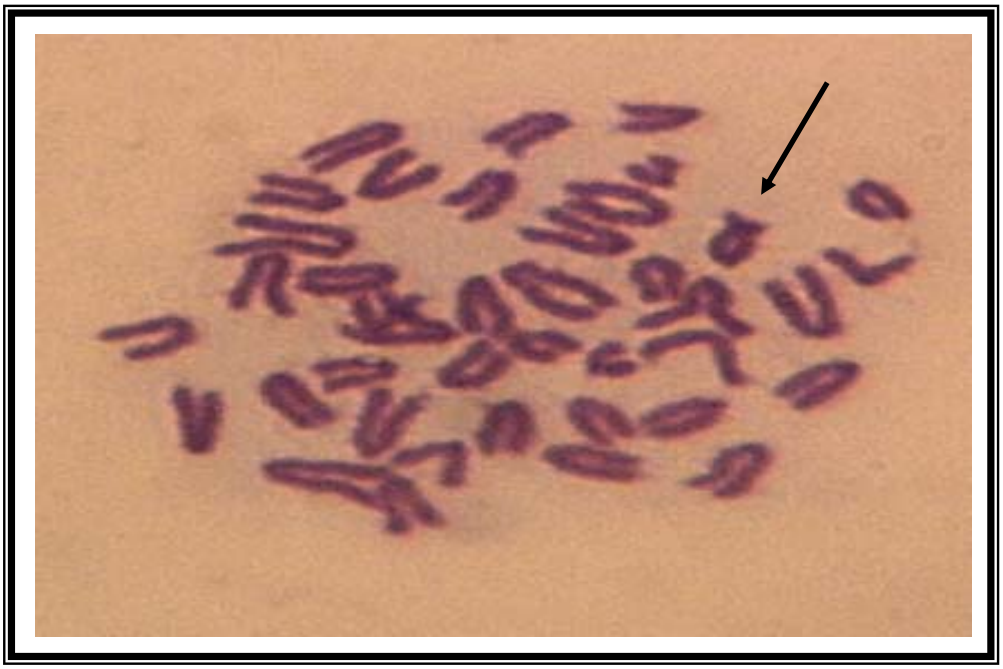
The results of this experiment indicated that the MTX concentration (1m
g/ml) increased CAs frequencies (chromatid break, chromosome break, acentric, dicentic, ring, and deletion) (Figure 3-3), which reached 1.31% after five days of MTX treatment, and these results were significantly different ($p \leq 0.05$) from the negative control and other treatments.

In cells treated with MTX, a progressive accumulation of strand break in mature DNA (post-replicate DNA) was detected (Kasahara *et al*., 1992). Hence, DNA strand breaks arise from spontaneous and normally repaired DNA lesions that are not repaired and there is also evidence that MTX influences the cellular topoisomerase II content and causes, therefore, an increase in DNA breaks (Holden *et al*., 1995).

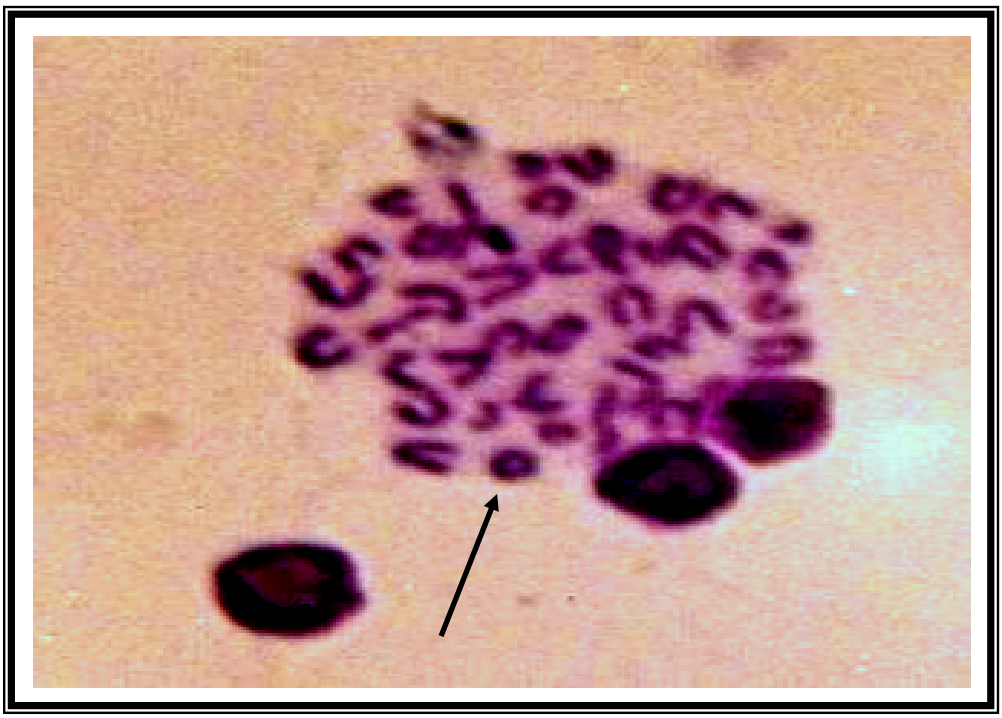
These results are in agreement with Al-Shimary (2004), who indicated that the MTX had the ability to induce the chromosomal abnormalities especially, ring chromosome, chromosome break, chromatid break, and dicentric chromosome.



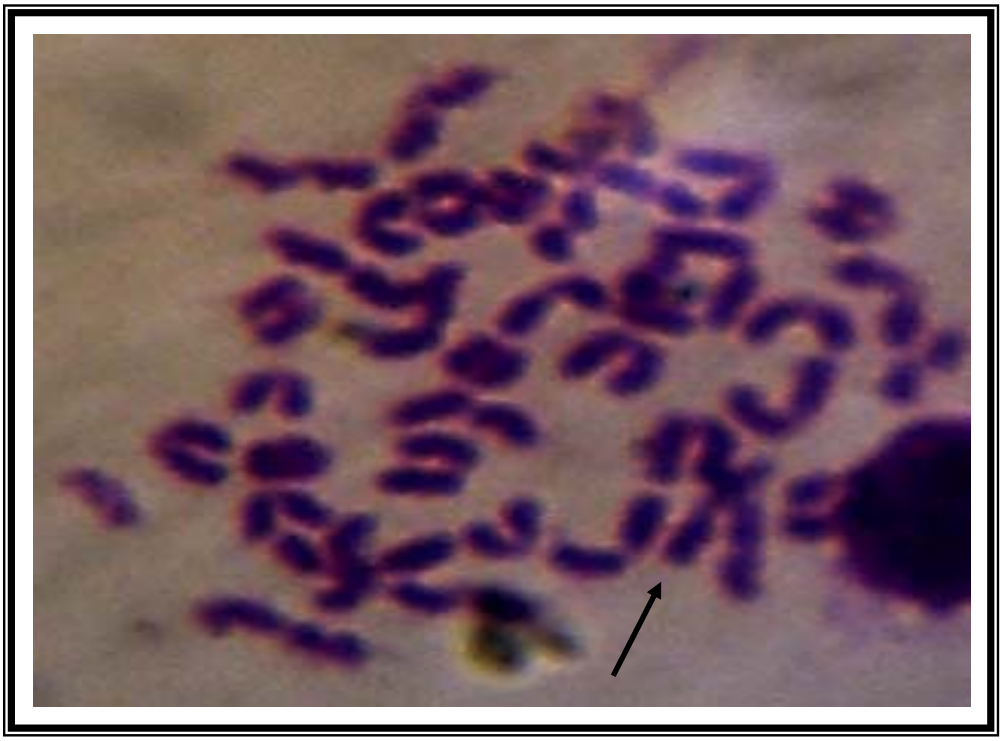
(A)



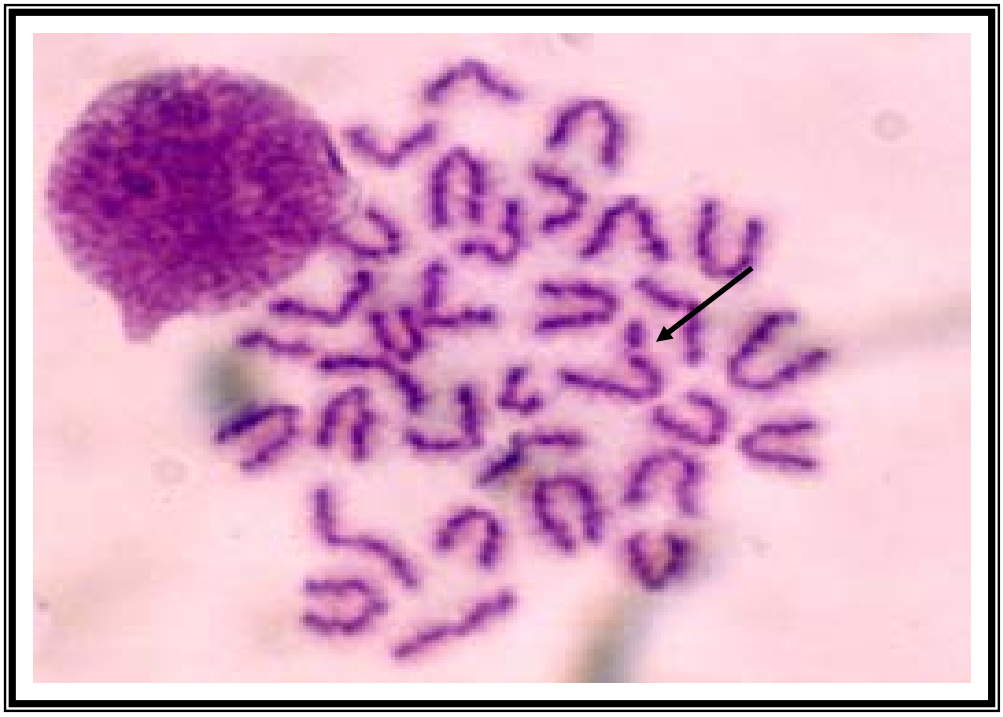
(B)



(C)



(D)



(E)

(Figure 3-3): Prophase and Metaphase of bone marrow cells from mouse treated with MTX, showing : normal chromosomes (A) ,dicentric Chromosome (B), ring chromosome (C),deletion (D) and chromatid break (E).(100 X).

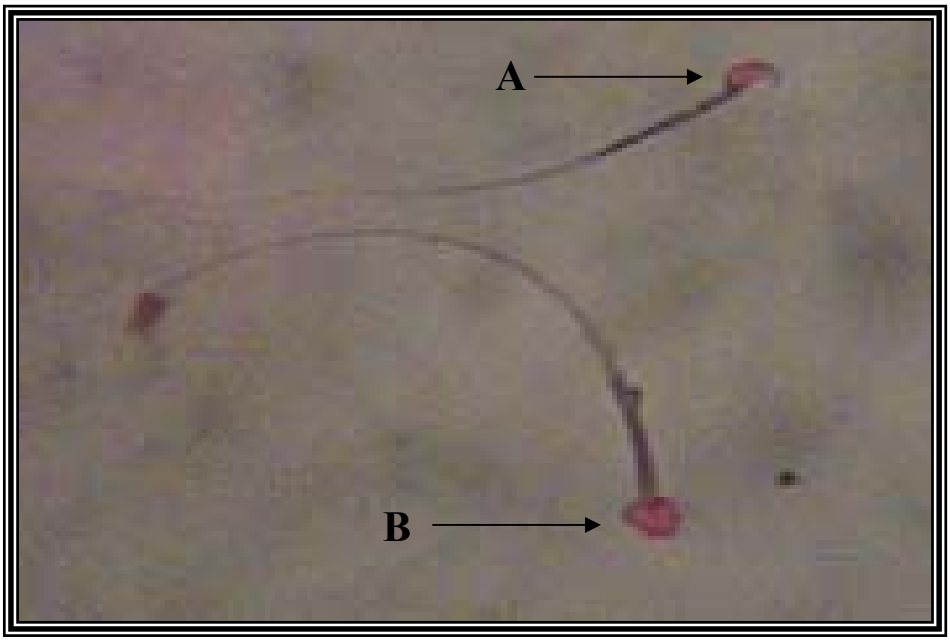
3.4.2 The effect of methotrexate on sperm morphology *in vivo*

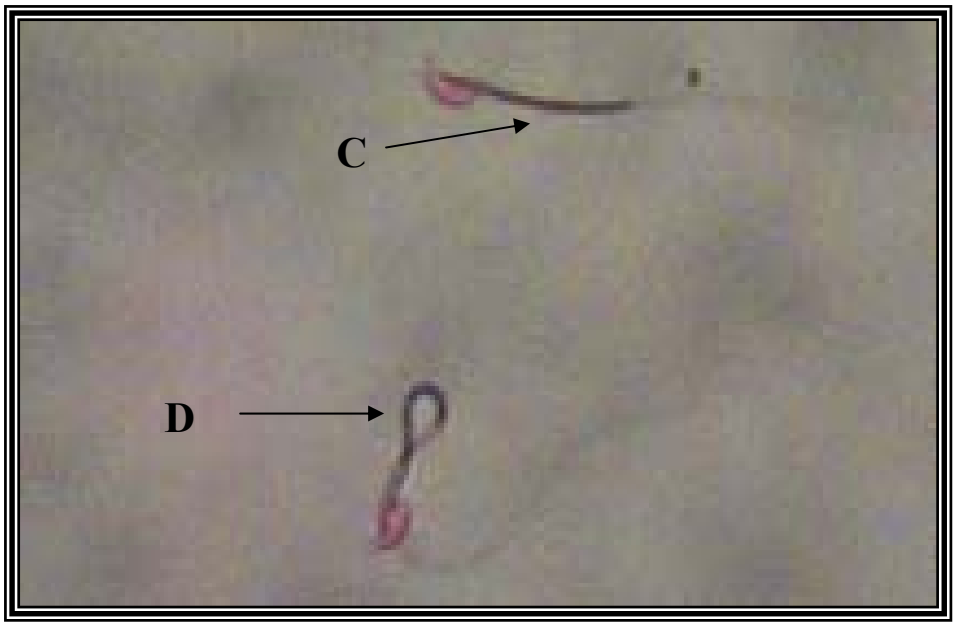
This experiment indicated that MTX had the ability to reduce the percentages of normal sperms from (64.5%) in the negative control to (47.7, 38.3, and 25%) after 7.21, and 35 days of treatment, respectively, and this reduction was significant ($P \leq 0.05$) compared with negative control (Table 3-8).

As a result of MTX treatment, the abnormalities of head and tail increased as in Figure 3-4. Head abnormalities increased from (19.83%) in the negative control to (27.5, 31.4, and 40%) after 7, 21, and 35 days of treatment, respectively, and this was significant ($P \leq 0.05$) in comparison with

negative control, also the abnormalities of tail increased from (15.67%) in the negative control to (24.8, 30.3, and 35%) after 7, 21 and 35 days of treatment, respectively, and this was significant ($P \leq 0.05$) in comparison with negative control (15.67%), as shown in (Table 3-8).

Male reproductive function is under hormonal control, and spermatogenesis process is under control of 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 dependant on testosterone and the final step of maturation of spermatids are dependant on FSH (Ganong, 1991), so the abnormal sperm morphology may reflect an 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 leads to production of abnormal sperms (Arab *et al*., 1989).





(Figure 3-4): Effect of methotrexate on sperm morphology, showing: normal sperm (A), sperm of amorphous head (B), sperm of banana like form head(C), sperm of coild tail(D) (40x)

Table (3-8): Effect of methotrexate on sperm head and tail morphology in vivo.

Treatment period	Normal sperms% M±SE	Head abnormality% M±SE	Tail abnormality% M±SE
Negative control	A 64.5±1.27	A 19.83±2.07	A 15.67±1.67
7 days MTX	B 47.7±4.04	B 27.5±2.88	B 24.8±1.32
21 days MTX	C 38.3±1.78	BC 31.4±1.78	C 30.3±1.03
35 days MTX	D 25±2.07	C 40.0±2	C 35±1.73

Numbers with different letters are significantly different (≤ 0.05) within the same column.

Conclusions and Recommendations

Conclusions

1. The main active compounds detected in the leaf extract of *Ocimum sanctum* are steroids and flavonoids.
2. *O. sanctum* methanolic extract induce abnormalities in sperm morphology like hummer head and coiled tail.
3. *O. sanctum* hexane extract has no effect on sperm morphology.
4. Methanolic extract of *O. sanctum* may be appeared with antimicrobial actions especially as antifungal.
5. Hexane extract of *O. sanctum* showed no antimicrobial activity.
6. *O. sanctum* extracts (methanolic and hexane) have no genotoxicity and no effect on the lymphocytes chromosome.

Recommendations

1. Further studies are needed for purification of active compounds with deferent techniques.
2. Further studies needed for the effects of *O. sanctum* extracts on cell line.
3. *In vitro* and *in vivo* study for pharmacological effects of *O. sanctum* extracts include immunological and cytological
4. Study the cytogenetic effects of *O. sanctum* on cells other than bone marrow cells.

References

References

A

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. Survey, 44:l- 17.

Ad'hiah, A. H.; G Hali, K. H. and El-Hassani, M. (2001). An epidemiological approach to bladder cancer in Iraq from 1976-1998. Al-Mustansirya J.of Sci. 11: 25-30.

Ad'hiah, A. H.; Al-Kashaly, S. S. and Abbas, T. A. A. (2002). Group A Streptococcus (*Streptococcus piygoenes*) and the mitotic activity of lymphoid organs in albino mice. The Eight Scientific Conferenemes of the Technical Education Committee, pp. 302-308.

Ad'hiah, A. H.; Sayhood, Y. D. and Shubber, E. K. (2004). Inhibiting the hematological and cytogenetic effects of tamoxifen by alcoholic extract of garlic (*Allium sativum*). Nucleus. 47; 10-16.

Ad'hiah, A. H.; Sulaiman, G. M. and Al-Zaidy, M. S. (2006). Some immunological evalutions of propolis in albino male mice. Journal of the Faculty of Medicine. (Accepted).

Aguayo, A.; Kantrarjan, H. and Manshout, T. (2002). Leukaemia and lymphoma (Hodgkin and non Hodgkin disease). Blood, 15: 2240 -2255.

Ahmed, M.; Ahamed, R.N.; Aladakatti, R.H. and Ghosesawar, M.G. (2002). Reversible anti-fertility effect of methanolic extract of *Ocimum sanctum* leaves on sperm parameters and fructose content in rats. J. Basic Clin. Physiol. Pharmacol., 13(1): 51-59.

Al-Abid, M.R. (1985) .zur zurammen der absuchlu B memtrame in phoenix dactily Grawuzburg University,Wurzburg F.R of Germany.

Al-Amiry, E.W.H. (1999). Enzymatic, cytogenetic, and drug resistance studies on blood from patients with breast cancer. M.Sc. thesis, College of Education for Woman, University of Baghdad (in Arabic).

Al-Bayatti, A.A.; Al-Duliemy, K.S. and Al-Nassir, I.J. (1997). The prognostic factor for childhood acute leukaemia. J.Fac.Med.Baghdad, 40:164-172.

Al-Duliemy, D.A. (2005). Anticlastogenicity and antileukaemic cell potencies of alcoholic extracts from grape (*Vitis vinifera*). M.Sc. thesis, College of Science, University of Baghdad.

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.; Fine, R.L. and Drak, J.C. (1986). The effect of methotrexate on intracellular folate pools in human MCF-7 breast cancer cells: Evidence for direct inhibition of purine synthesis .J.Biol.Chem., 261: 6478- 6485.

Allegra, C.J.; Hoang, K. and Yeh, G.C. (1987). Evidence for direct inhibition of denovo purine synthesis in human MCF-7 breast cells as a principle made of metabolic inhibition by methotrexate. J. Biol.Chem., 262: 13520 – 13526.

Allen, J.W.; Shuler, C.F; Menders, R.W. and Olatt, S.A. (1977). A simplified technique for *in vivo* analysis of SCE using 5-Bromodeoxyuridine tablets .Cytogenet. Cell. Genent., 18:231-237.

Al-Mohammed, N.T.; Al-Rawi, K.M.; Younis, M.A. and Al-Morani, W.K. (1986). PrincipleS of Statistics. Al-Mousl University (in Arabic).

Al-Shimary, A.O.A. (2004). Extraction and purification of lignan compound from flax seed *Linum usitatissimum* and study it's anti-mutagenicity on different systems .M.Sc. thesis, Genetic Engineering and Biotechnology ,Baghdad University (in Arabic).

Andersone, R.E.; Yoheic, I.; Nishi Yama, H. and Ishida, K. (1972). Pathogenesis of radiation-related leukaemia and lymphoma. Lancet, 1:1060-1062.

Al-Rubaiey, F. A. A. (2000). A study of the mutagenic and antimutagenic ability of some Iraqi medicinal plants. M.Sc. thesis, College of Eduction Ibn Al-Haitham University of Baghdad.

Al-samarae, K. W.; Al-Rekabi, S. and Ahmed, B. (2001). Effect of leaves extract *Withania somnifera* on the growth of some dermatophytes.

The second conference on medical and biological science. Faculty

of allied medical science. Zarka Private University, pp. 40.

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.

Ardito,G.; Lamberti, L. and ponzetto, p. (1980). Sister chromatid exchanges in cigarette-smoking .Mutat. Res., 78: 209-212.

B

Bain, J.B. (1999). Leukaemia Diagnosis, 2nd ed., Black well science. UK. Economics Company.

Baricevіv, D.; Sosa, S.; Loggia, R. D.; Tubaro, A.; Simonovska, B.; Kransa, A. and Zupancic, A, (2001). Topical anti-inflammatory activity of *Ocimum sanctum* L. leaves: the relevance of ursolic acid. Journal of Ethnopharmacology. 75: 132-152.

Berman, F. (2000). Herbs and dietary supplements in the prevention andtreatment of cardiovascular disease. *Prev. Cardiol*. **3**: 24–32.

Bishop, J.M. (1983). Cellular ecogenes and retrovirus. Ann.Rev. Biochem. 52:301.

Bloom, S.E. and Hsu, T.C. (1975). Differential fluorescence of sister chromatids in chicken embryos exposed to 5-BUdR. Chromosoma., 51:261-267.

Boles, J.M.; Dutel, J.; and Briere, J. (1984). Acute renal failure caused by

extreme hyper phosphatemia after chemotherapy. Cancer, 53:2424-2425.

Bruneton, J. (1995). Pharmacognosy, Photochemistry, And Medicinal Plants. Hatton, C.K (eds.) lavoisier publishers, Paris, pp 110.

C

Calixto, J. B. (2005). Twenty-Five years of research on medicinal plants in Latin America. A personal view. Journal of Ethnopharmacology. 100:131-134.

Caragay, A.B. (1992). Cancer-preventative foods and ingredients. Food Technol., 46: 65-68.

Catra, C.; Moretti, M. D. and Peana, A. T. (1996). Activity of the oil of *Ocimum sanctum* against *Botrytis cinere.* J. of Essential oil Res. 8: 399-404.

Cepek, P.; Hribalova, V.; Svadova, E.; Ebringerova, A; Sasinkova, V. and Masarova, J. (2003). Characterization of immunomodulatory polysaccharide from Ocimum sanctum L. Int. J. Biol. Macromol. 33: 113-119.

Chambers, H. F. (2001). Chemotherapeutic drugs. In: Basic and Clinical Pharmacology. Katzung, B.G. 8th ed. Lange Medical Books/McGraw-Hill. U.S.A.

Cronstein, B.N. (1997). The mechanism of action of methotrexate. Rhcum. Dis. Clin. N.Am. 23:739.

D

Dapkevicius, A.; Venskutonis, R.; Van- Beek, T. A. and Liussen, J. P. H. (1998). Antioxidant activity of extracts obtained by different isolation producers from some aromatic herbs grown in India. J. Sci. Food Agric. 77: 140-146.

Dharmani, P.; Kuchibhotla, V. K.; Maurya, R.; Srivastava, S.; Sharma, S. and Pali, G. (2004). Antiulcer activity.

Journal of Ethnopharmacology. 93 (2-3): 197-206.

E

Edward, C.R. and Bouchier, I.D. (1999). Davidson Principles And Practice Of Medicine, 8th ed. chapter 5:395-410.

Eisenberg, D.M.; Kessler, R.C.; Foster, C.; Norlock, F.E.; Calkins, D.R.; and Delbanco, T.L. (1993). Unconventional medicine in the United States. Preference, costs and pattens of use. N. Engl. J. Med., 328:246-252.

Ekachai-Khumphan; Darunee-Buripakdi-Lawso (2002). Acute toxicity, mutagenicity and antimutagenicity of ethanol Ocimum sanctum leaf extract using rat bone marrow micronucleus assay. Cyto. Bios. 32(4): 16-21.

Evans, H.J.; Neary, G. J. and Williamson, F. S. (1959). International Journal of Radiation Biol.. 1: 216-229.

Evan, H.J. (1976). Cytological methods for detecting chemical mutagens. In:chemical mutagens, principles and methods for their detection, VOl.4, Hollaender, A. (ed). Plenum press, New York and London, PP. 1-29.

F

Farrell, K.T. (1985). Spices Condiments And Seasonings. Westport, CT; AVI publishing company, USA, PP 170.

Feagen, B.G. ; Fedorak, R.N. ; Irvine, E.J. ; Wild, G. ; Sutherland, L. ; Steinhart, A.H. ; Greenberg, G.R. ; Koval, J. ; Wang, C.J. ; Hopkins, M. ; Hanauer, S.B. ; and MC Donald, J.W. (2000).

Ferreira, L.; Carvalho, J. C. T. and Maistro, E. L. (2003). Standardized *Solanum melongena* Extract Presents Protective Effects against Chromosomal Aberrations Induced by Doxorubicin in Wistar Rat Bone Marrow Cells. CYTOLOGIA. 68: 177-181.

Acomparison of methotrexate with placebo for the maintenance of remission in crohns disease. N. Engl. J. Med., 342:1627.

Fingold, S. M.; Nartin, W.J. and Scott, E. G. (1982). Anti- microbial susceptibility tests and assays in, Bialy and Scott's Diagnostic microbiology, 6th ed, chap. 36, pp. 385-404.

Fitoterapia. (2001). Anthelmintic activity. Journal of Ethnopharmacology. 72 (6): 669-670.

G

Gali-Muhtasib, H. U. and Affra, N. I. (2000). Chemopreventive effects of basil oil on skin popillomas in mice. Phytomedicine. 7: 129-136.

Galloway, S.M.; Armstronge, M.J.; Reuben, C. ; Colman, S .; Brown, B.; Cannon, C.; Bloom, A.D.; Nakamura, F.; Ahmed, M.; Duk, S.; Rimpo, J.; Margoline , G.H.; Resnik, M.A.; Anderson , G. and Zeiger, E.(1987). Chromosome aberration and sister chromatid exchanges in Chinese hamster ovary cells: Evaluation of 108 chemicals. Environ. Mol. Mutagen, 10: 1-175.

Ganong, W.F. (1991). Review of medical physiology. Connecticut: Appleton and lauge, 401: 94-95.

Gebhart, E. (1981). Sister chromatid exchange (SCE) and structural chromosomal aberrations in mutagenicity testing. Hum. Genet. 58: 235-245.

Geeta; Vasudevan, D.M.; Kedlaya, R.; Deepa, S. and Ballal, M. (2001). Activity of Ocimum sanctum (the traditional Indian medicinal plant) against the enteric pathogens. Indian J Med Sci. 55(8): 434-8, 472.

Genestier, L.; Paillot, R.; Guemeneur, L.; Izeradjene, K. and Revillard, J.P. (2000). Mechanisms of action of methotrexate. Immunophrmacology, 47:247-250.

Gescher, A. (2004). Mechanisms of action and toxicity of anti tumor drugs . M.Sc. thesis in molecular pathology and toxicology. Al-Mustansirya University.

Gilani, A. H. and Atta-ur-Rahman (2005). Trends in ethnopharmacology. Journal of Ethnopharmacology. 100:43-49.

Goldmen , J. and Tarig , M.(1999). Understanding Leukaemia And Related Cancer. Black well science, UK. PP: 3-45.

Goodenough, U. (1978). Genetics Helth. Rinechart and Winston, New York, pp.8.

Gosh, B. B.; Talukder, G. and Shorama, A. (1991). Effect of culture media on spontaneous residence of mitotic index, chromosomal aberration, micronucleus counts, sister chromatid exchange and cell cycle. Kinetics in principle blood lymphocytes of male and female donars. Cyto. Bios. 67: 71-75.

Gupta, S.K.; Prakash, J. and Srivastava, S. (2002). Validation of traditional claim of Tulsi, *Ocimum sanctum* Linn. as a medicinal plant. Indian J Expt Biol. 40:765-773.

Gubri-Fakim, A. (2005). Medicinal plants. Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine.

H

Hafez, B. and Hafez, E.S.E. (2000). Reproduction In Farm Animals . Awolters kluwer company, USA, pp.220.

Hammer, K. A.; Carson, C. F. and Riley, T. V. (1999). Antimicrobial activity of essential oils and other plant extracts. Journal of Applied Microbiology. 86: 985-990.

Harborne, J.B. (1973). Phytochemical Methods Science Paperable Active, chopman and Hall pub.

Haung, C.C.; Jan, J.C.; Pachdec, P.D. and Champman, V.M. (1990). Comparision of base line sister chromatid exchanges (SCE), cyclophosphamicle-ethylintrosourea (ENU), induce cells cycle delay and chromosome aberrations between peru and laboratory mice. Mutate. Res., 230:93-100.

Henshaw, D.L.; Etough, J.P. and Richardson, R.P. (1990). Causative factor in induction of myeloid leukaemia and other cancers in adults and children. Lancet., 335:1005-1012.

Hohmann, J.; Zupko, I.; Redei, D.; Csany, M.; Falkay, G.; Mathe, I. and Janicsak, G. (1999). Protective effects of the aerial parts of *Ocimum sancum* and its constituents against enzyme-dependant and enzyme- independent lipid peroxidation. Planta Medica. 65: 576-578.

Holden, S.A.; Teacher, B.A.; Northey, O. and Rosowsky, A. (1995). Antifolates can potentiate topoisomerase II inhibitors *in vito* and *in vivo* cancer chemo. Pharm., 36:165-171.

Hudson, F. and Hay, F. (1989). Practicle Immunology. Section 1; Blak well scientific publicasion, PP, 116.

Hughes, D. A. (2001). Dietaryn carotenoi 823-827.ds and human immune function. Nutrition. 1:

I

Internet (2004). www. Holy-basil.com.

Ishidate, M.J. and Sofuni, T. (1985). The *in vitro* Chromosomal Aberration Test Using Chinese Hamster Lung (CHL) Fibroblast Cells In Culture. In: progress in mutation research, VOI. 5, Ashby, J., (eds.), elsevier science publishers, Amsterdam-New York, Oxford, PP. 247-432.

J

Jensen, M.K. and Nyfros, A. (1979). Cytogenetic effect of MTX on human cells *in vitro*.Mutat.Res., 649:336-343.

John, S.L. (2000). Childhood Leukemia .2nd ed. Published in the United States by Oxford University press. Inc., New York.

Jolivet, J.; Cowan, K.H.; Curt, N.J. and Chabner, B.A. (1983). The pharmacology and clinical use of MTX. New Engl. J.Med., 309:1094-1104.

Juliani, H. R. and Simon, J. E. (2002). Antioxidant activity of basil. In trends

in new crops and new uses. (Janick & Whipkey eds.); ASHS

Press, Alexandrea, VA.

K

Kanazawa, M.; Staomi, Y.; Mizutani, Y.; Ukimura, O.; Kawauchi, A.; Sakai, T.; Baba, M.; Okuyama, T.; Nishino, H. and Miki, T. (2004). Isoliquiritigenin inhibits the growth of prostate cancer. Nature, 98:10.

Kantak, N.M. and Gogate, M.G. (1992). Effect of short term administration of Tulsi (*Ocimum sanctum* Linn.) on reproductive behavior of adult male rats. Ind. J. Physiol Pharmacol. 36: 109-111.

Kasahara, Y.N.; Miura, D.; Yagi, K. and Makita, T. (1992). Mechanism of induction of micronuclei and CAs in mouse bone marrow by multiple treatment of methotrexate. Mutat. Res., 280:117-128.

Kasinathan, S.; Ramakrishnan, S. and Basu, S. L. (1972). Antifertility effect of Ocimum sanctum L. Indian Journal of Experimental Biology. 10(1): 23-25.

Kavvadiase, D.; Monschein, V.; Sand, P.; Riederer, P. and Schreier, P. (2003). Constituents of basil (*Ocimum sanctum*) with *in vitro* affinity to human brain benzodiapine receptor. Journal of Agrichaltural Food chemistry. 69: 113-117.

Kennedy, D. O.; Pace, S.; Haskell, C.; Okello, E. J.; Milne, A. and Scholey, A. B. (2006). Effects of cholinesterase inhibiting basil (*Ocimum sanctum*) on mood, anxiety and performance on a psychological stressor battery. Neuropsychiatrypharmacology. 31: 845-852.

Klareskog, L.; Van Der Heijde, D.; De Jaeger, JP.; Goth, A.; Kalden, J.; Malaise, M.; Martin Mola, E.; pavelka, K ; Sany, J.; Settas, L.;

Wajdula, J.; Pederson, R.; Fatenejad, S. ; and Sanda, M. (2004). Therapeutic effect of the combination of etanercept and methotrexate compared with each treatment alone in patients with rheumatoid arthritis:double-bland randomised controlled trail. Lancet., 363:675-681.

L

Lambert, B.; Lindbald, M.; Norden, S. and Werelius, B. (1978). Increase frequency of sister chromatied exchange in cigarette smoker, Hereditas, 88:147-149.

Lawrence, B. M. (1992). Progress in essential oils perfumer and Flavorist.

London and New York: 70- 82.

Lamaison, J. L.; Petitjean-Freytet, C,; Duband, F. and Carnat, A. P. (1991). Rosmarinic acid content and antioxidant activity in French Lamiaceae. Fitoterapia. 62: 166-171.

Li, J.C. and Kaminskas, E. (1984). Accumulation of DNA strand breaks and methotrexate cytotoxicity. proc. Natl. Acad. Sci., 81:5694-5698.

Li, K.M.; Todoroviæ, R.; Deveanesan, P.; Higginbotham, S.; Kofeler, H.; Ramanathan, R.; Gross, M.L.; Rogan, E.G. and Cavalieri, E.L. (2004). Metabolism and DNA binding studies of 4-hydroxyestradiol and estradiol-3,4-quinone *in vitro* and in female ACI rat mammary gland *in vivo*. Carcinogenesis 25:289-297.

Liehr, J.G. (2001). Genotoxicity of the steroidal oestrogens oestrone and oestradiol: Possible mechanism of uterine and mammary cancer development. Hum Reprod Update. 7:273-281.

Lima, C. F.; Andrade, P. B.; Seabra, R. M.; Fernandes-Ferreira, M. and Pereira-Walson, C. (2005). The drinking of *Ocimum sanctum*

infusion improves liver antioxidant status in mice and rat. Journal of Ethnopharmacology. 97: 383-389.

Lu, Y.; Foo, L. and Wong, H. (1999). Basil cumarin, a novel caffeic acid trimmer from *Ocimum sanctum*. Photochemistry. 52: 1149-1152.

M

Mahajan, U.N.and Wasule, D.D. (2008). PHCOG MAG.: Research Article Sunscreen and Anti-Oxidant Activities of Herbal Gel Formulations. Phcog Mag. 4(13):12-18.

Martein, P.M.; Schasion, H. and Jonzer, J. (1999). Chronic myeloid leukaemia : No rearrangement of the break point cluster region . Cancer Genet. Cytogenet. 36: 227-229.

Martindale, H. (2002). The Complete Drug Reference. 3rd ed. Pharmaceutical press. Great Britain.

Martino-Roth, M. G.; Viegas, J. and Roth, D. M. (2003). Occupational genotoxicity risk evaluation through the comet assay and the micronucleus test. Genet. Mol. Res. 2: 410-417.

Moller, P. and Loft, S. (2002). Oxidative DNA damage in human white blood cells in dietary antioxidant intervention studies. American Journal of Clinical and Nutrition, 76: 303-310.

Mukheef, F.J. (1999). Cytogenetic study on leukaemia . M.Sc. thesis, College of Science, Al-Nahrain University.

N

Nash, R.A. ; Pineiro, L.A. ; Deeg, R. ; Fitzsimnons, W.E. ;Furlong, T. ; Hansen, J.A. ;Glooley, J. ;Maher, R.M. and Martin, P. (1996). FK 506 combination with methotrexate for the prevention of

graft-vs-host disease after marrow transplantation from matched unrelated denors. Blood. 88:36-34.

Nath, J.; Krishna, G.; Petersen, M. and Ong, T. (1988). Sister chromatid exchanges induced by triethyleneamine: *in vivo* and *in vitro* studies in mouse and Chines hamster bone marrow and spleen cells. Mutat. Res., 206:73-82.

National Committee for Clinical Laboratory Standard (1993). Methods for dilution antimicrobial susceptible tests for bacteria hat grow aerobically. National Committee for Clinical Laboratory Standard, Villanova, Pa.

Nayak, V. and Uma Devi, P. (2005). Protection of Mouse Bone Marrow against Radiation-Induced Chromosome Damage and Stem Cell Death by the Ocimum Flavonoids Orientin and Vicenin. Radiat. Res. 163: 165–171.

P

Panda, S. and Kar, A. (1998). Pharmacological Research 38(2): 107-110.

Pandit, P.; Singh, A.; Bafna, A.R.; Kadam, P.V. and Patil, M.J. (2008). Evaluation of antiasthmatic activity of *Ocimum sanctum*. Indian J. Pharm. Sci. 70:440-4.

Patton, J.L. (1967). Chromosome study of certain pocket genus perogenathus (Rodentia :Heteromyidea) Mamm. J., 48:27-37.

Paul, P. and Buul, V. (1977). Dose response relationship for radiation translocation in somatic and germs cells of mice. Mutat. Res., 45:61-68.

Pereira, R. S.; Sumita, T. C.; Furlan, M. R.; Jorge, A. O.C. and Ueno, M. (2004). Antibacterial activity of essential oils on microorganisms isolated from urinary tract infection. Rev. Saude Puplica. 38.

Peter, J.H. (1997). Myelocytic leukaemias. In: Principle of Hematology, harries – young (Ed.).P:296-316.

Pieters, L. and Vlietinick, A. J. (2005). Bioguided isolation of pharmacolicogically active plant components, still a valuable strategy for the finding of new lead compounds? Journal of Ethnopharmacology. 100: 57-60

Pitatevic, I.; Kuftinec, J.; Blazevic, N. and Kustrak, D. (1984). Seasonal variation of essential oil yield and composition of basil, *Ocimum sanctum*. J. Nat. Proc. 47: 409-412.

R

Rani, P. and Khullar, N. (2004). Antimicrobial evaluation of some medicinal plants for their anti-enteric potential against multi-drug resistant *Salmonella typhi*. Phytother. Res. 18(8): 670-673.

Rinsky, R.A and Young, R.J. (1981). Leukaemia in benzene workers. Am. J. of industrial Med., 2:217-245.

Rios, J. L. and Recio, M. C. (2005). Medicinal plants and antimicrobial activity.). Journal of Ethnopharmacology. 100: 80-84.

Robert, H.T. (1996). Principles of Genetics. 5th ed.The McGraw –Hill Company .USA.pp.112.

Ryue, S. Y.; Lee, C. O. and Choi, S. U. (1997). *In vitro* cytotoxicity of Tanshinones from Ocimum sanctum. Planta Med. 63: 339-342.

S

Saller, R.; Buechl, S.; Meyrat, R. and Schmidhauser, C. (2001). Combined herbal preparation for topical treatment of herps labialis. Forsch-Komplementarmed-Klass-Nalurheild. 8: 373-382.

Santos-Gomes, P. C.; Seabra, R. M.; Andrade, P.B. and Fernandes-Ferreira, M. (2002). Phenolic antioxidant compounds produced in vitro shoots of basil (*Ocimum sanctum* L.). Plant Science. 162: 981-987.

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(2): 72-76.

Sarkas, G.Y.; Al-Rawi, K.M. and Katii, J.M. (1980). Diagnosis Of Organic Compounds (Chemical Methods), Baghdad University.

Sato, M.; Sato, T. and Ose, Y. (1992). Modulating effect of tanshinones on mutagenic activity of Trp-p-1 and benzene (a) pyrene in *Salmonella typhimurium*. Mutation Reaserch. 265: 94-54.

Schroder, T. M. (1966). Humagenetic. 2: 287-3116.

Schroder, T. M. (1970). Chemical mutagenesis in mammals and man. Springer, Heideberg, pp. 214-219.

Seeley, R.R.; Stepheus, T.D. and Tate, P. (1996). Essentials Of Anatomy And Physiology. 2nd ed. Medical econmics company , USA, pp:110.

Seppa, N. (2004). Umbilical bounty. Cord blood shows value against leukaemia. Sci. news, 24:166-339.

Sethi, J.; Sood, S.; Seth, S. and Talwar, A. (2003). Protective effect of Tulsi (*Ocimum Sanctum*) on lipid peroxidation in stress induced by anemic hypoxia in rabbits. *Indian J Physiol Pharmacol.* 47(1):115-119.

Seitz, M. (1999). Molecular and cellular effects of methotrexate .Curr. Opin. Rheumatol., 11:226.

Shovlin, C.L.; Lamb, J.R. and Haslett, C. (1999). The Molecular And Cellular Basis Of Disease. In haslet , C. ; Hunter , A. J. and Boon, A.N. (Eds). Davidson. Principles and Practice of medicine 18th ed. Charchil Livingston. New York: 1-56.

Shubber, E.K. (1987). Sister chromatid exchange in lymphocytes from patients with schistosoma hematobium . Mutat . Res., 180:93-99.

Singh, S. and Majumdar, D.K. (1999). Evaluation of the gastric antiulcer activity of fixed oil of Ocimum sanctum (Holy Basil). Journal of Ethnopharmacology. 65(1): 13-19.

Singh, S.; Malhotra, M. and Majumdar, D. K. (2005). Antibacterial activity of Ocimum sanctum L. fixed oil. Indian journal of experimental biology. 43(9): 835-837.

Sivropoulou, A.; Nikolaou, C.; Papanikolaou, E.; Kokkini, S.; Lanaras, T. and Arsenakis, M. (1997). Antimicrobial, cytotoxic and antiviral activities of *Ocimum sanctum* essential oil. J. Agric. Food Chem.

45: 3197-3201.

Stahl, E. (1969). Thin-layer-chromatography a laboratory Handbook, 2nd Ed., translated by Ashworth. M. R. F. Spring- Verag, Berlin, Heidelberg and NewYork.

T

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.

Tian, Y.; Ishikawa, H.; Piao, F. Y.; Yamamoto, T.; Duan, Z. W.; Zhany, Y. M.; Ma, M. Y. and Cui, J. S. (2003). Micronucleus assay of human Capillary blood lymphocytes methods. Occup. Health. J. 45: 408-409.

Topham, J. C. (1980). The detection of carcinogen induced sperm head abnormalities mice. Mutation Research. 69: 149-155.

Tyler, V. (1994). Herbs of choice. The Therapeutic Use Of Phytomedicinal . Haworth press , New York , pp.66-67.

V

Vats, V. ; Yadav, S. P. and Grover, J. K. (2004). Effect on carbohydrate metabolism. Journal of Ethnopharmacology. 90 (1): 155-160.

Von Ledebur, M. and Schmid, W. (1973). Mutation Reasearch. 19: 109-117.

Vrinda, B. and Uma Devi, P. (2001). Radiation protection of human

lymphocyte chromosomes in vitro by orientin and vicenin. Mutation research/Genetic Toxiocology and Enviromental Mutagenisis. 498(1): 39-46.

Vujosevic, M. and Blagojevic, J. (2005). Antimutagenic effects of extracts from basil (*Ocimum sanctum*) in mamilane system in vitro. Acta. Vet. Hung., 52: 439-443.

W

Wang, M.; Kikuzaki, H. and Zhu, N. (2000). Isolation and structural elucidation of two new-glycoside from basil (*Ocimum sanctum*). Journal of Agricultural Food Chemistry. 48: 235-238.

Weinblatt, M.E.; Maier, A.L.; Fraser, P.A. and Colblyn, J.S. (1998). Long-term prospective study of methotrexate in rheumatoid arthritis: conclusion after 123 months of therapy .J. Rheumatol. 25:238.

Wolters, B. (1976). Serial experiments on transformation and degradation of secondary plant substances by microorganism. Planta Medica. 29: 41-53.

Wyrobek, A.J and Bruce, W.R. (1975) .Chemical indication of spermatities in mice. proc. Natl . Acad. Sci., 72:4425 -4429.

X

Xiong, L. L. (1993). Therapeutic effect of basil polysaccharide in the treatment of chronic hepetitis B Chung-Kuo-His-I-Chieh-Ho-Tsa-Chih. 13: 533-516.

Z

Zupko, I.; Hohmann, J.; Redei, D.; Falkay, G.; Mathe, I. and Janicsak, G. (2001). Antioxidant activity of leaves of Ocimum species in enzyme-dependant and enzyme- independent lipid peroxidation and their phenolic constituents. Planta Medica. 67: 336-368.

جمهورية العراق
وزارة التعليم العالي و البحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الأحيائية

التأثير المضاد المايكروبي والوراثي الخلوي لنبات الريحان الطبي على ذكور الفئران البيض

رسالة

مقدمة إلى مجلس كلية العلوم / جامعة النهرين

وهي جزء من متطلبات نيل درجة الماجستير علوم في التقانة الاحيائية

من قبل

حيدر جبار ابراهيم

بكالوريوس تقانة احيائية- جامعة النهرين (٢٠٠٣)

ذو الحجة 1429

كانون الاول ٢٠٠٨

بسم الله الرحمن الرحيم

وَعَلَّمَكَ مَالَمْ تَكُنْ تَعْلَمُ وَكَانَ فَضْلُ اللَّهِ عَلَيْكَ عَظِيماً

صدق الله العظيم

سورة النساء {113}

الاهداء

الى الذي تمنيت ان افرحه بمشاهدة هذا اليوم
والدي الغالي (رحمه الله)
الى املي وقدوتي في مجا ة كل الصعاب في الحياة
امي العزيزة
الى النبض الذي يجري في شراييني ومعنى الدنيا لدي
زوجتي الحبيبة
الى سندي وقوتي في الحياة
اخوتي احمد و علي

اهدي جهدي المتواضع

حيدر

الخلاصة

اجريت هذه الدراسة للكشف عن مختلف المركبات الفعالة للريحان الطبي, وفعالية مستخلص الميثانول والهكسان ضد بعض الاحياء المجهرية(خارج الجسم), والتاثير الوراثي الخلوي على ذكور الفئران البيض المختبرية(داخل الجسم).

اشتمل التاثير الوراثي الخلوي معامل الانقسام الخيطي والتغيرات الكروموسوميةز

الدراسة اشارت ايضا للتحقق من دور مستخلصات الريحان الطبي(الميثانولي والهكسان) في اختزال فعالية النطف لفئران المختبر البيض والتشوهات المظهرية في راس وذيل النطف.

الفعاليات ضد الميكروبية لمستخلصات الريحان الطبي(الميثانولي والهكسان) اجريت على انواع مختلفة من الاحياء المجهرية(*Staphylococcus aureus, Pseudomonas aeruginosa* و *Candida albicans*).

التراكيز المجربة في هذه الدراسة لمستخلص الريحان الطبي الميثانولي كانت 75,37.5 او 18.75 ملغم/مل. بينت النتائج ان لهذا المستخلص تاثير جيد على *Candida albicans* وتاثير خفيف على *Staphylococcus aureus* في التراكيز الثلاثة المستخدمة بينما مستخلص الهكسان لنبات الريحان الطبي لم يعطي اي نتائج في كل التراكيز المستخدمة على الاحياء المجهرية المجربة.

التاثيرات الوراثية الخلوية لمستخلصات نبات الريحان الطبي ظهرت بعد اليوم السابع من الحقن بثلاث تراكيز مختلفة.

الجرع المستخدمة للحقن بالميثوتركسيت كانت 0.6, 0.8, و1 ملغم/كغم بينما جرع الريحان الطبي كانت 200, 400 و600 ملغم/كغم.

وقد تم الحصول على النتائج التالية:

1. اظهر التحليل الكيميائي ان مستخلص الميثانول لاوراق نبات الريحان الطبي يحتوي على مركبات فعالة مثل flavonoids.
2. اظهر التحليل الكيميائي ان مستخلص الهكسان لاوراق نبات الريحان الطبي يحتوي على مركبات فعالة مختلفة مثل الزيوت الثابتة, والزيوت الطيارة.

3 اعطى الميثوتركسيت نتائج واضحة في تقليل معامل الانقسام وزيادة التغيرات الكروموسومية في خلايا نخاع العظم للفئران.

4. لوحظت زيادة التغيرات المظهرية في راس وذيل النطف عند المعاملة بالميثوتركسيت بعد 35 يوم من المعاملة.

5. ادى المستخلص الميثانولي لنبات الريحان الطبي الى زيادة معامل الانقسام وتقليل التغيرات الكروموسومية, بينما مستخلص الهكسان لنفس النبات لم يظهر اي تغيير.

6. ادى المستخلص الميثانولي لنبات الريحان الطبي الى زيادة التغيرات المظهرية على النطف مثل تكوين الذيل الملتف والراس المشابه للمطرقة,بينما مستخلص الهكسان لم يظهر اي تغيير.

7. المعاملة بالمستخلص الميثانولي لنبات الريحان الطبي على نطف ذكور فئران المختبر البيض اعطت النتائج بعد 35 يوم من المعاملة.

8. المعاملة بمستخلص الهكسان لنبات الريحان الطبي على نطف ذكور فئران المختبر البيض لم تعطي اي نتائج حتى وان زادت فترة المعاملة.