INTRODUCTION

Plants have been used as medicine for millennia. Out of estimated 250 000 to 350 000 plant species identified so far, about 35 000 are used worldwide for medicinal purposes. It has been confirmed by WHO that herbal medicines serve the health needs of about 80 percent of the world's population; especially for millions of people in the vast rural areas of developing countries. The use of plants as medicine goes back to early man. Evidences of this early association have been found in the grave of a Neanderthal man buried 60 000 years ago. Pollen analysis indicated that the numerous plants buried with the corpse were all of medicinal value . The earliest known medical document is a 4000-year-old Sumerian clay tablet that recorded plant remedies for various illnesses. By the time of the ancient Egyptian civilization, a great wealth of information already existed on medicinal plants. Among the many remedies prescribed were mandrake for pain relief, and garlic for the treatment of heart and circulatory disorders. This information, along with hundreds of other remedies, was preserved in the Ebers papyrus about 3500 years ago (Kong *et a l.*, 2003).

There have been about 60 new herb-derived drugs developed by Chinese scientists over the past four decades. An illustrative example is the discovery of artemisinin (Kong *et a l.*, 2003). Chemical studies on many species of *Artemisia* have been carried out in many places since the1930's, but China was the first to

discover it́s antimalarial activity and this yielded a new antimalarial drug in1972 (Evans *et al.*,1996).

Artemisinin is quite different from the old generation of antimalarial drugs because of it́s novel bioactive peroxide group, which is effective in treating chloroquine-resistant and severe cases without side effects, which is also a common feature of many Chinese herbal medicines (Kong *et al.*, 2003).

There are many herbal extracts and concoctions and plant products used to treat cancer. These include *Artemisia*, also known as wormwood is being researched as a safe, non-toxic, and inexpensive alternative for cancer patients (internet 20).

The clinical applications of artemisinin, in the treatment of malaria and cancer, could be one of the most important discoveries in the 21st century. Further research could lead to an effective, affordable, non-surgery and non-toxic treatment for cancer, malaria and other deadly diseases (internet 8).

Artemisinin had been analyzed for it́s activity against 55 cancer cell lines. It was most active against leukemia and colon cancer cell lines and active for melanomas, breast, ovarian, prostate, CNS, and renal cancer cell lines. Importantly, a comparison of artemisinin's cytotoxicity with those of other standard cytostatic drugs showed that it was active in molar ranges comparable to those of established anti-tumor drugs. These results and known low toxicity of artemisinin and it́s derivatives make them a promising novel candidate for cancer chemotherapy (Efferth, 2001).

In 2001, a WHO report concluded that artemisinin and it́s derivatives do not exhibit mutagenic or teratogenic activity.

Artemisinin enhances macrophage phagocytosis in mice. The herb *Artemisia* also is very effective against worms with reduction 80 - 90% within a week (internet 30).

Grendelmeier *et al.,* (2003), showed that the Artemisinin increased IgE-binding capacity were able to induce T-cell proliferation.

On the strength of those investigations, this study was proposed for: -

* Preparation of diethyl ether extract from *Artemisia herba-alba* to study the cytotoxic effect of extract on normal and tumor cell line.

* Evaluation the effect of extract in both *in vivo* and *in vitro* experiments including (transformation, phagocytosis and mitosis).

* Study the effect of extract on visceral organs.

LITERATURE REVIEW

2.1: The plant *Artemisia herba-alba* (wormwood) charactristics:

Artemisia hreba-alba is from the family compositae (Heinrich, *et al*., 1998). It is local name desert wormwood (Hussein, 1985). This plant is usually grown and widly spread in south East Asia and in most area of North Africa, Syria, Iran, Saudi Arabia and Arab Gulf (Al – Khazraji, 1991).

The silvery green leaves of this compact shrub give it a distinctive appearance when the rest of the vegetation is dry. It is unlike many members of the native flora, wormwood flowers in the middle of the summer. In addition to it̊ s medicinal value, it is a valuable fodder plant and in some areas wormwood is severely over grazed (internet 12).

Artemisia herba-alba is widely used in Iraq folk medicine for the treatment of diabetes mellitus (Al-khazraji, 1993), liver disorders, lowers fever, antiulcer (University of Arab countries for agriculture development, 1988), anti-bacterial, checks bleeding, anti-malarial, and the leaves are antiperiodic, antiseptic, digestive, febrifuge, while an infusion of the leaves is used internally to treat fevers, colds, diarrhea etc. Externally, the leaves are poultice on to nose bleeds, boils and abscesses (Pittler and Ernst, 1999).

Al-khazraji suggested that the aqueous extract of the leaves or barks produced a significant reduction in blood glucose level, while aqueous extract of root and methanolic extract of the aerial parts of the plants produce almost no reduction in blood glucose level (Al-khazraji, 1993; and Al-waili, 1986).

There are many species of *Artemisia* in addition to *Artemisia herba – alba*, such as: *Artemisia cinae , Artemisia camphorata , Artemisia absinthium* or *Artemisia absentium , Artemisia vulgaris , Artemisia maritima , Artemisia dracunculus , Artemisia chamissiparaissus, Artemisia glacialis, Artemisia mutellina, Artemisia spicata and Artemisia judiaca* . (Akeel, 2003; Giuseppe, 1993).

2.2: Historical studies of *Artemisia* plant and it̊ s active compounds:

The fascination of natural products, mostly used as a preparation from a plant with known medicinal properties, goes back to ancient times. The discovery of pure compounds as active principles in plants was first described at the beginning of the 19th century, and the art of exploiting natural products has become part of the molecular sciences (Keyser *et al*., 2003; and Tang and Eisenbrand, 1992). Until the beginning of the twentieth century, virtually all medicines were derived from natural sources, even today, at the start of the twenty-first century, 80% of the world's population still uses plants and plant extracts as their primary source of drugs and medicinal agents. There had been about 60 new herb-derived drugs developed by Chinese scientists over the past four decades, an illustrative example is the discovery of artemisinin (Kong *et al*., 2002).

Artemisia plant was first mentioned in 168 BC as a treatment for hemorrhoids (internet 2). In 341 AD was the first known description of *Artemisia* usefulness against malaria (Klayman, 1985; Greenberg, 1995; Hien and White, 1993; Khaw; De Smet, 1997; and Panosian, 1995), and then in 1596 AD was found that the use of *Artemisia* for chills and fevers, and it appears in other Chinese Materia Medica texts as a treatment for febrile illnesses, in addition, recent studies also indicate that some artemisinin derivatives have other bioactivities, including antiparasitic (against *Schistosoma japonicum*, *Toxoplasma gondii* and so on) and anticancer activities (internet 2; and Lefèvre *et al*., 2001).

Since the 1930's chemical studies on many species of *Artemisia* have been carried out in many places (Kong *et al*., 2002). But in the middle of this century and especially since the 1960s and 1970s, Chinese scientists have put considerable effort and resources into the search for new antimalarial compounds extracted from Chinese traditional herbs. Archaeological findings indicate that qinghao (*Artemisia annua L*.) has been used as a traditional remedy in China for over two thousand years. It's antimalarial principle was finally isolated in 1971 and named artemisinin or qinghaosu (meaning the principle of qinghao in Chinese), it's rapid action, low toxicity and powerful effect against *Falciparum* malaria (Li and Wu, 1998; Verpoorte, 1989 and 2000; Sidhu *et al*., 1998; and Greenberg 1995).

In 1976, the unique structure of the molecule characterized by an endoperoxide and an alternative O-C-O-C segment was identified. The specific lactone reduction discovered during the determination of the structure opened the way for the synthesis of artemisinin derivatives, and thereafter a series of more active and more oil or water soluble derivatives was developed.

Artemisinin derivatives are artemether and artesunate, are rapid acting antimalarials, effective against multidrug resistant *P. falciparum,* that have been used to treat over 3 million cases in South East Asia (Meshnick, 1998; Li and Wu, 1998; and Keyser *et al*., 2003). Other indications for malaria for the artemisinin drugs are currently under investigations. Without a final proof, other erythrocyte persisting parasites like *Babesia* are may be another interesting target parasite. But also *Toxoplasma gondii*, *Pneumocystis carinii* infections in mice have been treated successfully with artemisinin drugs (Keyser *et al*., 2003).

Artemisinin is quite different from the old generation of antimalarial drugs because of it's novel bioactive peroxide group, which is effective in treating chloroquine-resistant and severe cases without side effects, which is also a common feature of many Chinese herbal medicines (Kong *et al*., 2002).

In China in 1979, where in 2,099 patients infected with *P. viva* and *P. falciparum,* artemisinin had good therapeutic effects and improved or cured all patients. Furthermore, the treatment with artemisinin was without any obvious side effects. Body temperature of patients normalized within 72 hours, and asexual parasites were eliminated within 72 hours. (Rowen, 2002; Tawfiq *et al*., 1989; and China Cooperative Research Group, 1982).

In 1992, artemisinin has demonstrated it's cytotoxicity against tumor cells (Sun *et al*., 1992; Efferth *et al*., 2001).

For the past ten years, Hoang (2002) was using artemisinin in combination with several other herbs to treat cancer, and eliminate necrosis material from the body; for example, from wounds, from intestines of people who have ulcerative colitis, and from Crohn's disease. The efficacy of the artemisinin compound is very impressive for the treatment of breast cancer and possibly to prevent it because of direct anticancer activity, but also due to hormonal balancing properties of the artemisinin.

Rowen (2004) was used artemisinin to treat cancer for years. For over 400 patients treated in the past ten years, using artemisinin, they realized a 50 to 60 percent long-term remission rate. Artemisinin has proven itself to be nontoxic at the required dosage level for long periods of time; and that no significant toxicity in short-term use for malaria and at high dose has been reported either.

2.3. The active compounds of *Artemisia herba-alba:* -

The major chemical compounds of this plant are santonin, stigasteral, B-sitosterol (Khafagy, *et al*., 1971), camphor (Chakravarty, 1976), alkaloids, saponin, tannins, coumarin, flavonoid and artemisinin (Al-Khazraji, 1991). It is also have volatile oil such as thyjone and absinthin (Al-zubadi, *et al*., 1996).

2.4. The chemical properties of artemisinin:

Artemisinin is the natural extract of the active principle from the plant *Artemisia*, or wormwood. It has a background of use dating back thousands of years in Traditional Chinese Medicine (internet 2). It is not water-soluble, quickly absorbed and reaches it's peak concentration in the blood within 40 minutes. This form is broken down very quickly into it̊ s metabolites in the liver and excreted some what quickly, but still has some metabolic effects for as long as four hours absorption (Christina and White, 2002; and Li *et al.*, 1998).

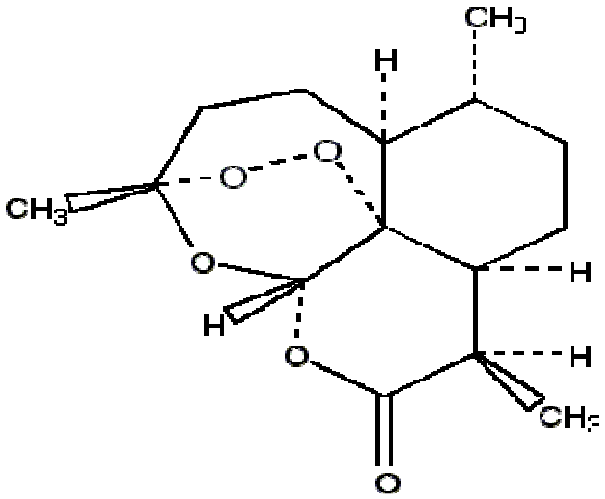
Artemisinin is a sesquiterpene lactone that bears a peroxide group with five oxygen atoms and unlike most other antimalarial drugs that lacks a nitrogen-containing heterocyclic ring system (Rosenthal, 2003; Jiang *et al.*, 1997; and Ames, 1985). It has endoperoxide linkage; this peroxide bridge is the key to artemisinin's antimalarial activity (Meshnick, 1994; and Navaratnam *et al.*, 2000).

The peroxide bridge, two of them in a peroxide bridge system over a seven-member ring and two others in a lactone ring structure, which it is responsible for artemisinin's powerful activity against *Plasmodium falciparum*, the malaria parasite, including some chemo-resistant strains (internet 2 and Burk *et al*., 2003). It is poorly soluble in water and decomposes in other protic solvents, probably by opening of the lactone ring, it is unaffected by them up to 150 C° and it is poorly soluble in oil, and it shows a remarkable thermal stability (Ziffer *et al*., 1997; internet 12; and internet 8).

The peroxide moiety of artemisinin appears to be indispensable for chemotherapeutic activity. When artemisinin treated with borohydride to give dihydroartemisinin, a lactol is formed in which the integrity of the peroxide group retained and the schizonticidal activity enhanced ten fold. Although it is desirable that a single epimer is used, there seems to be no evidence that the a- and ß-epimers differ greatly in antimalarial activity (Zhang, 1992; Burk *et al*.,2003; Posner 2000; Posner *et al*., 1999 and internet 8) .

Artemisinin is described as a white crystalline powder. The chemical structure of artemisinin is C15H22O5 and it's chemical name is (3R, 5aS, 6R, 8aS, 9R, 12S, 12aR)-Octahydro-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano [4.3-j]-1, 2-benzodioxepin-10 (3H)-one. Artemisinin molecular weight is 282.3, it's melt at the range 150-153 C°, and it is soluble in ethanol diethel ether and hexane (internet 13).

Artemisinin structure is:-



Christina and White (2002) described that the artemisinin have several other derivatives available, the three forms that are presently being used by physicians will be discussed: artemisinin, artesunate and artemether. All three of the forms are broken down at various rates in the body into several metabolites, the principal one being DHA. All three forms are absorbed fairly quickly after oral intake, but each reaches a peak concentration and has effects that last different lengths of time.

2.5: Artemisinin derivatives:-

2.5.1: Artesunate:-

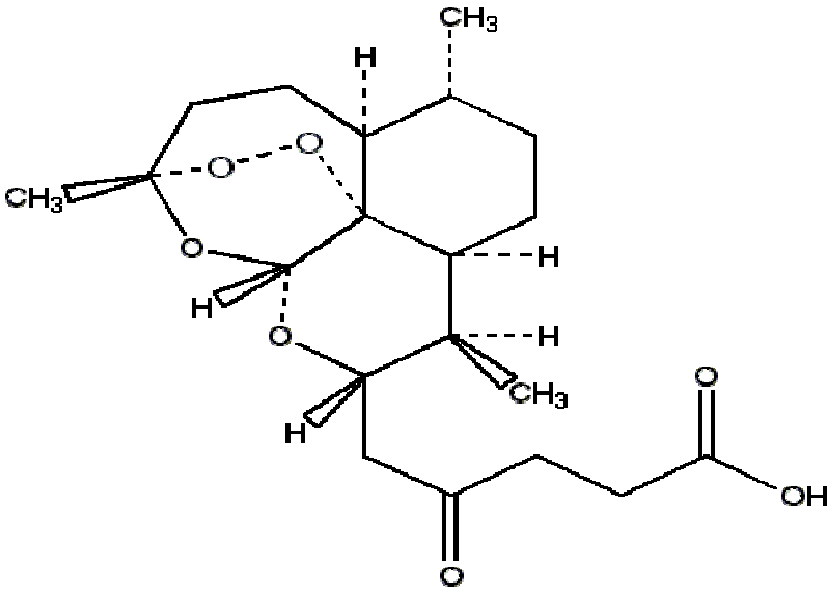
It is a semi-synthetic form that is more water-soluble and may be the most active and the least toxic, but it has the shortest life within the body (internet 14).

Van Agtmael *et al*. (1999) found that the artesunate synthesized by reacting DHA and succinic acid anhydride in an alkaline medium. This type of reaction yields an ester linkage in alpha configuration. This form tends to break down easily when being stored in hot climates, which is certainly a

consideration for treating malaria (Borstnik *et al*., 2002; and internet 14) and it is also cytotoxic to cancer cell lines (Woerdenbag *et al*., 1993; Effreth *et al*., 2003; and internet 14).

The study of Olliaro *et al*. (2001) described that when the pH (acid or alkaline state) of the stomach was more acid the rate of conversion of oral artesunate into its metabolite DHA was increased. This study indicated that some portion of the DHA that is formed within minutes in an acidic stomach environment is absorbed directly. The remaining amount of the parent compound artesunate that makes it to the blood stream will be converted to DHA and other metabolites more slowly in the more alkaline environment of the blood plasma. One study reported that the absorption of artesunate is about 61%, and it has the shortest activity period in the body of the three forms.

Artesunate structure is:-

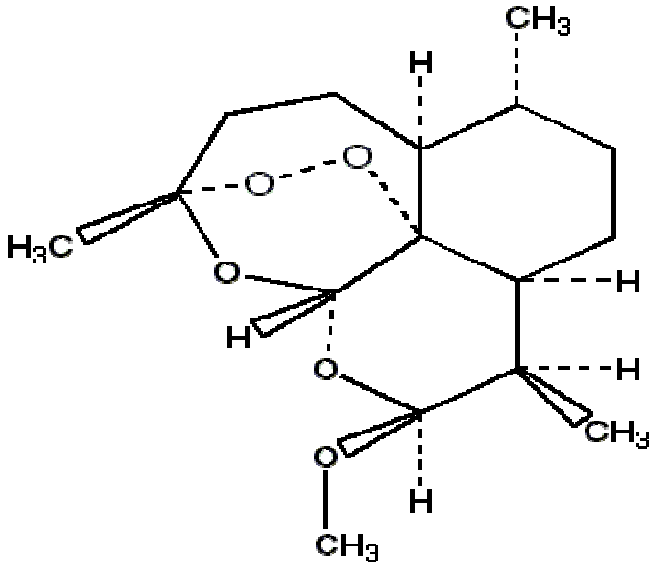


It is molecular weight 38.4, Chemical name (3R, 5aS, 6R, 8aS, 9R, 10S, 12R, 12aR)-Decahydro-3, 6,9-trimethyl-3, 12-epoxy-12H-pyrano [4,3-j] 1,2-benzodioxepin-10-ol, hydrogen succinate; Melting point is 144 °C, and it is very slightly soluble in water, very soluble in dichloromethane R, freely soluble in ethanol and acetone (internet 15).

2.5.2: Artemether: -

Artemether is a white anhydrous crystalline powder, is a derivative of artemisinin and is more chemically active than it s parent. Technically, it's chemical name is: [3R-(3a, 5aB, 6B, 8aB, 9a, 10a, 12B, -12aR)]-Decahydro-10-methoxy-3, 6, 9-trimethyl-3, 12-epoxy-12H-pyrano [4,3-jl-1, 2-benzodioxepin, and in most pharmacopoeias in which it is now listed, it's pharmacological class is "antimalarial." (It's molecular formula is C16-H26-O5, and it has a molecular weight of 298.38 (Internet 8). practically it is insoluble in water; very soluble in dichloromethane R and acetone R; freely soluble in ethyl acetate R and dehydrated ethanol R. Melting range 86 °C.

Artemether structure is:-



Artemether is synthesized from DHA using methanol and a catalyst in acidic medium. This results in the production of predominantly beta-artemether. The alpha-epimer is also present and causes a difficult purification of the product.

The pure alpha product behaves differently and has a melting point of 100 C° whereas the bêta-epimer has a melting point of 84-86°C. Both the alpha and the beta-epimers are active antimalarials. It is the purification

and separation of alpha from beta that leads to low yields and hence this increases the costprice of artemether significantly (internet 15).

Artemether is a semi-synthetic from more fat-soluble form that is the longest lasting in the blood stream. Due to the fat-soluble nature of this compound, it passes more readily through the blood brain barrier to reach cancers in the nervous system. It also has the most toxicity (but this is related to rather high dosages, which are not necessary). It's metabolized fairly rapidly to DHA, and blood tests show that after 6 hours the DHA metabolite has higher plasma concentrations than the parent compound artemether (Rowen, 2002, 2004; internet 14; and White, 2002).

White (2002) showed that artemether is longer acting compounds and may be better for cancer treatment, because that the cancer cells in a tumor do not divide all at the same time; therefore he believes it is best to have some of the compound in the blood stream for longer periods of time.

The presence of it's endoperoxide bridge is essential for it̊ s function. This generates a single oxygen molecule and leads to the formulation of free radicals. While it does not last very long in the body, it is metabolised in the liver to a demethylated derivative DHA, which has a half-life surviving 10 hours more than artemether. As a result of free radicals, DHA morphologically changes the parasites' membranes. Artemether has no clinical neurotoxicity and no major side effects. However, monotherapy often results in relapse and coma resolution time is significantly delayed. On rare occasions, there can be a decrease in reticulocyte count and changes in ECG patterns (Van Agtmael *et al*., 1999; and Karbwang *et al.*, 1998*)*.

2.6. The chemical properties of terpenes:

Terpenoids are widely distributed in nature, mostly in the plant kingdom. They may be regarded as derivatives of oligomers of isoprene, $CH_2=\underset{\displaystyle CH_3}{\overset{\displaystyle |}{C}}-CH=CH_2$, usually joined head to tail. Terpene hydrocarbons are

Classified as the follows:

Monotepenes $C_{10}H_{16}$

Sesquiterpenes $C_{15}H_{24}$

Diterpenes $C_{20}H_{32}$

Triterpenes $C_{30}H_{48}$

Tetraterpenes $C_{40}H_{64}$

Polyterpenes $(C_5H_8)_n$

Abundant sources of terpenoids are the essential oils. They consist of a complex mixture of terpenes or sesquiterpenes, alcohols, aldehydes, ketones, acids, and esters. There are four general methods for the extraction of essential oil:

1- Expression.
2- Steam distillation.
3- Extraction with volatile solvents.
4- Resorption in purified fats.

The separation of individual components is accomplished by vacum fractionation and by chromatographic methods. The unsaturated hydrocarbons are conveniently separated as their crystalline addition products with hydrochloric acid, hydrobromic acid, or nitrosyl chloride (Ikan, 1969).

Chemically, terpenoids are generally lipid-soluble and are located in the cytoplasm of the plant cell. Essential oils sometimes occur in special glandular cells on the leaf surface, while carotenoids are especially associated with chloroplasts in the leaf and with chromoplast in the petal. Terpenoids are normally extracted from plant tissues with light petroleum, ether or chloroform and can be separated by chromatography on silica gel or alumina using the same solvents. Aconsiderable numbers of quite different functions have been described to plant terpenoids. Their growth regulating properties are very well documented; two of the major classes of growth regulators are the sesquiterpenoid abscisins and the diterpenoid-based gibberellins. The important contribution of carotenoids to plant color is well known and it is almost certain that these C_{40} terpenoids are also involved as accessory pigments in photosynthesis. The important of mono- and sesquiterpenes in providing plants with many of their distinctive smells and odors is also familiar to most scientists. Less is generally known of the role of terpenoids in the more suitable interactions between plants and animals, e.g. as agents of communication and defense among insects, but this is now an area of active research. Finally, it should be mentioned that certain non- volatile terpenoids have been implicated as sex hormones among the fungi (Harborne *et al*., 1973).

2.7: Artemisinin cytotoxicity on different tumor cell lines: -

Artemesia has been used by Rowen (2002) in the past to treat intestinal parasites. It is also considered a safe malaria treatment. When Rowen discovered a report by Drs. Lai and Singh, which indicated that the herb "might provide a safe, non-toxic, and inexpensive alternative for cancer patients", he started using

it with cancer patients. Lai and Singh (1995), had found it's use dramatically killed breast-cancer cells and leukemia cells while leaving normal breast cells and white blood cells unscathed. Rowen (2002) also reported that the artemisinin have anticancer activity in a wide variety of laboratory cultured cancer cells. Cancers resistant to common chemotherapy drugs showed no resistance to artemisinin (internet 17 and internet 19).

Moore *et al*. (1995) found that oral administration of an artemisinin analog and ferrous sulfate retarded the growth of implanted fibrosarcoma tumors in the rat (several patients with different types of cancers have begun treatment with artemisinin and it's analogs with promising results. Singh and Verma (2002) feel that this emerging newtherapy has promise to prevent and treat different types of cancers since it works via a simple mechanism which is common to all cancer cells (i.e. an increase in iron influx).

Hoang (2002) reports that 50-60 percent of 400 cancer patients have achieved long-term remission utilizing artemisinin together with a comprehensive integrative cancer strategy. Among these patients is a 47 -year-old female who, presented with terminal liver cancer from hepatitis B and abdominal ascites (massive swelling from liver failure), was just days or weeks from death. Today; two-and-a-half years later, she is alive and well with no signs of any disease. Singh and Verma (2002) are currently following many cancer patients. While not reporting remissions or apparent cures, he says all patients are responding and have at least stabilized. He has found no type of cancer unresponsive to artemisinin derivatives in his studies.

The cytotoxic activity of nine terpenoids and flavonoids isolated from *Artemisia annua* was tested in vitro on several human tumor cell lines. These compounds are artemisinin, deoxyartemisinin, artemisinic acid, arteannuin-B, stigmasterol, friedelin, friedelan-3 beta-ol, artemetin, and

quercetagetin 6,7,3',4'-tetramethyl ether. Friedelane-type triterpenoids were isolated for the first time from this plant. Artemisinin and quercetagetin 6,7,3',4'-tetramethyl ether showed significant cytotoxicity against P-388, A-549, HT-29, MCF-7, and KB tumor cells (Zheng, 1994).

Cancer could be like the malaria parasite, if just one cell remains, it can find it's way back. Thus, as in malaria, although the parasite is cleared in a few days, prolonged treatment best prevents relapse (internet 19; and Hoang 2002).

In another study, Lai and Singh (1995) noted even more amazing results involving leukemia cells. They mentioned that the cancer cells were destroyed very quickly within a few hours when exposed to holotransferrin (which binds with transferring receptors to transport iron into cells) and DHA (a more water-soluble form of artemisinin). They further explained that it might be because of the high concentration of iron in the leukemia cells.

An earlier study with human leukemia cells demonstrated 100% cancer cell destruction in half the time (8 hours) as the breast cancer cells, probably due to the rapid cell division and higher iron concentration of leukemia cells (internet 22; Ingels 2001; and Efferth *et al.*, 2001).

This amazing herb was also examined for it̓ s activity against 55 cancer cell lines. It was found to be the most active against colon cancer and active against melanomas, breast cancer, prostate cancer, bone marrow, CNS, ovarian, and renal cancer, gastro-intestinal carcinoma, fibrosarcoma and peritoneal carcinosis. It was also reported that artemisinin's effectiveness was comparable with other standard drugs used to combat cancer. As such, these results and the low toxicity of artemisinin had made this herb to be a potential for cancer chemotherapy (Efferth, 2001; Giuseppe, 1993; Beekman *et al.*,1998; Moore *et al.*, 1995 and Oda *et al.*, 2004).

The studies on artemisinin and his derivatives show their effectiveness on other different types cancer such as pancreatic cancer, brain tumors, tumor of tongue and lymphoma (internet 7; Dhingra *et al.,* 2000; Efferth *et al.*,2002 ; Li *et al.,* 2001; and Beekman *et al.,* 1997).

Compared to normal cells, cancer cells sequester relatively large amount of iron mainly in the form of holotransferrin .Artemisinin has been shown to cause rapid and extensive damage and death in cancer cells and have relatively low toxicity to normal cells. Importantly, a comparison of artemisinin's cytotoxicity with those of other standard cytostatic drugs showed that it was active in molar ranges comparable to those of established anti-tumor drugs. In one study regarding the activity of 22 drugs on leukemia CCRF-CEM cells lines, artemisinin showed both antileukemia activity if applied alone and modulation activity in combination with daunorubicin in multidrug-resistant (MDR) cells. Artemisinin and it's derivatives have been found to inhibit the proliferation of cancer cells and increased cytotoxicity of perarubicin and doxorubicin in P-glycoprotein-overexpressing and in MRP1-overexpressing, but not in their corresponding drug-sensitive cell lines (internet 20; Efferth *et al*., 2002 and Reungpatthanaphong, *et al*., 2002).

Jung *et al.* (1994) found that the artemisininin is virtually non-toxic (LD50 = 4228 mg/kg orally administered to mice) and without carcinogenicity.

A cancer treatment method of inhibiting or killing cancer cells has been disclosed where in compounds having an endoperoxide moiety that is reactive with iron are administered under conditions which enhance intracellullar iron concentrations. Representative endoperoxide compounds are artemisinin and it's analogs (internet 8). DHA and a rapid cell death, as evidenced by a decrease in cell counts, were observed (Lai and Singh, 1995).

Singh and Lai (2001) showed that the artemisinin becomes cytotoxic in the presence of ferrous iron. Since iron influx is high in cancer cells, artemisinin and it's analogs selective kill cancer cells under conditions that increase intracellular iron concentrations. Artemisinin analog effectively killed radiation-resistant human breast cancer cells *in vitro*. The same treatment had considerably less effect on normal human breast cells. Administration of artemisinin like drugs may be a simple, effective, and economical treatment for cancer.

Also the breast cancer cell research resulted in a 28% reduction of breast cancer cells treated only with artemisinin, and a staggering 98% decrease in breast cancer cells that were treated with artemisinin and an iron-enhancing molecule, transferrin, within 16 hours. The same treatments had no significant effect on normal human breast cells (internet 24).

Small-cell Lung Carcinoma Cells (SCLC). Artemisinin was tested for the effects on drug-sensitive (H69) and multi-drug resistant (H69VP) SCLC cells, pretreated with transferrin to increase the intracellular iron level. Low doses of artemisinin were cytotoxic to SCLC cells. The cytotoxicity of artemisinin for H69VP cells was ten-fold lower than for H69 cells, indicating that artemisinin is part of the drug resistance phenotype. Pretreatment of H69 did not alter the IC_{50} for artemisinin, however, in the artemisinin-resistant H69VP cells, pretreatment with transferrin lowered the artemisinin IC_{50} to near drug-sensitive levels. Desferrioxamine inhibited the effect of transferrin on the IC_{50} for artemisinin in drug-resistant cells but did not have an effect on artemisinin cytotoxicity in drug-sensitive cells. These data indicate the potential use of artemisinin and transferrin in drug-resistant SCLC. (Sadava *et al*., 2002*).*

Ying *et al.* (2001) found a modification of artemisinin structure led to discovery of a novel class of antitumor compounds. The artemisinin derivatives

containing cyano and aryl groups showed potent antiproliferative effect *in vitro* against leukemia and human lung carcinoma cell lines.

A protocol clinic trials for 50 canines diagnosed with metastatic osteosarcoma to the lungs is being considered following *in vitro* testing at Georgetown University and Washington Cancer Institute. Toxicity data do not suggest that systemic toxicity occurs at therapeutic dosages. It is believed that the use of oral artemisinin protocol will produce clinically measurable cytotoxic effects in the tumor cells of canines with metastatic disease. (Krutz *et al*., 2002).

A series of artemisinin-related endoperoxides was tested for cytotoxicity to Ehrlich ascites tumor cells (EAT). Artemisinin showed cytotoxicity to the EAT cells and it̊ s derivatives exhibited a somewhat more potent cytotoxicity at higher concentrations than those needed for *in vitro* antimalaria activity (Woerdenbag *et al.,* 1993; and Beekman *et al.,*1997).

Heo and Jun (2003) described that the blood-Brain Barrier (BBB) & Alzheimer's disease (AD) were affected by methanolic extract from *Artemisia* which showed a highest inhibitory effect on acetylcholinesterase *in vitro*. The study demonstrated that an alkaloid of *Artemisia asiatica,* metabolized to small molecule in digestive tract and passed through the BBB, be an acetylcholinesterase inhibitor with a blocker of neurotoxicity induced by a beta in human brain causing AD.

AIDS and HIV a series of artemisinin related trioxanes has been prepared and assayed in vitro for anti-HIV activity. One of these compounds, 12-n-butyldeoxoartemisinin shows a good antiviral activity against HIV-1. (Jung *et al*., 1994).

The researches was also found that the artemisinin used in the treatment of hepatitis B and lyme disease, 320 cases of chronic hepatitis B were treated with artemisinin derivatives resulted in the recovery of liver function (internet 22).

Singh and Verma (2002) have been described that the treatment of a laryngeal squamous cell carcinoma case with the water-soluble artemisinin analog (artesunate).

Artemisinin is a novel anti-cancer drug with demonstrated results in killing cancer cells. While artesunate injections and orally were administered to the patient over a period of nine months. The tumor was significantly reduced (by approximately 70%) after two months of treatment. Overall, the artesunate treatment of the patient was beneficial in prolonging and improving the quality of life, so artemisinin and it' s analogs offer promise for cancer therapy (Singh and Verma, 2002; and Beekman *et al.*,1998).

2.8: The effect of *Artemisia* extract on immune system:

Grendelmeier *et al*. (2003), showed that artemisinin increased IgE-binding capacity and were able to induce T-cell proliferation.

Artemisinin enhances macrophage phagocytosis in mice. The herb *Artemisia* also is very effective against worms with reduction 80 - 90% within a week (internet 27).

Artemisia vulgaris was found to be affective in different human leukocyte antigen (HLA) class II alleles. The results reveal that the DRB1*01-DQB1*0501 genotype is strongly associated with a positive response to *Artemisia* in the population studied (Torio *et al.,* 2003).

The ethanolic extract of *Artemisia inculta* has been screened for anti-inflammatory, analgesic and antipyretic activities on suitable experimental models. It decreased the prothrombin time in rats. It failed to produce any

analgesic or antipyretic activity on the hot plate reaction time and yeast induced hyperexia tests in mice. It also didn't produce any effect on the platelet aggregation and fibrinogen level in the rats. Amongst the phytoconstituents detected in this plant, flavonoids may be responsible for the observed anti-inflammatory effect of the ethanolic extract (Mossa *et al.*, 1987).

Maxican *Artemisia* species, which was used in folk medicine, have anti-inflammatory properties, analgestic and anti-migraine activities (Heinrich *et al.*, 1998).

The aerial part of *Artemisia capillaris* showed antiplatelet aggregation activity and have a significant activity against HIV replication in H9 lymphocytic cells (Wu *et al*., 2001).

In 2001, a WHO report concluded that artemisinin and it̊ s derivatives do not exhibit mutagenic or teratogenic activity.

The polysaccharide fraction of the leaves of *Artemisia princeps* was had antithrombin activity. In human plasma, the polysaccharide accelerated the formation of thrombin-HC II complex. The stimulating effect on HC II-dependent antithrombin activity was almost totally abolished by treatment with chondroitinase AC I, heparinase or heparitinase, while chondroitinase ABC or chondroitinase AC II had little or no effect. These results suggest that the polysaccharide is a glycosaminoglycan-like material with properties that are quite distinct from heparin or dermatan sulfate (Hayakawa *et al*., 1995; Yamada *et al*., 1991; and Zhao *et al*., 1994).

2.9. Histological studies: -

Spleen-The spleen is enclosed by a dense connective tissue capsule**,** from which extend connective tissue trabeculae deep into the interior of the spleen. The main trabeculae enter the spleen at the hilus, branch throughout the organ, and carry with them trabecular arteries and veins. Trabeculae that are cut in transverse section have a round or nodular appearance.

The spleen is characterized by lymphatic nodules; these constitute the white pulp of the organ. The lymphatic nodules contain germinal centers, which progressively decrease in the number as the individual ages. Passing through each lymphatic nodule is central artery, which usually displaced to one side, thus losing the central position . Central arteries are branches of trabecular arteries which become ensheathed with lymphatic tissue as they leave the trabeculae. This sheath also forms the lymphatic nodules the trabeculae. This sheath also forms the lymphatic nodules, which then constitute the white pulp of the spleen.

Surrounding the lymphatic nodules and intermeshed with the trabeculae is a diffuse cellular meshwork, which collectively form the red or splenic pulp; it exhibits a red color in fresh preparation. Red pulp cotains venous sinuses and splenic cords; these appear as diffuse stands of lymphatic tissue between the venous sinuses. The cords form a spongy meshwork of reticular connective tissue, which is usually obscured by the density of other tissue.

The spleen does not exhibit a cortex and a medulla, as seen in lymph nodes; however, lymphatic nodules are found throughout the spleen. The spleen contains venous sinuses, in contrast to lymphatic sinuses seen in lymph nodes, but the spleen does not have subcapsular or trabecular sinuses. The capsule and trabeculae in the spleen are thicker than those in the lymph nodes and contain some smooth muscle cells.

Liver-In the primate or humane liver, the connective tissue septa between individual hepatic lobules are not as conspicuous as in the pig's liver. The liver sinusoids are continuous from one lobule to the next. Despite these differences, portal areas containing the interlobular branches of the portal veins, hepatic arteries, and bile ducts are visible around the peripheries of different lobules.

In the center of each hepatic lobule is the central vein. The hepatic sinusoid are seen between the hepatic plates the radiate from the central veins toward the periphery of the hepatic lobule. Numerous branches of interlobular vessels and bile ducte are seen within the portal areas of a given hepatic lobule.

Kidney-The kidney is subdivided into an outer region, the cortex and an inner region, the medulla. Externally, the cortex is covered with a connective tissue capsule and the perirenal connective and adipose tissues.

In the cortex are found convoluted tubules, glomeruli, strainght tubules and medullary rays. The cortex also contains renal corpuscles (glomerular or Bowman's capsules and glomeruli), adjacent proximal and distal convoluted tubules of the nephrons, and the interlobular arteries and veins. The medullary rays contain straight portions of nephrons and collecting tubules. Medullary rays do not extend to the kidney capsule because of a narrow zone of convoluted tubules.

The medulla is composed of a number of renal pyramids. Each pyramid is situated with itó s base adjacent to the cortex and itó s apex directed inward .The apices of renal pyramids form the papilla, which project into a minor calyx. The

medulla also contains the loops of Henle (straight or descending proximal tubules, thin segments, and straight or ascending distal tubules) and collecting

tubules. The collecting tubules join each other in the medulla to form large papillary ducts.

The papilla is usually covered with a simple columnar epithelium. As this epithelium reflects on to the outer wall of the calyx, it becomes transitional epithelium. A thin layer of connective tissue and smooth muscle is found under this epithelium, which then merges with the connective tissue of the renal sinus.

In the renal sinus, between the pyramids, are branches of the renal artery and vein, the interlobar vessels. These vessels enter the kidney and then arch over the base of the pyramid at the corticomedullary junction as the arcuate vessels. The arcuate vessels given rise to smaller, interlobular arteries and veins. The arcuate arterier pass radially into the kidney cortex and given off numerous afferent glomerular arteries to the glomeruli.

Testis-The testis is enclosed in a thick, connective tissue capsule, the tunica albuginea. Internal to this capsule is a vascular layer of loose connective tissue, the tunica vasculosa. This layer merges with the stroma of the testis, the interstitiall connective tissue, which is also rich in blood vessels. The fibers of the interstitial connective tissue bind and support the seminiferous tubules.

The seminiferous tubules are long, highly convoluted tubules in the testis that are normally observed cut in various planes of sections. These tubules are lined with a specialized stratified epithelium called the germinal epithelium, which consist of the spermatognic and supportive or Sertoli cells. These cells rest on a thin basement membrane.

Located in the interstitial connective tissue that surrounds the seminiferous tubules are groups of endocrine cells, the interstitial cells (of Leydig). These cells secrete the male sex hormone, testosterone (Lea and Febiger, 1993).

2.10. The effect of *Artemisia* extract on visceral organs: -

Penissi and Piezzi (1999) have demonstrated that dehydroleucodine (DhL), a lactone isolated from *Artemisia douglasiana* Besser, prevents gastroduodenal damage they observed an increase in the adherent mucus layer thickness in the experimental samples. This confirms that one of the main mechanisms involved in the cytoprotective action of the drug is mucus secretion.

The aqueous-methanolic extract of *Artemisia maritima* have been used against liver damage (Janbaz and Gilani, 1995; Batty *et al.*, 1998).

Shiratori *et al.*(1994) found that the artemisinin could prevent the recurrence of LN and protect kidney function.

Properties of artemisinin are bitter and cold, entering the kidney, liver, and gallbladder channels. It is noted for clearing summer heat, clearing fevers from deficiency, cooling blood and stopping nosebleeds, and for checking malarial disorders and relieving heat (Zhong *et al.*, 2002).

MATERIAL AND METHODS

3.1. Materials and solutions

3.1.1. The experimental instruments: -

The instruments that used in the experiments are as the following list:

Instrument	Company
Autoclave	Tomy, Japan
Centrifuge	Griffin and George, Britan
Hood	Heraeus, Germany
ELISA reader	Organon Teknika, Austria
Inverted microscope	Leitz, Wetzlar, Swizerland
Incubator	Memert , Germany
Light microscope	Lomo, Russia
Magnetic stirrer	Stuard Scientific, Germany
Routary	Bachi, Swizerland
Shaxulat	Labconco, U.S.A

3.1.2. Sort of plant: -

The plant *Artemisia herba-alba* obtained from Dr. Raad Al-Maula private herbarium.

3.1.3. Stock solutions for cell cultures: -

*** Antibiotics**

Penicillin crystalline G (Sigma chemical Co. USA)	1000000 IU
Streptomycin (Sigma chemical Co. USA)	1 gm
D. W.	100 ml

The solution sterilized by filtration through millipore filter 0.22 μm, dispensed in aliquots and stored at -20 C^{o}.

*** DMSO solutions**

I- For *in vivo* study: -

1ml from DMSO diluted with 1ml PBS, then 0.5 ml from diluted DMSO was adding to 4.5 ml PBS, 0.2 ml from the last dilution of DMSO added to 9.8 ml PBS, finally 0.2 ml used for injection after adjacent pH to 7 and sterilized by 0.22 μm millipore filter.

II- For *in vitro* study: -

2 ml DMSO diluted with 1 ml PBS, then 1 ml from diluted DMSO added to 4 ml PBS, 0.4 ml from last dilution of DMSO was adding to 9.6 ml PBS, finally 0.1 ml used for culture after adjacent pH to 7 and sterilized by 0.22 μm millipore filte

* **Extract stock solution**

1.5 gm from the plant extract (*Artemisia herba-alba*) dissolved in 2.5 ml DMSO, mixed continuously until the extract completely dissolved with DMSO.

I- For *in vivo* study: -

1 ml from stock solution diluted with 1 ml PBS, then 0.5 ml from the diluted extract added to 4.5 ml PBS, finally 0.2 ml from the last dilution dissolved in 9.8 ml PBS. Adjacent pH to 7 and sterilized with 0.22 μm millipore filter, and then 0.2 ml (0.2 mg / ml) was used for injection.

II- For *in vitro* study: -

2 ml from stock solution diluted with 1 ml PBS , then 1 ml from the diluted extract added to 4 ml PBS , finally 0.4 ml from the last dilution dissolved in 9.6 ml PBS . Adjucent pH to 7 and sterilized with 0.22 μm millipore filter , and then 0.1 ml (0.2 mg / ml) used for culture .

* Heparin (Rotex Medica , Germany)

20 IU per / ml have been used used .

* L – Glutamin (Sigma chemical company, Germany)

2 gm of L – glutamin dissolved in 100 ml D. W. and 1.5 ml used for 100 ml tissue culture medium .

* PHA

Crude PHA obtained from Iraqi center for cancer and medical cytogenetic research. Dispensed in to 2 ml aliquots and stored at -20C^{o}.

* Serum (Sigma chemical company, Germany)

Fetal calf serum (F.C. S) inactivated by heating at 56 C^{o} for 30 minutes in a water bath, then dispensed in to 20 ml aliquots and stored at -20C^{o} .

* Tissue culture medium pH 7.2

RPMI (Sigma chemical company, Germany)	10 gm
Hepes (Sigma chemical company, Germany)	3 gm
Sodium bicarbonate	2 gm
Antibiotic	10 ml
F. C. S	10 ml
D. D. W .eqiuvalent to	1000 ml

Mixed well , sterilized by filteration through 0.22 µm millipore filter. Dispensed in to 20 ml aliquots and stored at -20 C^{o} .

* Trypsin(Sigma chmical company, Germany)

Trypsin solution prepared by dissolving 1 gm of trypsin powder in 100 ml of PBS and sterilised by filtration, dispensed in to 20 ml aliquots and stored at -20 C^{o} **.**

* Versen (BDH chemicals LTD . England)

A 0.05 % solution of EDTA (ethylene diamine tetra acetic acid, disodium saft) prepared by adding 0.2 gm EDTA in 400 ml of PBS. sterilized by autoclaving for 10 minutes . The solution dispensed in 20 ml aliquots in to universal containers and stored at 4 C^o .

* Trypsin – Versen solution

Trysin solution	10 ml
0.05 % Versene solution	10 ml

Mixed thoroughly and used.

* PBS

NaCl	8 gm
KCl	0.2 gm
KH_2PO_4	0.2 gm
Na_2HPO_4	0.15 gm
D.D.W	1000 ml

Sterilized by autoclaving.

3.1.4. Stock solutions for lymphocyte transformation assay and cytogenetic assay: -

* Hypotonic solution

Hypotonic solution (0.075 M KCl) prepared by dissolving 0.575 gm of KCl in 100 ml D. W. The stoke solution stored at 4 C^o.

* Fixative

The fixative employed for transformation studies was a freshly made mixture of absolute methanol and glacial acetic acid in the ratio of 3:1 (V/V).

* Sorenson buffer

Sorenson buffer prepared by dissolving 9.47 gm of Na_2HPO_4 and 9.8 gm of KH_2PO_4 in to 1000 ml D. D. W. The stock solution stored at 4 C^o.

* Giemsa stain (BDH chemical LTD .England)

It prepared by adding 2 gm of Giemsa powder to 100 ml absolute methanol , stirring for 2 hrs. at 50 C^o , incubated at 37 C^o for 24 hrs. ,and filtered before use .

One ml of filtered stain diluted in 4 ml of sorenson's buffer immediately used in staining for 2–5 minutes . Wash the stained slide by the same buffer also.

*** Colcimid (Sigma chemical company. Germany)**

Colcimid (N - methyl – N – deathly colchicines) as sterile lyophilized powder prepared at a concentration of 0.1% W/V solution the stock solution stored at 4 C^o.

3.1.5. Stock solutions for phagocytosis test: -

*** *Staphylococcus aureaus* suspension**

Pure bacterial culture harvested with sterile saline, bacterial suspension made in concentration of 1×10^6 bacterial cell per ml. Dispensed in to 0.5 ml aliquots, store in – 20 C^o.

*** Normal salin**

NaCl	8.5 gm
D.W	1000 ml

3.1.6. Stock solutions for cytotoxicity tests: -

*** Neutral red stain (BDH chemical LTD. England)**

Neutral red	10 gm
PBS	100 ml

Mixed thoroughly and used.

*** Eluant solution**

0.1 M from NaH_2PO_4 dissolved in absolute ethanol 1:1 volume.

3.1.7. Stock solution for vaginal smear test: -

* Methylen blue

5 gm methylene blue dissolved in 100 ml D.W.

3.1.8. Stock solutions for histology procedure: -

* Haematoxilin stain

haematoxilin (panreac quimica SA (Barcelona) . Espana)	1mg
Absolute ethanol	10 ml
K_2SO_4. AL_2 $(SO_4)_3$	20 gm
Hg_2O	0.5 gm
Acetic acid	8 ml
D. W. equivalent to	200 ml

K_2SO_4.AL_2 $(SO_4)_3$ dissolved with D.W on a flame, on the other wise the haemotoxilin stain dissolved with the absolute ethanol, then mixed together by boiling and added Hg_2O to the mixture. The mixture left boiling until it becomes dark purple, cold and added acetic acid. The stain filtered with Whitman filter paper (number 1) before used.

* Eosin stain (BDH chemical LTD. England)

Eosin	1 gm
D.W	99 ml

Mixed thoroughly until the stain completely dissolved and then filtered with Whitman filter paper (number 1).

*Egg-albumin

1 thymol: 1 glycerol: 1 albumin

* Acid – alcohol

Ethanol	70 %
HCl	1 %

3.1.9. Stock solution for testis cytogenetic test: -

*** Colcimide**

0.5 gm of colcimide dissolved in 0.5 ml PBS, mixed thoroughly until it completely dissolved and then used directly.

3.1.10. Experimental animals: -

Male (8-10 weeks old, 20-25 g in weight) BALB/c mice used in this study supplied by Biotechnology Research Center. These animals housed in plastic cages and maintained in hygienic conditions. The animals were feeding on special formula food pellets and supplied with water by special bottles.

The components of mice food were as the following: -

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

3.2. Methods

3.2.1 Animals administration: -

A total 112 male BALB/c mice have been used in this study. These mice divided in two groups used for *in vivo* and *in vitro* experiments.

I- *In vivo* assessment: -

In this experiment 92 mice have been used. The mice divided in to 3 groups, first group has 34 mice that injected Ip with 0.2 mg /ml of extract for 10 days. While the second group has 29 mice injected Ip with DMSO for 10 days as a control 1. The third group was control 2 that has 29 non-treated mice.

II- *In vitro* assessment: -

Total 20 mice used in this experiment that divided in to 3 groups. The first group has 10 mice treated with 0.2 mg/ml from extract. The second group was control 2 that has 5 mice treated with DMSO. While the third group has 5 non-treated mice that used as a control 2.

3.2.2. Plant extraction procedure: -

Artemisinin extracted from the plant *artemisia harba – alba* by shaxulat at low temperature (Keyser *et al*., 2003) as following: -

5 gm from the plant weighted for each thimble (4 thimbles used in each run) , 25 ml from diethyl ether solvent put in each beaker. The extraction have been done at 40 C^{o} for 4 hrs.each run, when the extraction finished, the extract solution filtered at Whitman filter paper (number1) and then evaporated by the rotary to remove undesirable solvent. The residual

powder have been weighted and dissolved with a suitable solvent (DMSO). Mixed thoroughly until the extract completely dissolved, and then diluted the solution with PBS to obtain the required concentration for used, adjacent pH at 7, sterilized with 0.22 nm millipore filter and kept in 4 C^{o} ready for used.

3.2.3. Determination the effective dose of the extract: -

Injected 10 mice with five concentrations of extract (0.1, 0.2, 0.4, 0.8, 1.6 mg/ml), 2 mice injected for each concentration. 5 mice were Injecting with DMSO as control 1 and 5 non-treated mice used as control 2. After 24 hrs. the mice killed and lymphocyte transformation assay has been done.

3.2.4. Lymphocyte transformation and cytogenetic assay: -

This procedure was done according to the method that described by Alex and Leonard (1980) and Verma and Babu (1989) with modifications.

I- *In vivo* assessment: -

Cell culture: -

Blood samples were taking by insulin syringe coated with heparin from treated mouse heart before cervical dislocation. After cervical dislocation each mouse washed with 70% ethanol and the spleen removed by using sterile technique. Single spleen cell suspensions prepared by fine mincing of spleen in 2ml of tissue culture medium (PRMI 1640 supplemented with 10 % F.C.S), wait for few minutes to allow large particles to be settled and take the supernatant. 0.25ml have been taken from spleen cells suspension and 0.25ml from blood sample, each sample cultured

in a test tube contain 2.5ml tissue culture medium (RPMI 1640 supplemented with 10% F.C.S) with 0.3 ml PHA and other test tube for the same sample with out PHA. Incubated at $37\,C^0$ for 72 hours.

Harvesting: -

After 71 hours and 30 mins. from incubation 0.1ml of colcemid was adding for each test tube and incubated for 30 minutes at $37\,C^0$, centrifuged at 2000 rpm for 10 minutes, resuspended the cells pellet in few drops from prewarmed hypotonic solution (0.075 M kcl) at 37 C^0 with continuous shaking, then the volume completed to 5ml by adding more prewarmed (0.075 M kcl) gradually with constant shaking. The cells suspension incubated at 37 C^0 for 30 minutes with occasional shaking. The cells have been collected by centrifugation at 2000 rpm for 10 minutes, discarded the supernatant and take the pellet. Cells pellet fixed by adding drop wise from freshly made cold fixative (methanol and glacial acetic acid, 3:l v/v) with continuous agitation until the volume completed to 5ml, then cell suspension was left for 30 minutes in the refrigerator. Cells collected by centrifugation and added another freshly made fixative as above, fixative changed 3 times , and after final change , the cells resuspended in 3ml of freshly made fixative and stored in the refrigerator before spreading on slides .

The cells suspension removed from refrigerator and centrifuged at 2000 rpm for 10 minutes. Discarded the supernatant and the cells resuspended in appropriate amount of freshly made fixative to make the suspension thinly cloudy. With a Pasteur pipette, 2 to 3 drops of cells suspension dropped from 30 cm on to wet, chilled, grease – free slides and allowed to dry at room temperature.

Slides were stained with freshly made giemzas stain (1 part giemazs stain to 4 parts Sorenson's buffer) for 5-10 minutes. The slides washed by Sorenson's buffer, allowed to air dry at room temperature and examined under light microscope by oil immersion lens, and count at least 1000 cells both transformed and non – transformed lymphocytes, mitotic and non – mitotic lymphocytes.

Control 1 made by using mice treated with DMSO. While the control 2 made by using normal mice.

To calculate the percentage of transformed cells by using the formula: -

$$\% \text{ Transformed cells} = \frac{\text{Transformed cells}}{\text{Total}} \times 100$$

To calculate the percentage of mitotic cells by using the formula: -

$$\% \text{ Mitotic index} = \frac{\text{Mitotic cells}}{\text{Total}} \times 100$$

II-*In vitro* assessment: -

Blood samples were taken and spleen single cell suspension prepared by the same method of *in vivo* which described before.

0.25 ml was taken from spleen cells suspension and 0.25 ml from blood sample, each sample cultured in a test tube contain 2.5 ml tissue cultured medium (RPMI 1460 supplemented with 10 % F.C.S) with 0.3 ml from PHA and 0.1 ml (2 mg/ml) from the extract, the same sample cultured in another test tube without PHA and incubated for 72 hours at $37 C^0$.

control 1 samples made by adding 0.1 ml from DMSO. While the control 2 samples made by using normal mice.

After incubation used the same *in vivo* harvesting procedure that described before.

To calculate the percentage of the transformed cells on the basis of the formula: -

$$\% \text{ Transformed cells} = \frac{\text{Transformed cells}}{\text{Total}} \times 100$$

To calculate the percentage of the mitotic cells on the basis of the formula: -

$$\% \text{ Mitotic index} = \frac{\text{Mitotic cells}}{\text{Total}} \times 100$$

3.2.5. Testis Cytogenetic study: -

This done according to the method applied by Evans *et al*., (1964) with modifications.

Treated mice injected IP with 0.25ml freshly prepared colcimid solution. After 2 – 3 hr. from injection the mice killed and put the testis in a petri dish, mince the testis gently with 5 ml PBS, centrifuged for10 minuets at 2000 rpm, resuspanded the pellet with 37 C^o prewarmed 0.075 M KCL solution by gradual drops with shaking until it completed to 5 ml, then incubated for 15 minuets at 37 C^o with shaking.

After that completed the same steps of blood and spleen cytogenetic procedure which described above.

3.2.6. Phagocytosis procedure: -

I-*In vivo* assessment: -

0.25 ml blood sample obtained from treated mouse heart by insulin syringe coated with heparin. Blood sample collected in a sterile test tube and added 0.25 ml from *staphylococcus aureus* mixed gently then incubated at $37 C^0$ for 30 minutes (Furth, *et al*., 1985).

After incubation blood smears made, left to dry, fixed with methanol by dropping 3-5 drops to cover the smear and left to dry. Before staining, immerse the fixed cells in Sorenson's buffer for 5 minutes, left to dry, stained with freshly made giemsa stain (1 part giemsa stain to 4 part Sorenson's buffer) for 5-10 minutes, rinse the slides with Sorenson's buffer and left to dry (Hudson and hay, 1989).

The slides examined under light microscope by using oil. Immersion lens and count 100 cell phagocytic and non – phagocytic cells.

control 1 samples made by using mice treated with the DMSO. While the control 2 samples made by using normal mice.

I- *In vitro* assessment: -

0.25 ml blood sample obtained from normal mouse heart by insulin syringe coated with heparin, blood sample collected in a sterile test tube, added 0.1 ml (2mg / ml) from extraction, mixed gently, incubated at 37 C^0 for 30 minutes, after that added 0.25 ml from *staphylococcus aurous*, mix gently, reincubated at 37C^0 for 30 minutes, blood smears were made, left to dry, stained with giemsa stain as in the *in vivo* procedure which described before.

3.2.7: Cytotoxicity assay: -

A- Cytotoxic effect of extract on mouse embryo fibroblast cells: -

I – Fibroblast cells preparation: -

In this experiment pregnant mouse 11-13 days used for preparation of fibroblast primary culture. To determine the day zero of pregnancy vaginal smears was done. This test was done as the following: -

One drop of sterile normal saline placed by the loop in the vagina, aspirated this loop several times, transformed the mixture of sterile normal slain and scraping vaginal cells on to a clean slide, dried in the air, stained

with methylene blue for 3- 5 minutes, washed with D. W., dried in the air, and then examined under the light microscope (40 X).

Presence of large cornfield cells and ruminants of epithelial cells considered that this female within estrus stage and mating with active male should be taking place. Vaginal plug is resulted within 16 – 24 hours. Occurrence of vaginal plug considered the first day of gestation (Fakhrildin *et al.*, 2003).

At 11-13 days of pregnancy the mouse killed by cervical dislocation and swab the ventral surface liberally with 70 % alcohol removed the Utri that filled with embryos by sterile technique and placed in a sterile Petri dish contain 10 – 20 ml PBS and washed with PBS 2 – 3 times to remove the blood. Take the intact Uteri and transfer to another Petri dish, dissected out the embryos by treated the uterus with sterile forceps and scissors, the embryos freed from the membranes and placenta, placed one side of the dish to bleed, then transferred to another Petri dish, wash 2 – 3 times with PBS to remove the blood, chopped finely, placed the pieces in a universal with 10 – 20 ml PBS, allowed the pieces to be settled, removed the supernatant fluid, repeated the washing 2 – 3 times. Transfer the pieces to trypsinization flask that contain magnetic bar and 50 ml of trypsin (0.25 % trypsin), stir at about 200 rpm for 15 min at $37 C^0$, allow pieces to be settled, collected supernatant, centrifuged at 2000 rpm for 10 minutes, resuspened the pellet in to 10 ml tissue culture medium (RPMI 1640 supplemented with 10 % F.C.S), added fresh trypsin to pieces and continue to stir and repeated the steps until no further disaggregating apparent.

Primary Culture: -

Collected the cells suspension that centrifuged and kept in RPMI with 10 % F. C. S cultured the cells on tissue culture flasks and incubated at 37 C^0 for 4 days.

Harvesting: -

After 4 days of incubation the cells grew as a monolayer. To remove monolayer detachment, discarded the medium, added PBS (5ml / 25 CM^3) to the side of the flask opposite the cells, rinse the cells and discarded PBS. Added trypsin versen (3ml / 255CM^3) to the side of the flask opposite the cells, turned the flask over to cover the monolayer completely, leave 15 – 30 second and made sure that the monolayer has not detected, cells round up and the monolayer should slide down the surface, added RPMI medium (0.1 - 0.2 ml /CM^2) supplemented with 10 % F.C. S, supplemented with 10 % F. C. S dispersed cells by repeated pipetting over the surface bearing the monolayer. Finally, pipette the cell suspension up and down for a few times and then collected the cell suspension in a sterile universals.

II- Cytotoxicity test: -

In this test micro titer plates (96 wells flat bottom) have been used. For plant extract and control 1 (DMSO) and their replicative lines, started with column 2 to column 12, added 50 µl medium (RPMI 1640 supplemented with 10 % F.C.S) while control 2 line and it's replication 50 µl medium added from column 1 to 12. 50 µl from plant extract was adding to column 1 and 2, and to all it's replicative lines, then from column 2

double fold dilutions have been made, the same steps used to make control 1 lines

but 50 µl from DMSO was adding instead of plant extract and then made dilution by the same way. Finally 150 µl from fibroblast cells suspension was adding to all wells, incubation at 37 C^0 for 4 days.

Reading the results: -

After 4 days incubation, 50 µl was added from natural red stain to each well and reincubated at 37C^0 for 2 hours, discarded the stain, gently washed with PBS and discarded gently, added 200 µl from the eleunate solution to each well, red by ELISA reader at OD 492 nm which record the results.

This procedure done according to the method that applied by Freshney (1994).

B- Cytotoxic Effect of extract on S.U.99 plasmacytoma cell line: -

S.U.99 plasmacytoma cell line that used in this study supplied by biotechnology research center.

This done according to the method applied by Naradra and Henry (2001) with modifications.

Removed the S.U.99 attachment to the flask surface by gently pipeiting with sterile technique. Added 12 µM (1mg/ml) from human halotransferrin and incubated at 37C˚ for 2 hrs. After incubation cultured the S.U.99 cells with extract as in the fibroblast cells procedure that described

before.

The control 1 was done by culture the S.U.99 cells with halotransferrin and DMSO while the control 2 was done by cultured the S.U.99 cells with halotransferrin only.

3.2.8: Histological examination: -

This test was done according to the method adopted by Bancroft and Stevens (1982).

Samples (spleen, kidney, liver and testis) obtained from treated mice that prepared for histological studies by the following steps: -

II – Fixation: -

Samples fixed in 10 % formalin for 24 hours after that exchanged with new one.

II – Dehydration: -

The samples placed in 70 % ethanol over night. Dehydration was done by four changes of (80 %, 90 %, 100 %, 100 %) ethanol for 2 hours for each concentration.

III – Clearing: -

Samples placed in toluene for 2 – 4 hours to clear the tissue.

IV - Embedding: -

Sample embedded in melting paraffin (melting point of paraffin was 58 C°) for 4 hours at 60 – 70 C° in oven.

Samples blocked in paraffin wax, sections were cutting by microtome 4- 5 μm in thickness, placed in water bath at 37 c for few minutes and then covered a clean slide with egg- albumin by finger that make the section stick on the slide surface.

V – Haematoxiline – Eosin staining: -

Tissue section on slides dewaxed by toluene for 30 minutes, washed by 3 changed (90 %, 80 %, 70 %) of absolute ethanol for 10 minutes for each concentration, washed in D.W. for 5 minutes, stained with haematoxylin for 5 – 10 minutes, washed with D.W. and washed with acid alcohol for few minutes until the sections became lightly red, washed with tap water for 5 minutes, the slides then placed in eosin for 2-5 minutes, washed with tap water for 2 – 3 minutes.

After staining the sections dehydrated in (70 % for 1 minute, 80 % for 2 minutes, 90 % for 5 minutes, first 100 % for 10 minutes and second 100 % for 10 minutes) of absolute ethanol, put the slides in first toluene for 10 minutes and then put in the second toluene for another 10 minutes finally leave the slides to be dried, and then covered by cover slip with Canada balsam put in incubator over night at 37 C°.

3.2.9: Statistical analysis: -

The obtained data were statistically analyzed by using analysis of variance (ANOVA) test to compare between different percentages of each experiment to observe the level of significance (0.05) (Gill, 1978; Steel and Torrie, 1980).

RESULTS: -

4.1. Extract effective dose assay:-

The appendix (1) showed no effect of extract at 0.1 mg/ml on the spleenocyte blastogenic response percentage value. While, the concentrations (0.2, 0.4, 0.8 and 1.6 mg/ml) descrided an increasing in the blastogenic response percentage value have been detacted in comparesion with control 1 and 2 ($b<0.05$) (appendix 1).

4.2. Immunological study: -

4.2.1. Lymphocyte transformation assay

I-*In vivo* assesment:-

Figures 2 and 3 illustrate the reactivity of spleenocyte transfomation with and without PHA. The results cleared that the plant extract increased the blastogenic response in both stimulated and non-stimulated splenocyte cells compared with control 1 and 2 ($b<0.05$) (appendix 2). While the

results of blood lymphocyte transformation was demonsrating in the figure 6 and 7 that showed the extract also have been increased the blastogenic response in stimulated and non-stimulated cells compared with control 1 and 2 ($b<0.05$) (appendix 3).

II- *In vitro* assesment:-

The resulte of this expariment represented in the figers (4, 5, 8 and 9). The reactivity of norml spleenocyte cultures incubated with extract showed an increasing in the blastogenic response in both stimulated and non-stimulated cells in comparesion with control 1 and 2 ($b<0.05$) that showed in the figures (4 and 5) (appendix 8).Also, the plant extract resulted in an effect on blood lymphocyte cells by increasing the blastogenic response in stimulated and non-stimulated cells compared with control 1 and 2 that showed in the figure (8 and 9) ($b<0.05$) (appendix 9).

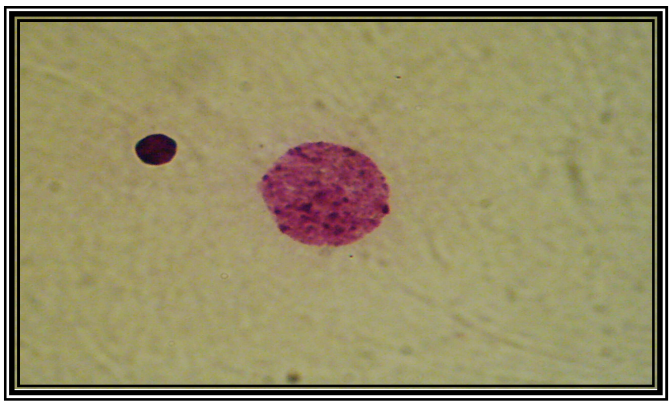


Figure 1: - Cytosmear of lymphocyte culture (three days in culture). Showing large pale cells are blastogenic lymphocytes and small dark are original lymphocytes. Giemsa stain, 100 X.

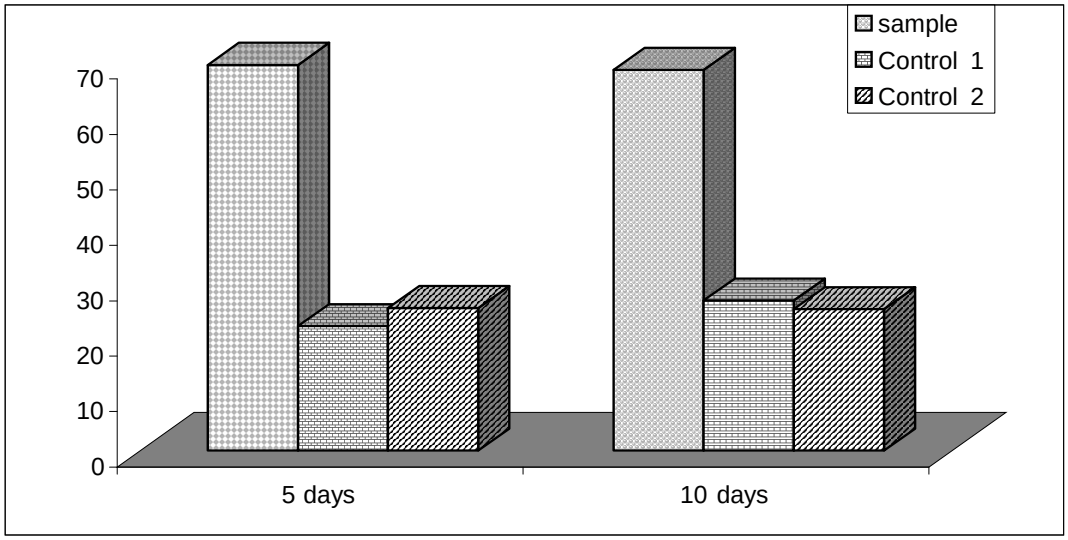


Figure 2 :The effect of extract on spleen lymphocyte transformation with PHA (*in vivo*).

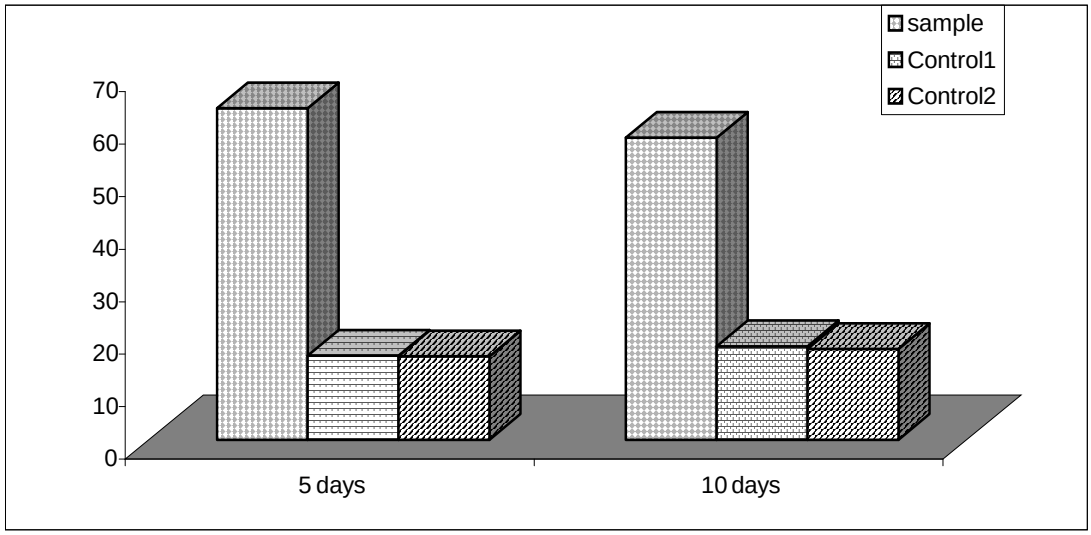


Figure 3: The effect of extract on spleen lymphocyte transformation without PHA (in *vivo*).

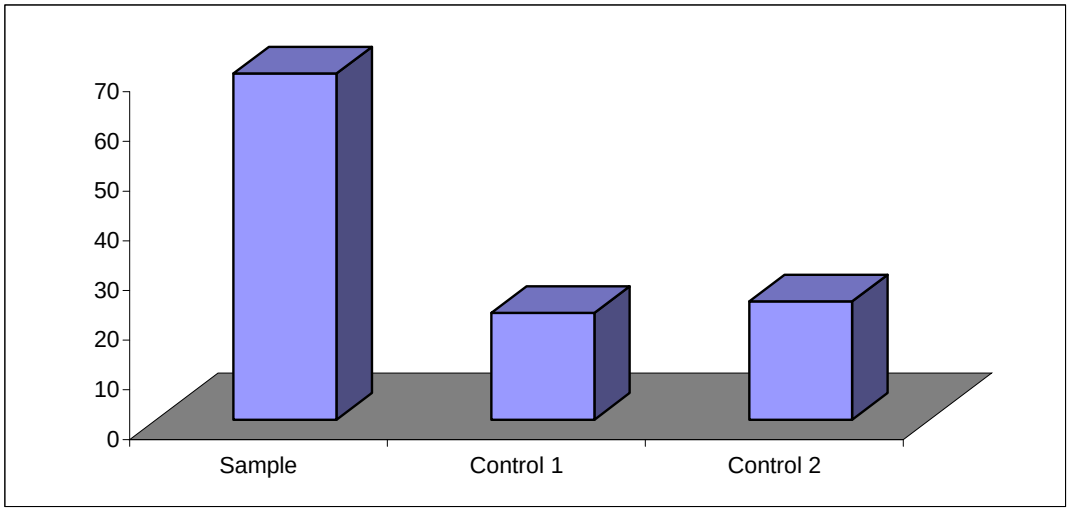


Figure 4: The effect of extract on spleen lymphocyte transformation with PHA (*in vitro*).

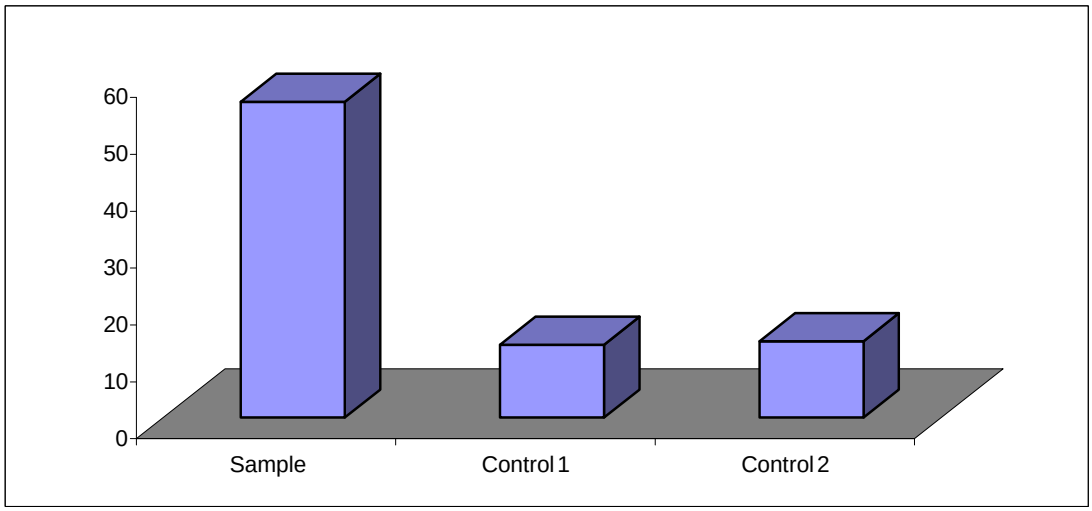


Figure 5: The effect of extract on spleen lymphocyte transformation without PHA (*in vitro*).

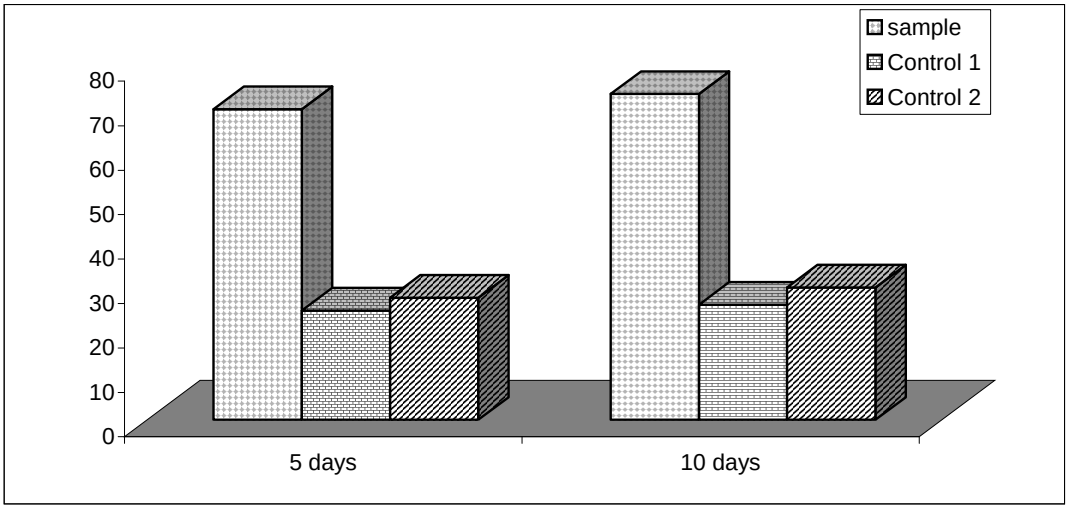


Figure 6: The effect of extract on blood lymphocyte transformation with PHA (*in vivo*).

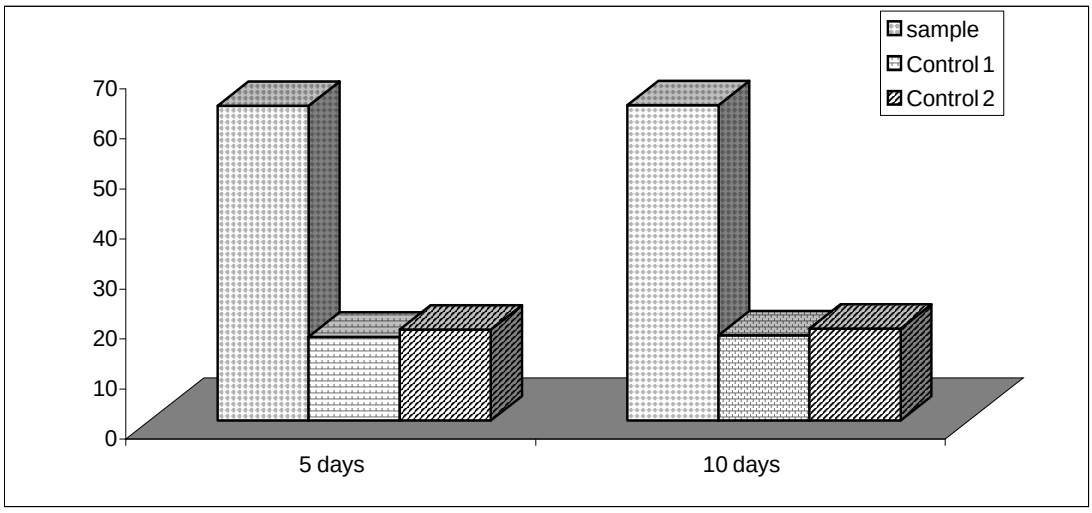


Figure 7: The effect of extract on blood lymphocyte transformation without PHA (*in vivo*).

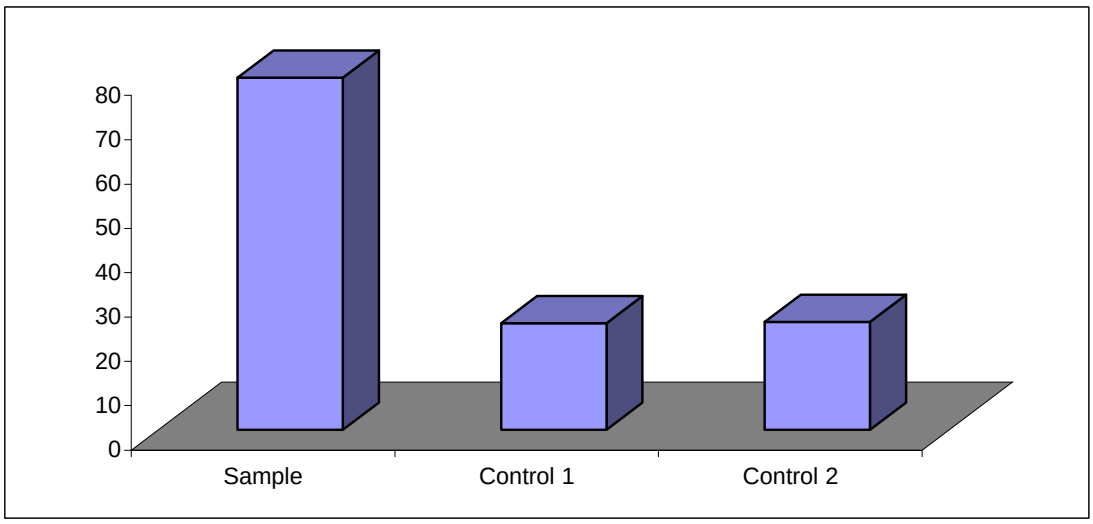


Figure 8: The effect of extract on blood lymphocyte transformation with PHA (*in vitro*).

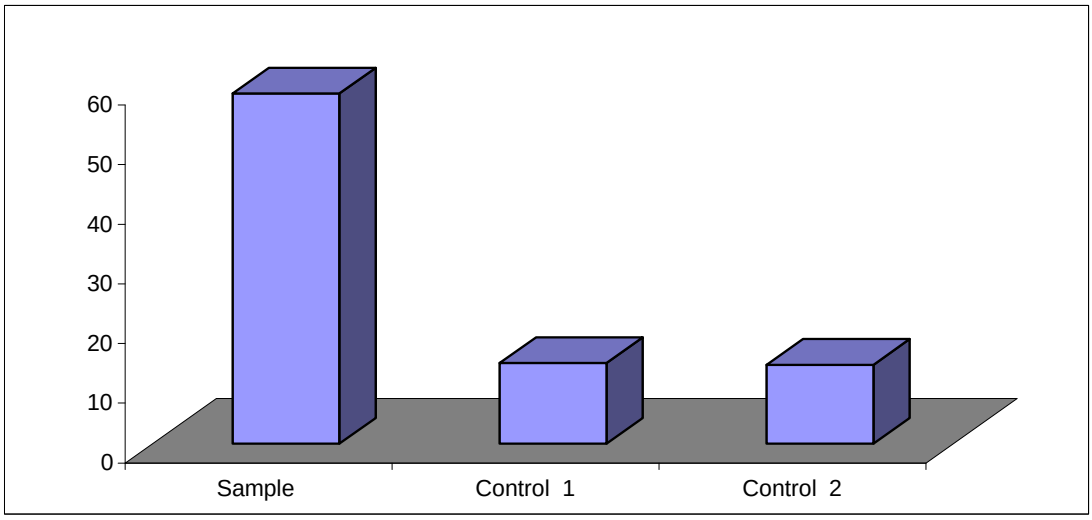


Figure 9: The effect of extract on blood lymphocyte transformation without PHA (*in vitro*).

4.2.2. Phagocytosis assay :-

I- *In vivo* assesment :-

Figure (11) illuminate the phagocytic function of blood phagocytic cells toward bacterial cells. The results showed increase in the phagocytic percentage value compared with control 1 and 2 ($b<0.05$) (appendix 7).

II- *In vitro* assesment :-

The results of phagocytic function of blood phagocytic cells toward bacterial cells described an increasing in the phagocytic percentage value that showed in the figure (12) compared with control 1 and 2 ($b<0.05$) (appendix 12).

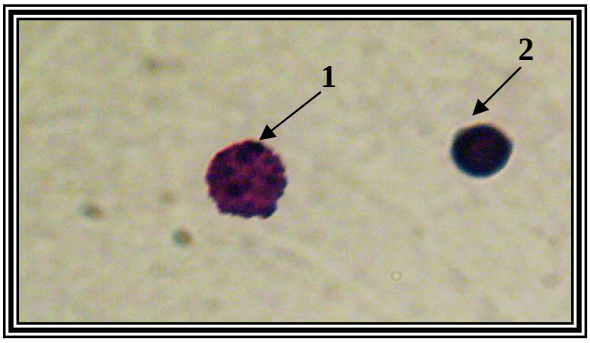


Figure 10 :- Bloob film of phagocytosis test samples showing:

1- phagocytic cells engulfed bacterial cells (*Staph. aureus).*

2- Non phagocytic cells.

Giemsa stain, 100 X.

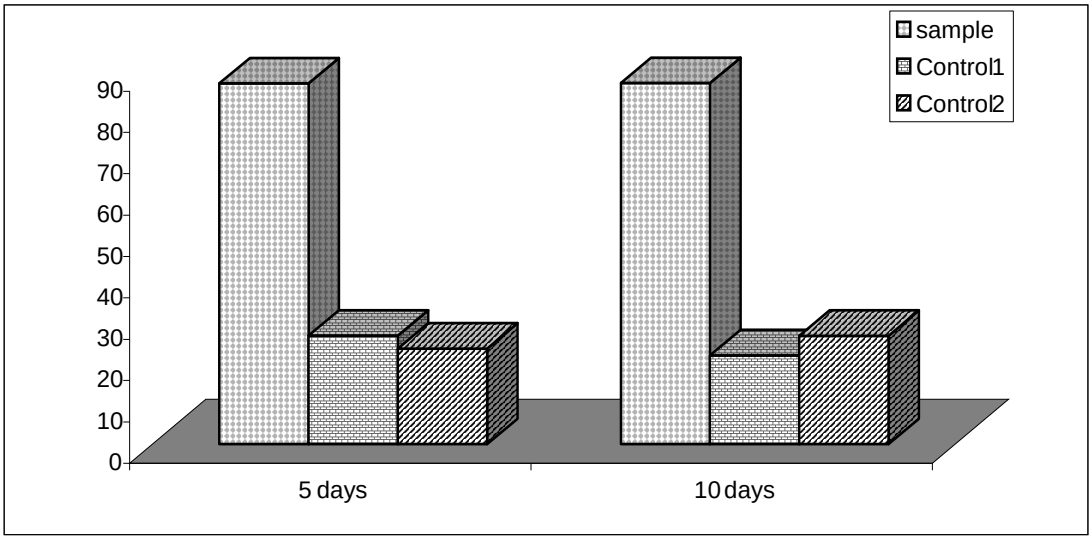


Figure 11: The effect of extract on blood phagocytic cells (*in vivo*).

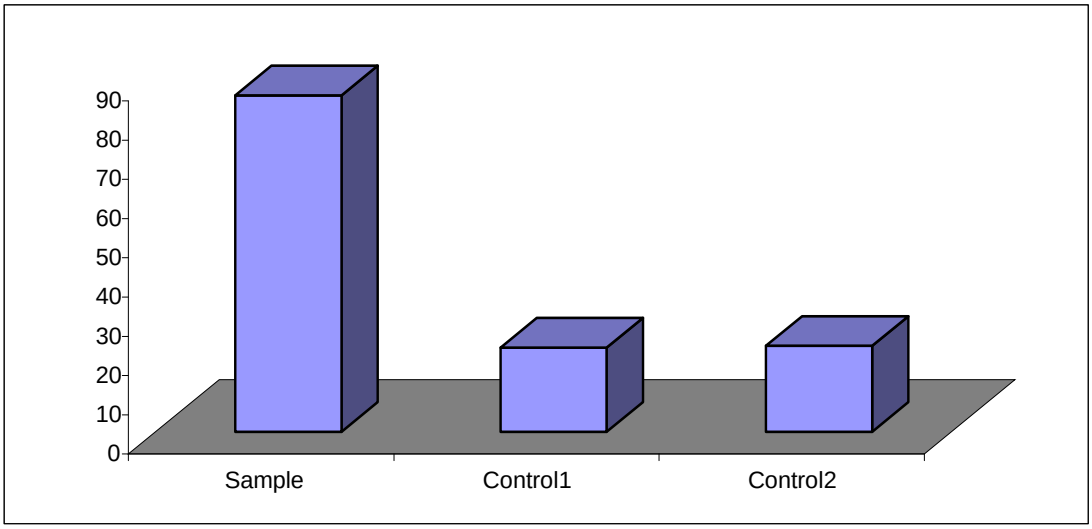


Figure 12: The effect of extract on blood phagocytic cells (*in vitro*).

4.3. Cytogenetic study: -

I- *In vivo* assesment:-

The results of this experiment was represent in the figures (13,14,17 and 18) . Figures 13 and 14 was described the result of spleen lymphocyte mitosis with PHA and without PHA . It showed that the plant extract increased the mitotic response percentage value in stimulated and non – stimulated spleenocyte compared with the control 1 and 2 ($b<0.05$) (appendix 4) .

Depending on the result of blood lymphocyte mitosis with PHA and without PHA . Also, there was an increasing in the mitotic response percentage value of the stimulated and non – stimulated blood lymphocyte cells that showed in the figures (17 and 18) compared with control 1 and 2 ($b<0.05$) (appendix 5).

Figure 21 reveald the effect of the extract on spermatogenesis of mice testis. The results were showing an increase in the mitotic response percentage value of cells compared with control 1 and 2 ($b<0.05$) (appendix 6).

II- *In vitro* assesment:-

The figures (15,16, 19 and 20) described the results of the mitosis test.
The figures 15 and 16 showed increasing in the mitosis response percentage value of the splenocyte of stimulated and non stimulated cells compared with control 1 and 2 ($b<0.05$) (appendix 10).While the figures 19 and 20 were describing the results of the plant extract effect on blood lymphocyte cells mitosis that showed increasing in the mitotic response percentage value in stimulated and non –stimulated blood lymphocyte cells compared with control 1 and 2 ($b<0.05$) (appendix 11) .

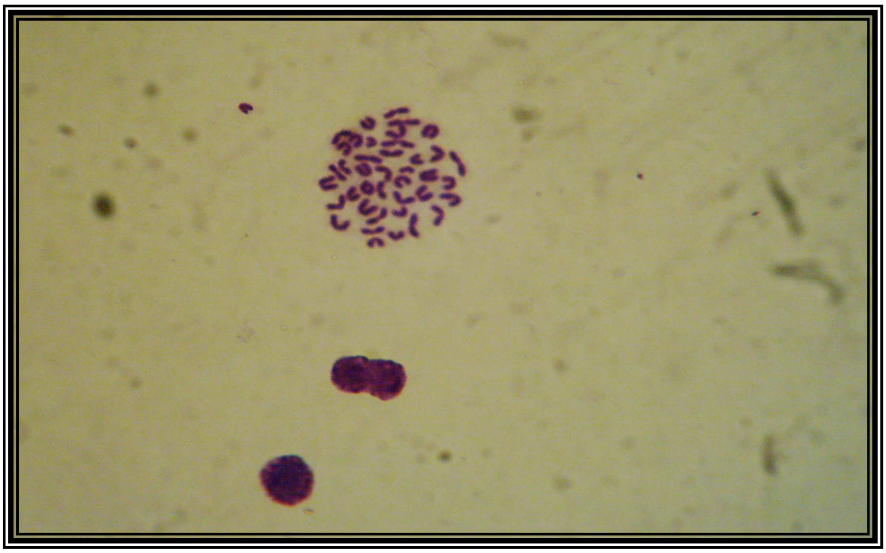


Figure 13:- Cell chromosomes at metaphase stage .

Giemsa stain, 100 X.

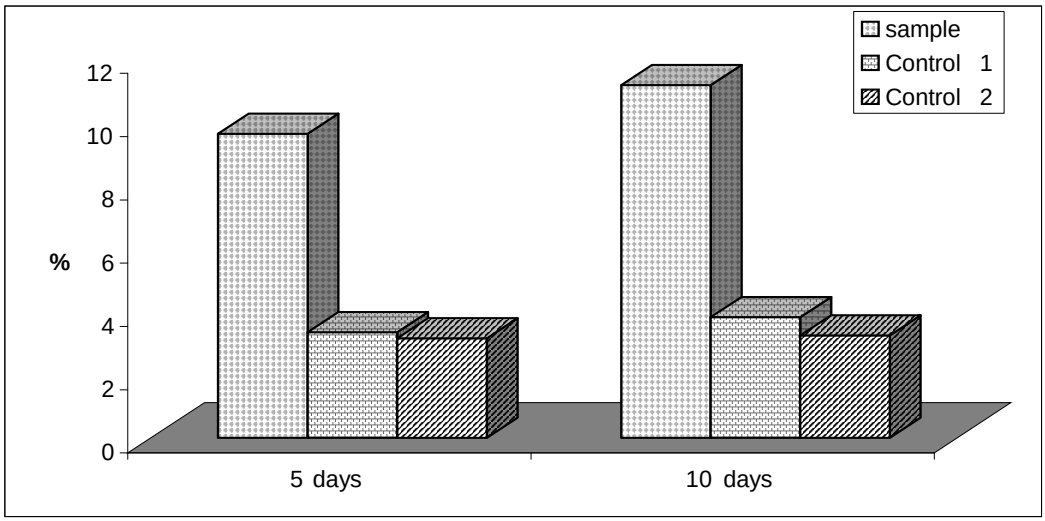


Figure 14: The effect of extract on spleen lymphocyte mitosis with PHA (*in vivo*).

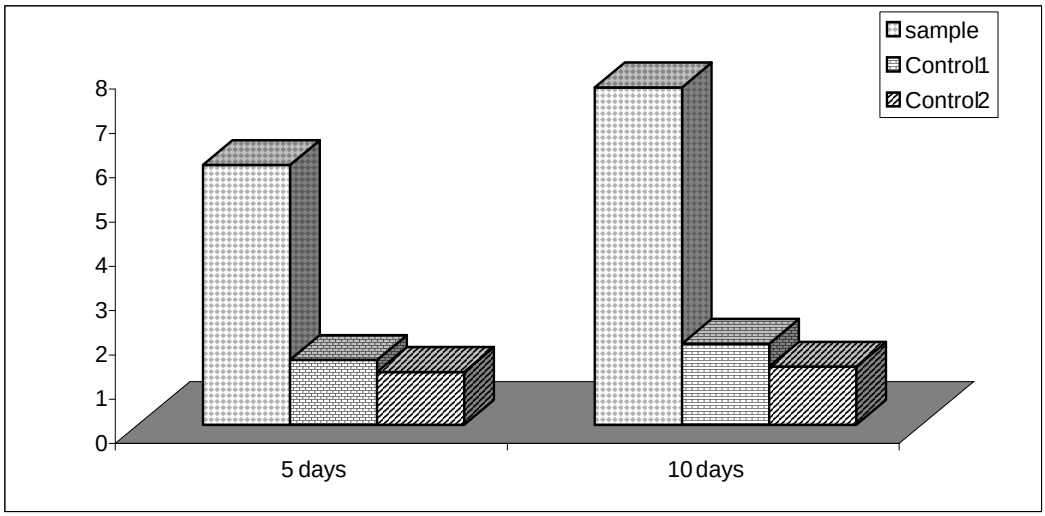


Figure 15: The effect of extract on spleen lymphocyte mitosis without PHA (*in vivo*).

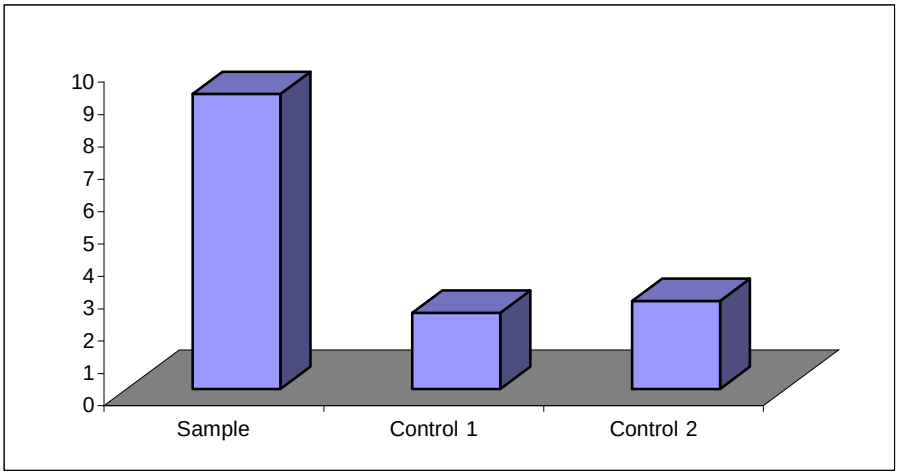


Figure 16: The effect of extract on spleen lymphocyte mitosis with PHA (*in vitro*).

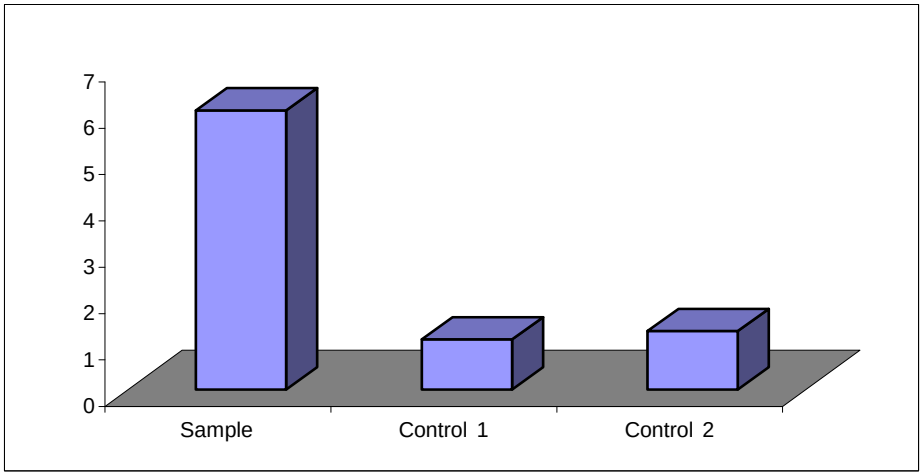


Figure 17: The effect of extract on spleen lymphocyte mitosis without PHA (*in vitro*).

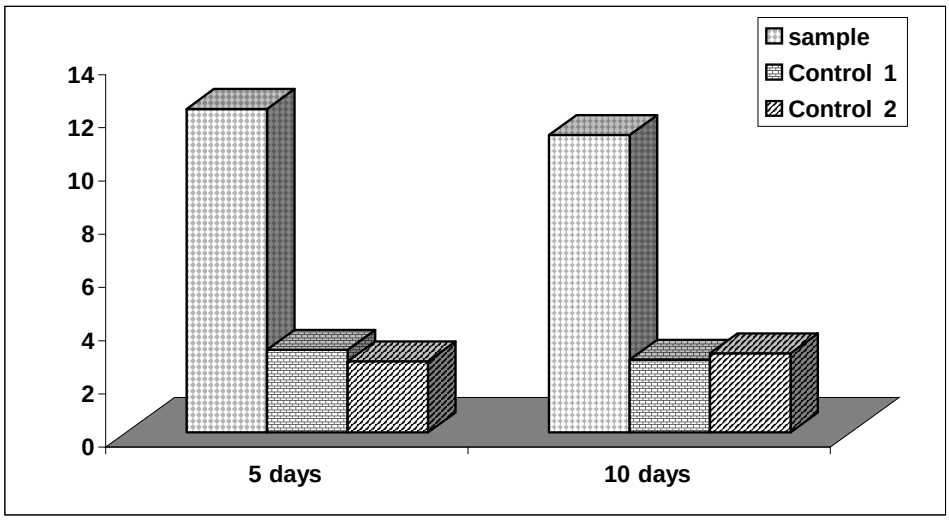


Figure 18: The effect of extract on blood lymphocyte mitosis with PHA (*in vivo*).

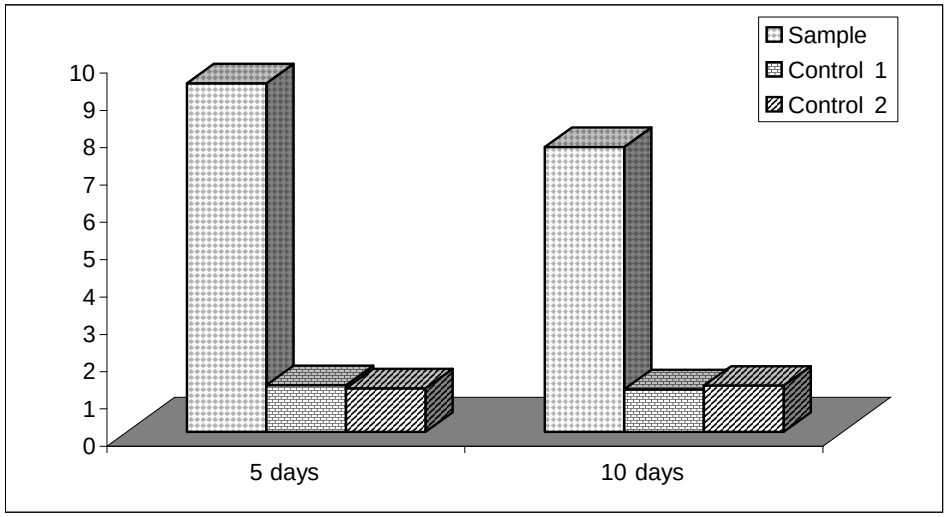


Figure 19: The effect of extract on blood lymphocyte mitosis without PHA (*in vivo*).

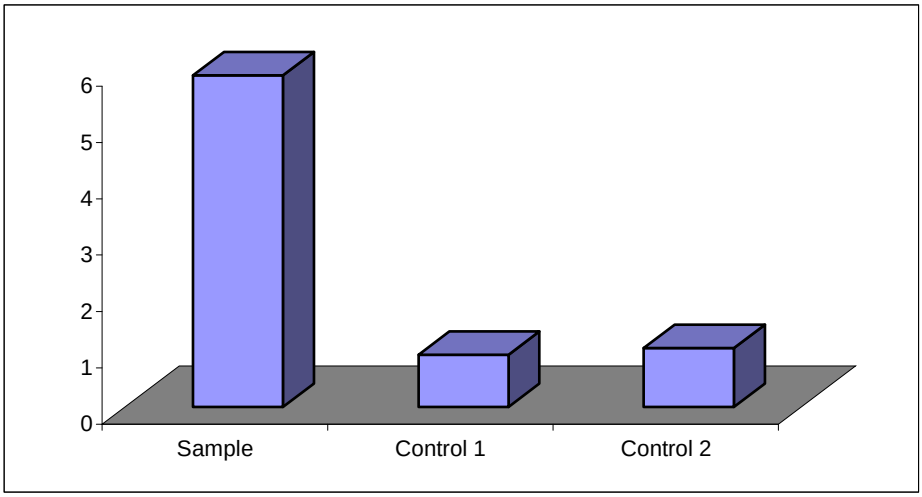


Figure 20: The effect of extract on blood lymphocyte mitosis with PHA (*in vitro*).

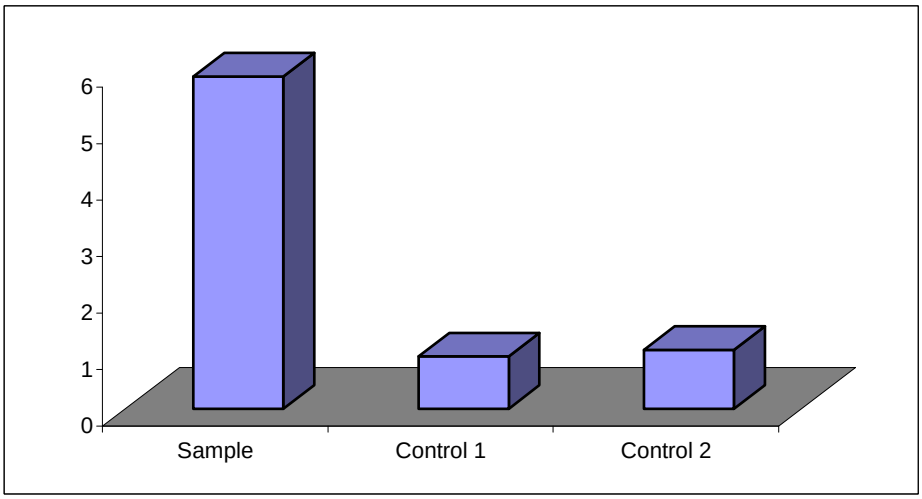


Figure 21: The effect of extract on blood lymphocyte mitosis without PHA (*in vitro*).

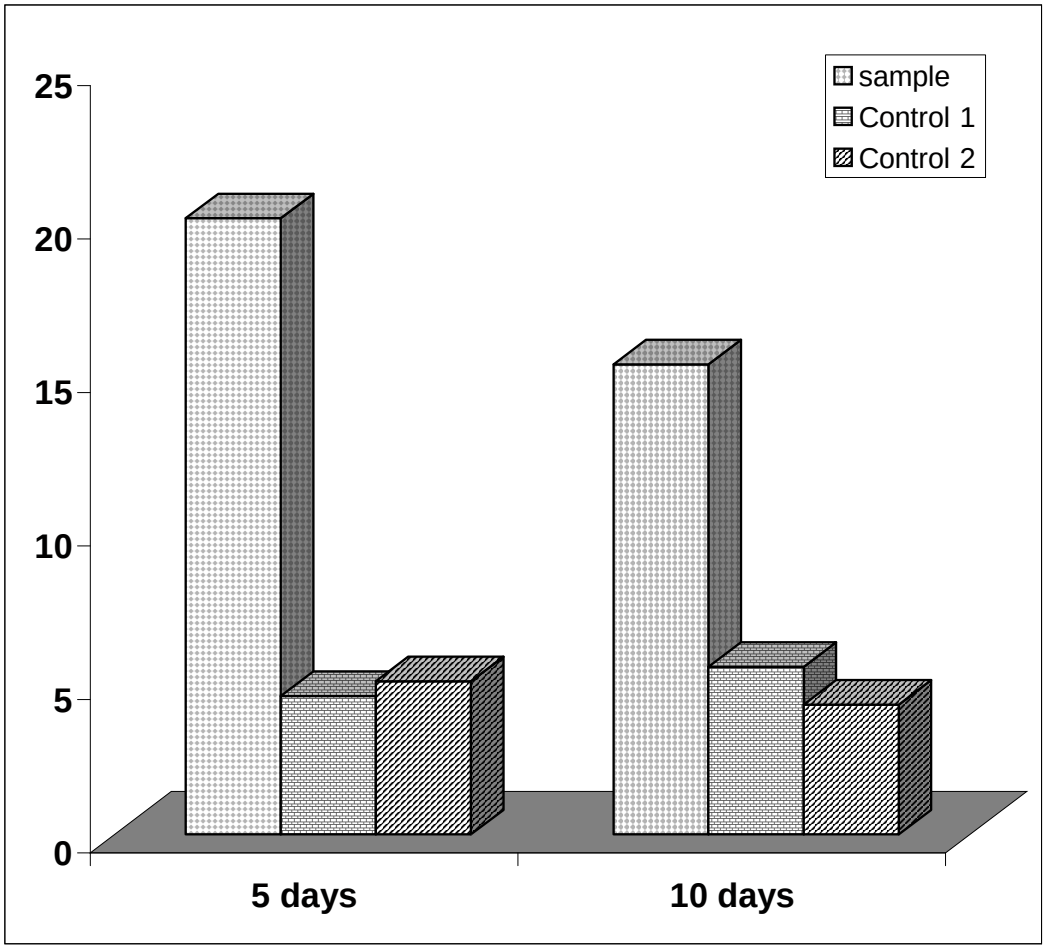


Figure 22: The effect of extract on testis cells mitosis (*in vivo*).

4.4. Cytotoxicity assay: -

I- Fibroblast cells assay

The results of this expirement cleared that the extract has a stimulatory effect on fibroblast cells growth rate at the last two concentrations (60 and 45 µg/well), while in the other concentrations no effect of extract on the cells growth rate have been detected in comparsion with control 1 and 2 ($b<0.05$), that showed in the figure (22).

II- Plasmacytoma cell line procedure

The resultes indicated that the plant extract has an inhibitory effect on S.U.99 cell line growth rate in all different times (after 24 hrs., 48 hrs., 72 hrs. and four days) of incubation that showed in the figures (23, 24 25 and 26) compared with control 1 and 2 ($b<0.05$) .

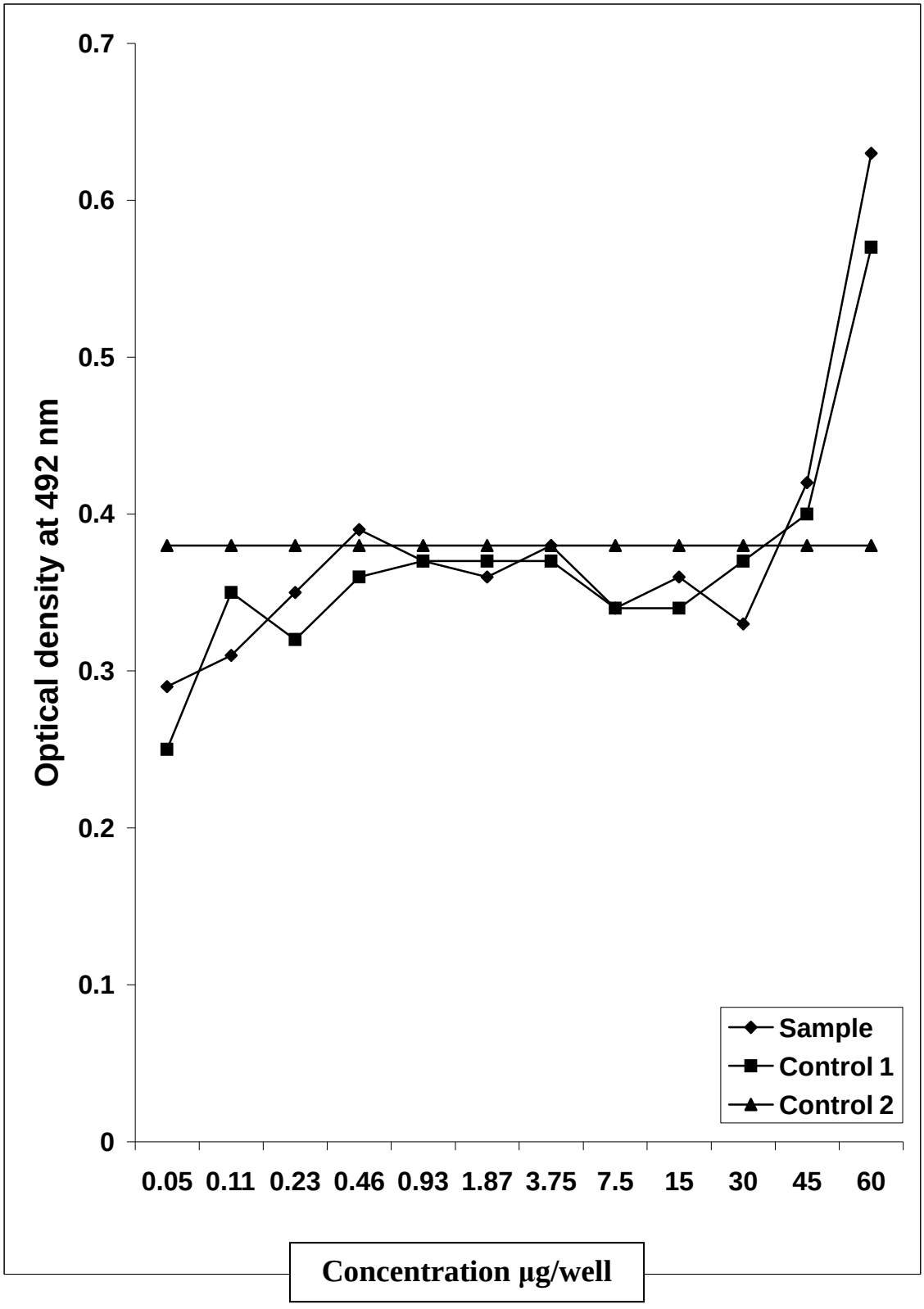


Figure 23: Cytotoxic effects of extract on mice fibroblast cells after 4 days of incubation.

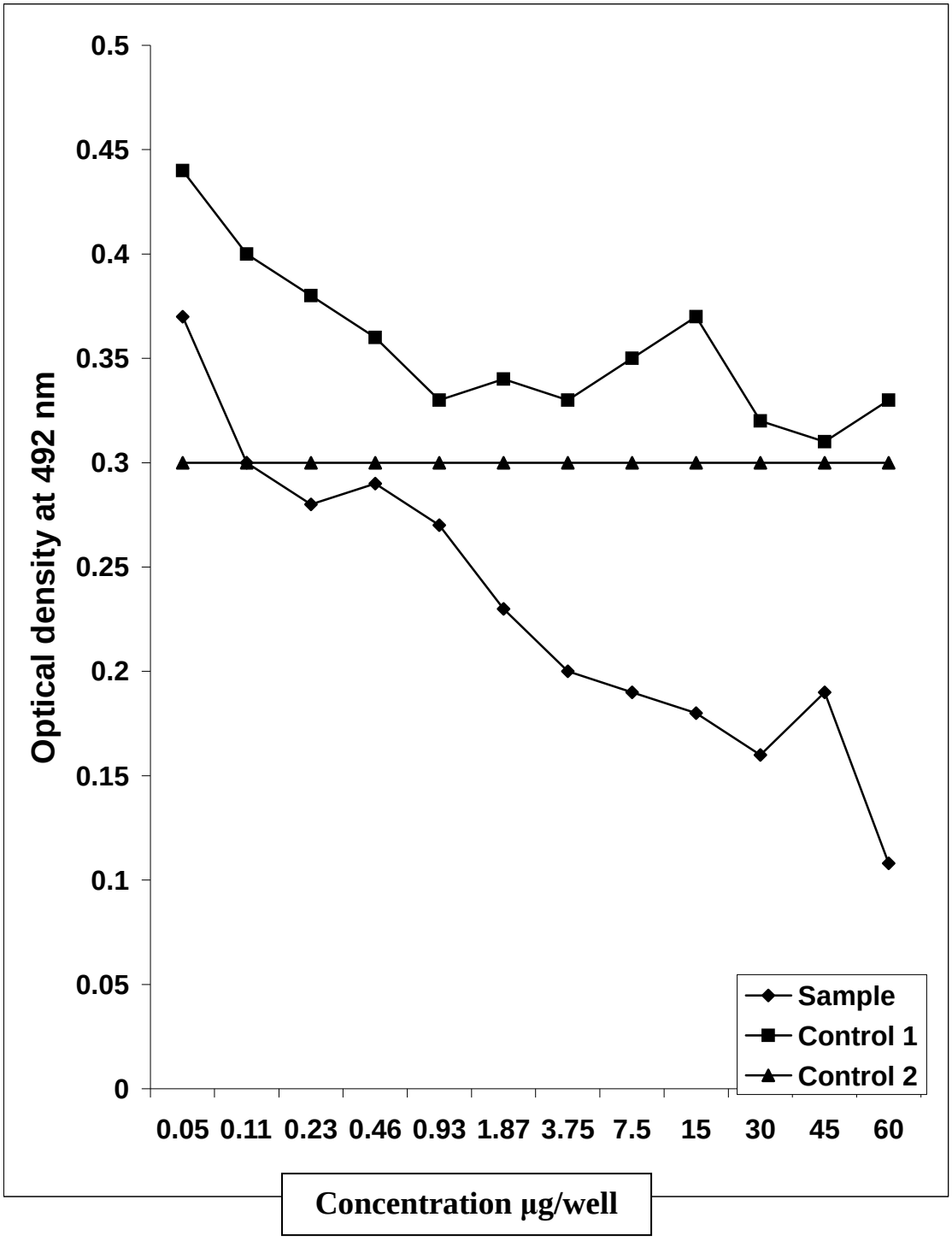


Figure 24: Cytotoxic effects of extract on S.U.99 cell line after 24 hours of incubation.

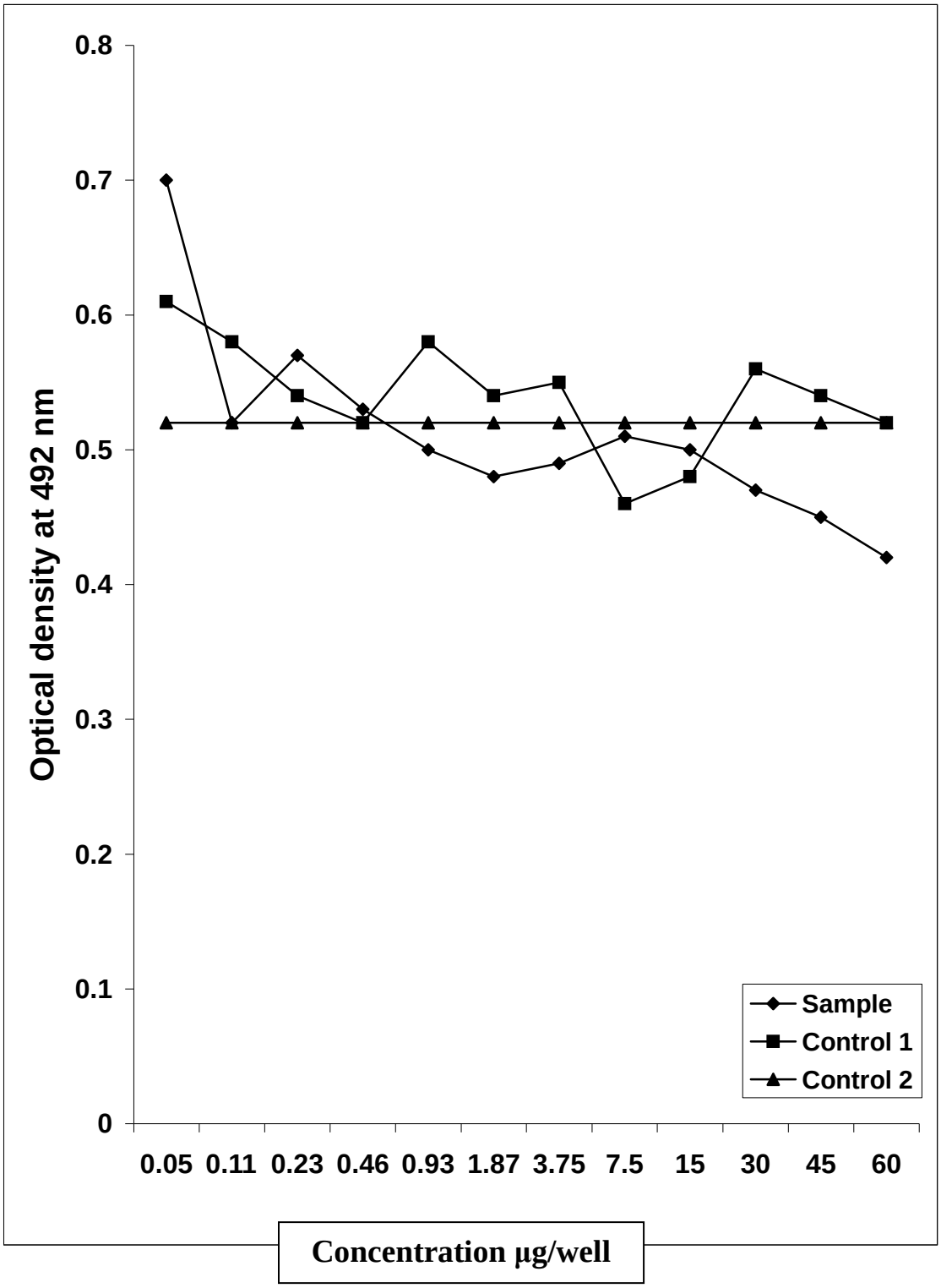


Figure 25: Cytotoxic effects of extract on S.U.99 cell line after 48 hours of incubation.

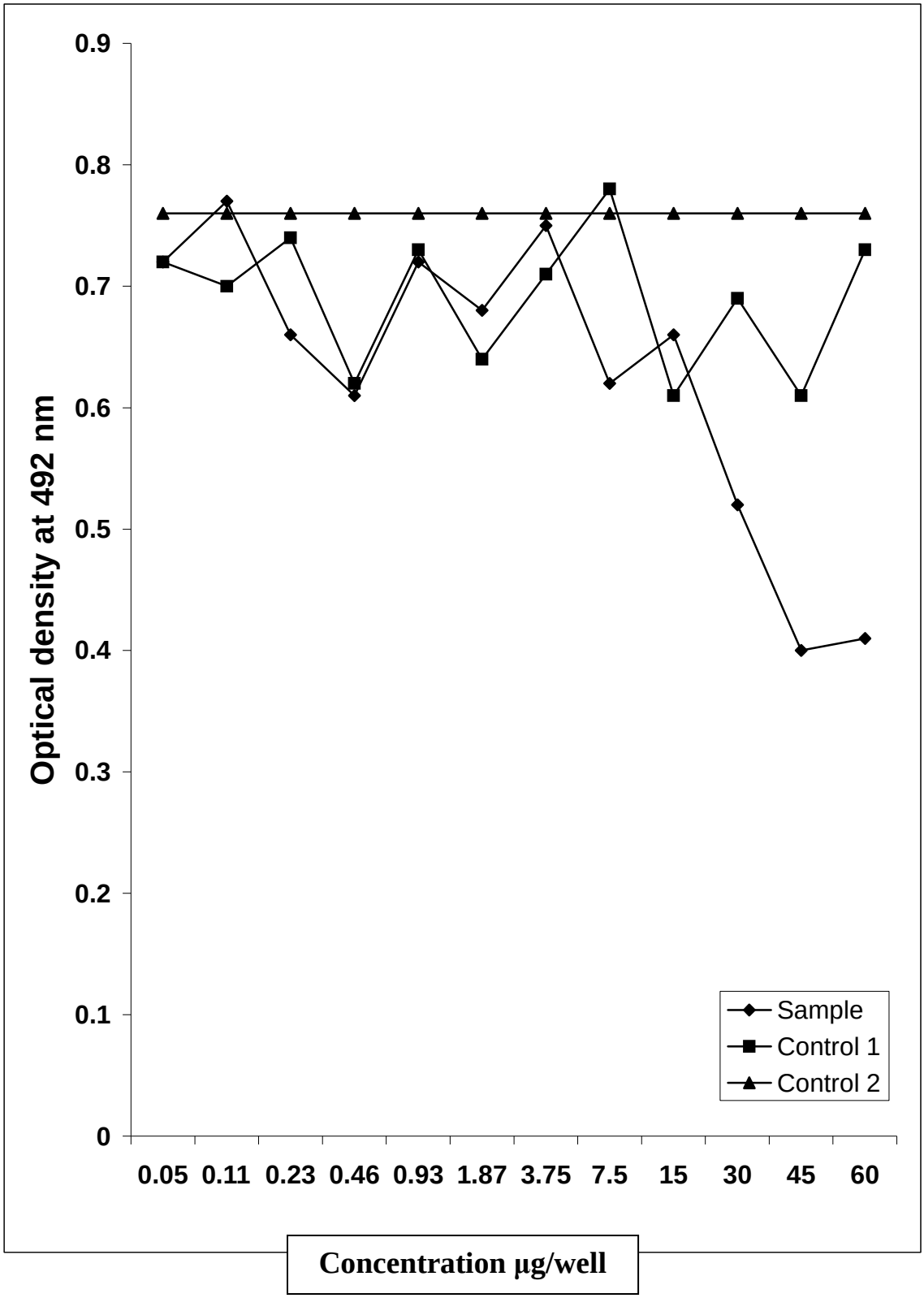


Figure26: Cytotoxic effects of extract on S.U.99 cell line after 72 hours of incubation.

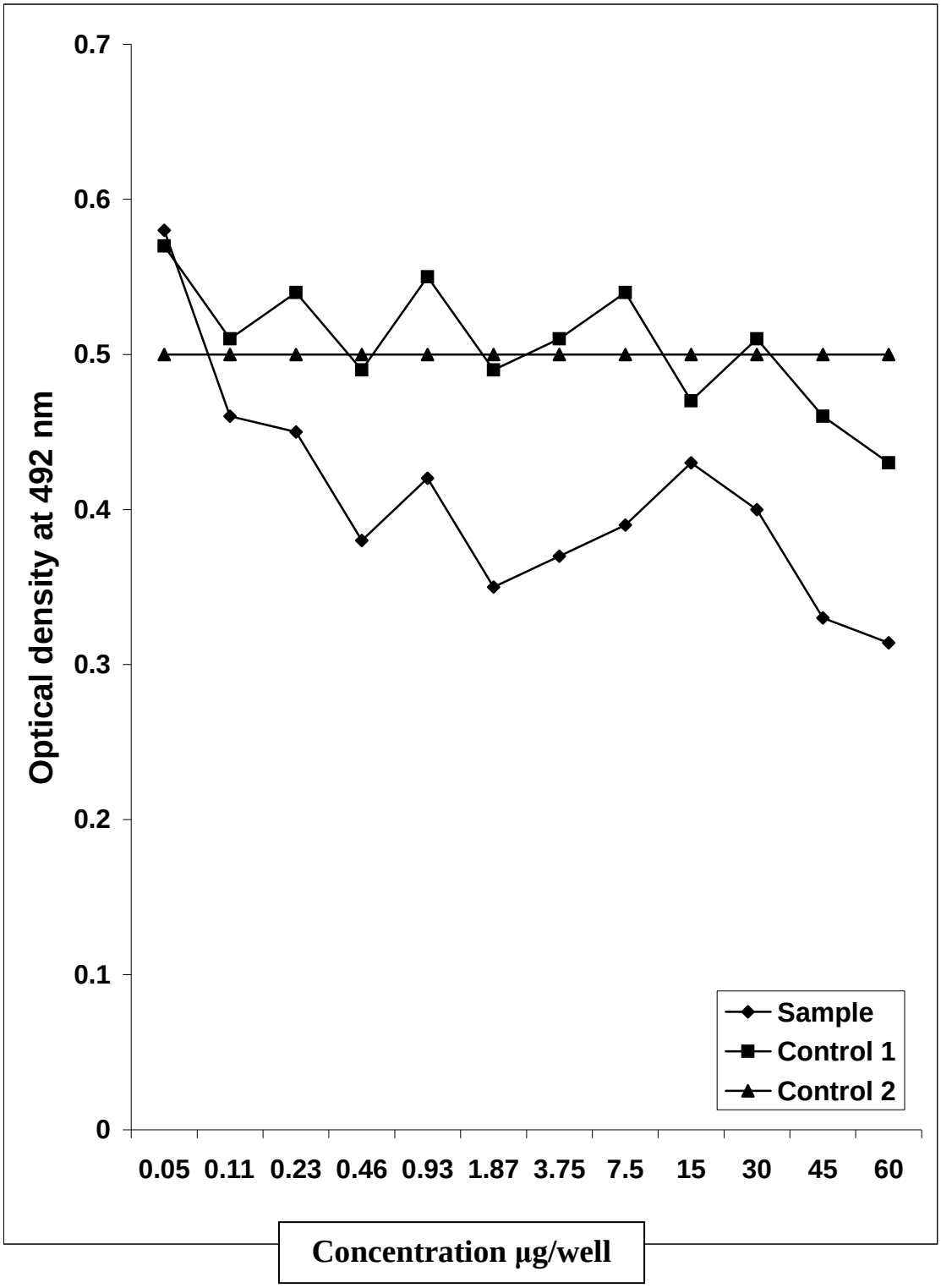
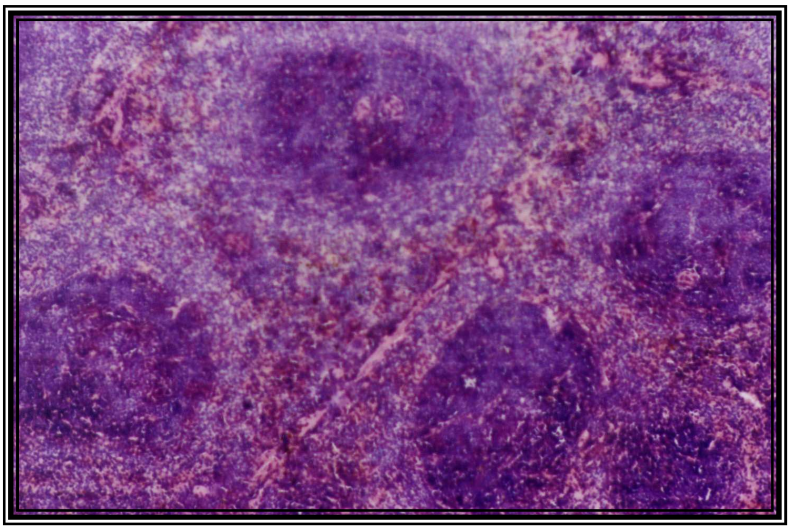


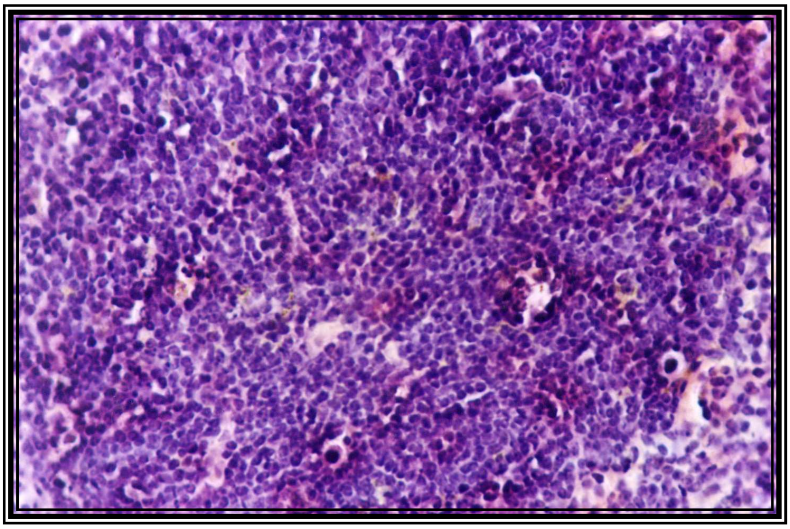
Figure 27: Cytotoxic effects of extract on S.U.99 cell line after 4 days of incubation.

4.5. Hitological studies: -

This study detremined that was no damage on the level of tissues (spleen , liver , kidney and testis)or any precipitation of the extract in side these tissues that showed in the figures (28, 29, 30and 31) .

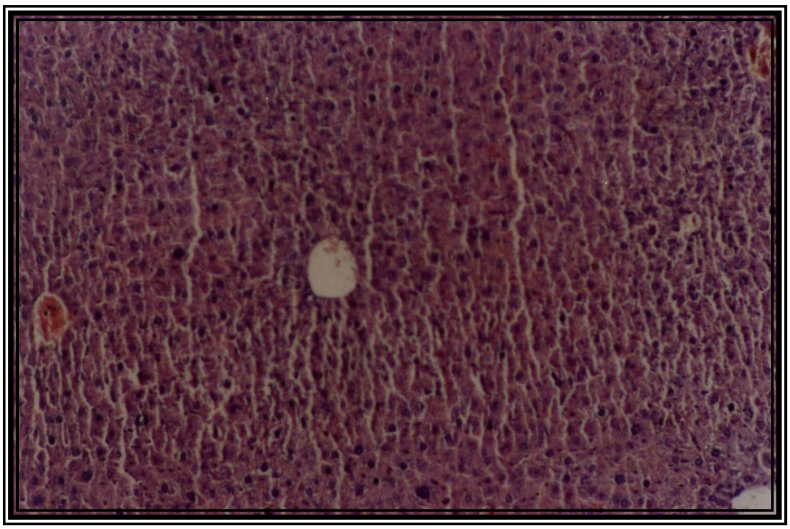


A - At 40 X..

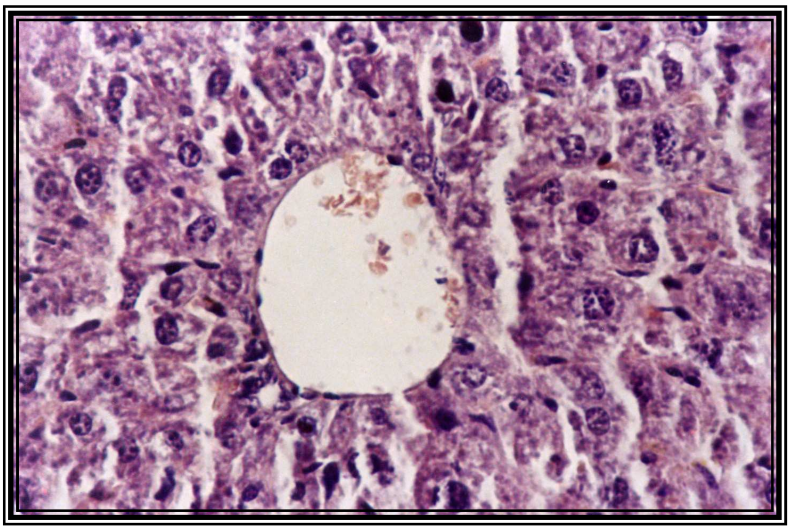


B - At 100 X..

Figure 29:- Histological section of spleen stained by H & E .

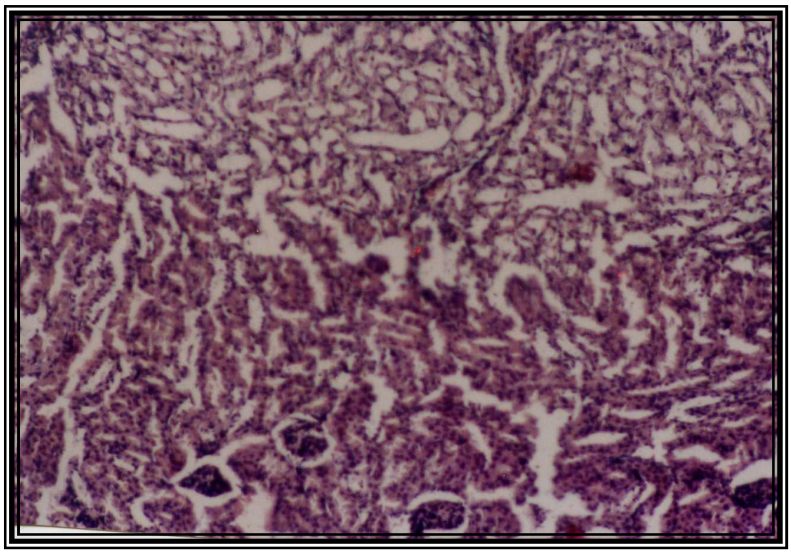


A- At 40 X.

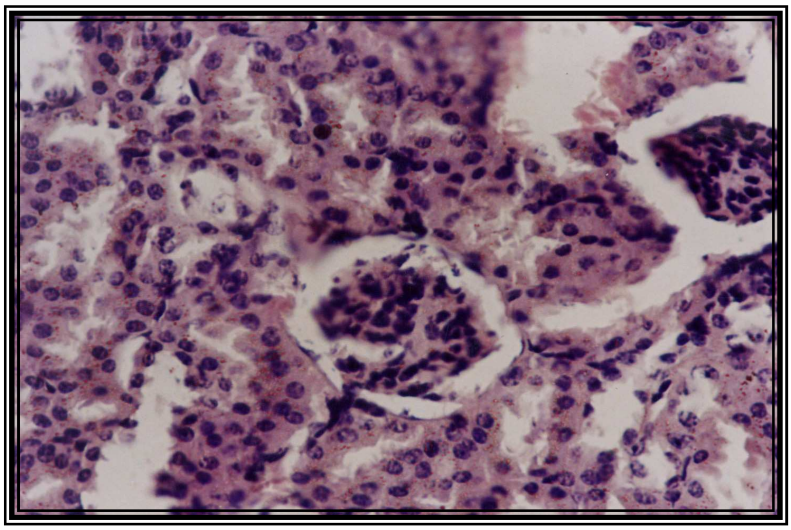


B- At 100 X.

Figure 30:- Histological section of liver stained by H & E.

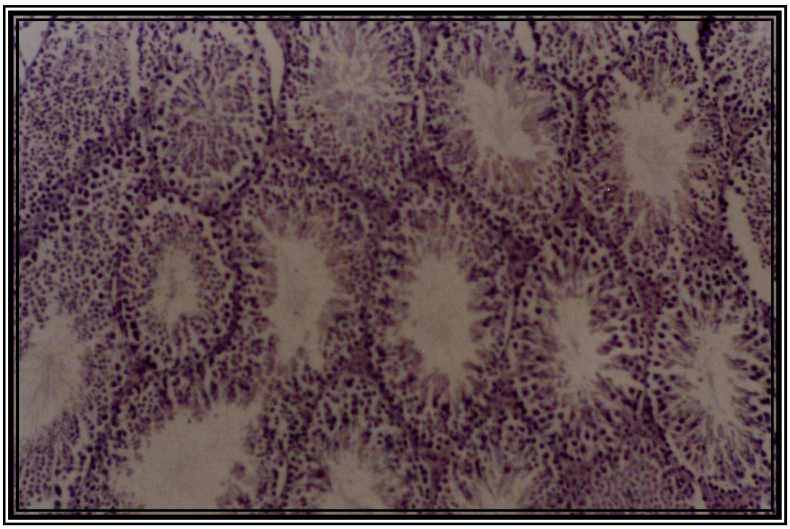


A- At 40 X..

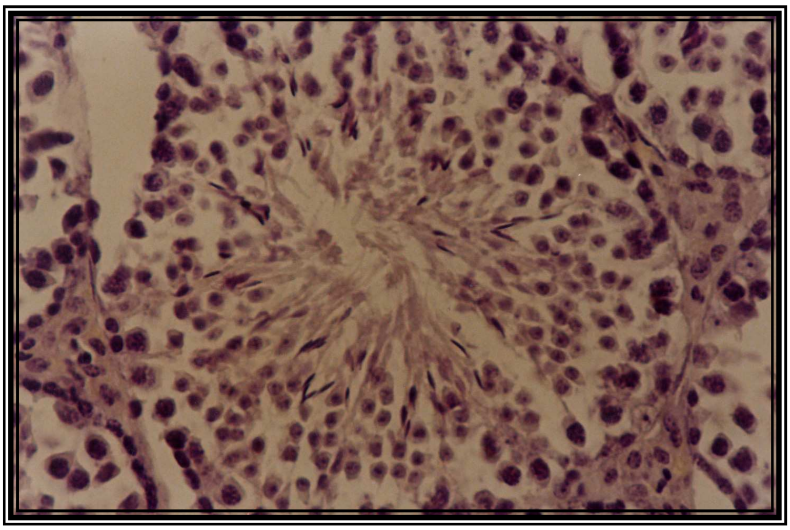


B- At 100 X.

Figure31:- Histological section of kidney stained by H & E.



A- At 40 X.



B- At 100 X.

Figure 32:- Histological section of testis stained by H & E.

Discussion :-

The result of I.p injection of male BALB\c mice with *Artemisia herba-alba* diethyl ether extract as a cytotoxic effect after 5 and 10 days , showed that induced T lymphocyte prolifferation (both *in vivo* and *in vitro*) in stimulated and non stimulated cells with mitogen (PHA) compared with control 1and control 2. This result was agreed with the results of Grendelmeier *et al.* (2003) , that showed the artemisinin increased IgE-binding capacity were able to induce T-cell proliferation .

There is substantial evidence for a role for T cells in both the development and suppression of IgE responses. This led to a suggestion that a defect in T cells, and in particular suppressor T cells could be involved with any defect in IgE response . Because , the cell mediated immunity is correlate with T cell response , therfore we found according to the results that the extract has the ability to increased Cell mediated immune response (Roitt *et al.*,1998).

While the results was disagreed with Mossa *et al.* (1987) found that the ethanolic extract of *Artemisia inculta* has been screened for anti-inflammatory, analgesic and antipyretic activities on suitable experimental models and he found that the flavonoids may be responsible for the observed anti-inflammatory effect of the ethanolic extract . While Heinrich *et al.*,(1998) found that the

Maxican *Artemisia* species , which was used in folk medicine , have anti-inflammatory properties, analgestic and anti-migraine activities .

This disagreement was a result of using different types of *Artemisia* species with different kind of extraction , so it will appeare a different active compounds which have different mechanism of action on immune respons that different from the curd diethyl ether extract of the localy Iraqi plant (*Artemisia herba-alba*) that used in folk medicine for many years .

The phagocytosis results also showed an increase in the cells phagocytic function in both expariments *in vivo* and *in vitro* . This resuts agreed with a research puplished by (Tang 2000) which found that the artemisinin enhances macrophage phagocytosis in mice , while Wenisch (1997) found that the effect of DHA , artemisinin, artemether and artesunate on phagocytic function was enhancing the reactive oxygen response of neutrophils but depress their phagocytic ability at therapeutic blood level .

There are two mechanisims of actions of phagocytosis which described as that the phagocytosis is associated with marked increase in activity of hexose monophosphate shunt providing NADPH and the contact of microbe results in activation of NADPH oxidase of plasma membrane . This results in formation of superoxide ions e.g. hydrogen peroxide , singlet oxygen hydroxyl radicals all of which are powerful microbicidal agent. The combination of peroxide , myeloperoxide and halide ions constitute potent helogenating system which is bactericidal and viricidal. While these low pH , Lysosome , lactoferrin and cationic protein which are not oxygen dependent function as bacteriostatic or bactericidal . The proteolytic and hydrolytic enzymes digest the killed organisms. There is also an extracellular release of lysosomes during phagocytosis which may play an amplifying role (Joshi and Osamo , 1994).

So according to first mechanisim of phagocytosis , and according to the mechanism of action of the extract which are depend on increase the secreation

of hydrogen peroxide , singlet oxygen hydroxyl radicals from neutrophils which are killing the parasites and effect on cancer cells, so increase the seacretion of superoxide ions reffer to increase the phagocytic function .

The increasing in the phagocytic response ,that cleared in the results, will cause an increasing in the humaral immune respnse (Roitt *et al.*,1998).

According to the results of lymphocyte transformation and phagocytosis experiments, we suggestion that the extract has an immune stimulatory response by stimulating both cell mediated immunity and humaral immunity.

The results of mitosis (spleen , blood and testis) in both *in vivo* and *in vitro* experiments showed an increase in the mitotic percentage value according to the control 1 and control 2 . The increas in mitotic results may reffer that the plant extract containe colchicine-like compound which causing an increase in mitotic activity of the treated cells (Zhuravskaia 2000).Artemisinin is not affected on spermatogenesis (internet 18) .

In 2001, a WHO report concluded that artemisinin and its derivatives do not exhibit mutagenic or teratogenic activity.

The extract showed an control 1 effect on fibroblast growth on the two first concentrations and then the growth decreased to the normal level compared with control 1 and control 2. Kaji (1990) showed that the *artemisia* leaf stimulated endothelial cell proliferation by increasing the production of basic fibroblast growth factor (bFGF)rather than by an increase in the number of bFGF receptors and the content of glycosaminoglycans in the cell layer .

S.U.99 cell line growth rate was affecting with the extract that decreased the cells growth in the all time of incubation because of, all cancer cells have sequester iron , and the mechanism of action of artemisinin and it’s drevetives depended on the presence of iron in cells at high concentration ,so, according to Lai and Singh 2002, it is believed to work because when artemisinin or any of it’s derivatives comes into contact with iron, a chemical reaction ensures,

spawning charged atoms that chemists call free radicals. Cells need iron to replicate DNA when they divide, and since cancer is characterized by out-of-control cell division, cancer cells have much higher iron concentrations than do normal cells. What Lai did was to pump up cancer cells with even more iron and then introduce artemisinin to selectively kill them. Lai theorizes that more aggressive cancers such as pancreatic and acute leukemia — which are characterized by more rapid cell division and thus higher iron concentrations — may respond even better.

Cancer cells are notoriously deficient in antioxidant enzymes - both forms of superoxide dismutase, the manganese form in mitochondria, and the copper zinc form in the cell cytoplasm are generally low in cancer cells. Cancer cells are grossly deficient in catalase and glutathione peroxidase, both of which degrade hydrogen peroxide. It is these deficiencies in antioxidant enzymes which lead to the use of many of the common chemotherapeutics which are superoxide generators.

The higher iron fluxes, especially associated with the reproductive phase of tumor cells, should render these cells even more susceptible to oxidative damage via hydrogen peroxide and superoxides. Normally, the profound catalase deficiency in cancer cells is credited with creating vulnerability to oxidants, in relationship to IV vitamin C or IV hydrogen peroxide. However, since all of these protective antioxidant enzymes are most often deficient in transformed cells, the oxidant vulnerability should be enhanced dramatically, and further so, due to enhanced unbound iron during cell division. (internet 6; and Vattanaviboon *et al*., 2001).

For histological examination also found no effect of extract on the level of tissues (spleen , liver , kideny and testis) and there were no changes or extract priciptation inside this tissues .

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Appendix (1)

The effect of extract with different concentrations on spleenocyte blastogenic response

I-with PHA

	Mice number	0.1mg/ml %	0.2mg/ml %	0.4mg/ml %	0.8mg/ml %	1.6mg/ml %
Sample	M1	20. 9	73. 1	60. 4	55. 7	50. 2
	M2	26. 1	68. 4	67. 1	60. 3	55. 9
	M3	30. 0	63. 8	70. 1	52. 4	51.7
	M4	32. 5	70. 5	63. 7	66. 3	59.1
	M5	28. 7	67. 5	67. 8	57. 2	53.7
	M6	33. 2	62. 9	65. 3	63. 2	49. 9
	M7	29. 3	65. 7	57. 9	59. 1	57. 1
	M8	22. 4	71. 3	60. 8	50. 3	54. 6
	M9	27. 3	67. 2	58. 6	51. 8	51..3
	M10	21. 6	72. 3	64. 6	61. 3	53. 4
	Mean ± SE	a,A 27. 19 1. 38	b,A 68. 27 1. 1	c,A 63. 63 1. 2	d,A 57. 76 1. 65	d,A 53. 72 0. 96
Positive Control	M1	20. 7	32. 3	28. 6	19. 9	26. 1
	M2	31. 5	34. 7	23. 9	20. 4	21. 2
	M3	27. 4	28. 1	24. 6	27. 2	24. 1
	M4	23. 8	23. 4	21. 5	28. 1	26. 7
	M5	22. 9	30. 7	23.6	25. 3	20. 9
	Mean ± SE	a,A 25. 26 1. 89	a,B 29. 84 1. 93	a,B 24. 44 1. 15	a,B 24. 18 1. 7	a,B 23. 8 1. 2
Negative control	M1	28. 4	28. 4	28. 4	28. 4	28. 4
	M2	30. 1	30. 1	30. 1	30. 1	30. 1
	M3	25. 7	25. 7	25. 7	25. 7	25. 7
	M4	22. 9	22. 9	22. 9	22. 9	22. 9
	M5	31. 2	31. 2	31. 2	31. 2	31. 2
	Mean ± SE	a,A 27. 66 1. 5	a,B 27. 66 1. 5	a,B 27. 66 1. 5	a,B 27. 66 1. 5	a,B 27. 66 1. 5

Differences (a b, c and d) are significant (b<0.05) to comparison rows between different sample concentrations.

Differences (A and B) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

II- without PHA

	Mice number	0.1mg/ml %	0.2mg/ml %	0.4mg/ml %	0.8mg/ml %	1.6mg/ml %
Sample	M1	13. 6	60. 3	50. 6	47.4	40. 5
	M2	18. 2	59. 7	57. 9	50. 3	46. 1
	M3	12. 9	62. 3	49. 8	43. 5	47. 4
	M4	15. 8	58. 5	53. 1	48. 1	43. 2
	M5	13. 5	65. 1	52. 6	44. 7	40. 7
	M6	17. 2	58. 9	56. 7	43. 9	45. 2
	M7	16. 7	60. 5	52. 7	41. 6	48. 1
	M8	15. 6	54. 9	55. 7	50. 1	42. 8
	M9	14. 7	62. 4	50. 1	46. 3	38. 8
	M10	13. 4	58. 9	51. 2	40. 9	42. 6
	Mean ± SE	a,A 15. 16 0. 57	b,A 60. 15 0. 86	c,A 53. 04 0. 89	d,A 45. 68 1. 04	d,A 43. 54 0. 97
Positive control	M1	11. 9	18. 3	12. 5	17. 3	15. 3
	M2	15. 4	11. 9	13. 9	13. 5	17. 2
	M3	13. 8	16. 4	17. 8	16. 4	19. 2
	M4	17. 3	14. 7	18. 5	15. 3	12. 7
	M5	12. 5	14. 9	13. 9	14. 9	17. 6
	Mean ± SE	a,A 14. 18 0. 98	a,B 15. 24 1. 05	a,B 15. 32 1. 18	a,B 15. 48 0. 64	a,B 16. 4 1. 11
Negative Control	M1	17. 8	17. 8	17. 8	17. 8	17. 8
	M2	18. 6	18. 6	18. 6	18. 6	18. 6
	M3	16. 2	16. 2	16. 2	16. 2	16. 2
	M4	13. 9	13. 9	13. 9	13. 9	13. 9
	M5	14. 6	14. 6	14. 6	14. 6	14. 6
	Mean ± SE	a,A 16. 22 0. 89	a,B 16. 22 0. 89	a,B 16. 22 0. 89	a,B 16. 22 0. 89	a,B 16. 22 0. 89

Differences (a b, c and d) are significant (b<0.05) to comparison rows between different sample concentrations.

Differences (A and B) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (2)

The effect of extract on spleenocyte blastogenic response (*in vivo*).

After 5 days of injection

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	71. 3	60. 3	21. 4	15. 9	23. 7	14. 6
M2	68. .9	67. 1	23. 3	12. 7	27. 3	17. 2
M3	75.1	67. 2	25. 2	16. 9	20. 8	15. 6
M4	72. 5	63. 8	22. 4	14. 7	24. 1	12. 8
M5	65. 2	61. 2	20. 9	18. 9	28. 6	16. 9
M6	64. 6	59. 5	21. 6	17. 2	30. 1	18. 2
Mean ± SE	a 69. 6 1. 69	a 63. 18 1. 38	b 22. 46 0. 64	b 16. 05 0. 88	b 25. 76 1.42	b 15. 88 0. 8

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

After 10 days of injection

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	70. 8	50. 2	32. 6	20. 4	25. 3	19. 6
M2	72. 3	55. 6	29. 9	17. 8	24. 7	17. 8
M3	67. 4	60. 3	23. 6	16. 5	22. 8	13. 6
M4	69. 1	58. 9	25. 1	18. 1	23. 4	15. 1
M5	65. 1	59. 1	25. 4	17. 7	29. 3	20. 1
M6	67. 5	61. 3	26. 2	16. 4	27. 9	17. 8
Mean ± SE	a 68. 7 1. 05	a 57. 56 1. 67	b 27. 13 1. 34	b 17. 81 0. 59	b 25. 56 1. 04	b 17. 33 1. 03

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (3)

The effect of extract on blood lymphocytes blastogenic response (*in vivo*).

After 5 days of injection

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	74. 7	66. 7	25. 6	20. 1	34. 5	30, 6
M2	71. 8	64. 2	28. 1	17. 9	32. 6	25. 3
M3	66. 2	60. 3	23. .5	15. 9	22. 8	13. 6
M4	68. 5	61. 9	25. 1	18. 1	21. 2	11. 9
M5	68.4	63. 7	21. 2	12. 9	26. 5	12. 9
M6	69. 8	60. 8	24. 1	15. 3	27. 2	14. 8
Mean ± SE	a 69. 9 1. 21	a 62. 93 0. 98	b 24. 6 0. 94	b 16. 7 1. 03	b 27. 46 2. 14	b 18. 18 3. 18

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

After 10 days of injection

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	78. 9	57. 5	24. 7	17. 2	25. 8	14. 9
M2	75. 6	60. 8	23. 5	15. 8	26. 3	16. 5
M3	71. 2	65. 8	26. 1	18. 3	29. 2	20. 0
M4	70. 5	63. 1	23. 9	17. 1	31. 4	21. 3
M5	70. 2	63. 8	29. 3	17. 7	34. 2	20. 1
M6	73.5	67. 3	27. 9	16. 2	31. 8	17. 6
Mean ± SE	a 73. 31 1. 39	a 63. 05 1. 41	b 25. 9 0. 94	b 17. 05 0. 37	b 29. 78 1. 34	b 18. 41 1. 01

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (4)

The effect of extract on spleenocyte mitosis (*in vivo*).

After 5 days of injection

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	9. 8	6. 3	3. 39	1. 7	2. 5	0. 6
M2	8. 5	5. 7	3. 8	2. 3	2. 8	0. 7
M3	6. 5	4. 8	2. 8	0. 9	3. 3	1. 3
M4	8, 2	6. 7	3. 4	1. 8	2. 7	0. 8
M5	13. 2	6. 6	2. 8	0. 7	4. 1	2. 1
M6	11. 5	5. 1	2. 6	1. 4	3. 5	1. 7
Mean ± SE	a 9. 61 0. 98	a 5. 86 0. 12	b 3. 13 0.19	b 1. 46 0.41	b 3. 15 0. 24	b 1. 2 0. 24

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

After 10 days of injection

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	13. 9	10. 0	4. 1	2. 2	2. 8	0. 8
M2	12. 6	7. 1	3. 7	1. 9	3. 7	1. 6
M3	10. 6	7. 4	2. 8	0. 8	2. 5	0. 6
M4	8. 4	5. 5	3. 5	1.4	2. 7	0. 8
M5	9. 0	7. 1	4. 2	2. 1	3. 6	1. 8
M6	12. 4	8. 6	4. 6	2. 6	4. 1	2. 3
Mean ± SE	a 11.15 0. 88	a 7. 61 0. 62	b 3. 81 0. 25	b 1. 83 0. 26	b 3. 23 0. 26	b 1.31 0. 27

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (5)

The effect of extract on blood lymphocyte mitosis (*in vivo*).

After 5 days of injection

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	13. 5	11. 2	4. 8	2. 1	3	2. 5
M2	11. 8	8. 9	3. 5	1. 6	2. 2	0. 8
M3	8. 3	6. 5	2. 6	0. 8	2. 6	0. 9
M4	10. 4	8. 1	3.1	1. 2	2. 1	0. 7
M5	15. 7	11. 76	2. 5	1. 1	3. 2	1. 2
M6	13. 2	9. 6	2.1	0. 7	2. 9	0. 9
Mean ± SE	a 12. 15 1. 05	a 9. 34 0. 79	b 3. 1 0. 39	b 1. 25 0. 21	b 2. 66 0. 18	b 1.16 0. 27

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

After 10 days of injection

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	13. 1	10. 1	2. 9	1. 2	4. 1	2. 1
M2	10. 8	7. 5	3. 6	1. 9	3. 8	1. 6
M3	9. 6	6. 4	1. 8	0. 6	2. 7	1. 1
M4	11. 3	7. 2	2. 2	0. 7	3. 1	1. 4
M5	10. 7	6. 2	3. 2	1. 5	2. 3	0. 7
M6	11. 5	8. 4	2. 7	0. 9	1. 8	0. 5
Mean ± SE	a 11. 16 0. 47	a 7. 63 0. 58	b 2. 73 0. 26	b 1.13 0. 2	b 2. 96 0. 35	b 1. 23 0. 24

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (6)

The effect of extract on mice spermatogenesis (*in vivo*).

After 5 days of injection

Mice number	Sample %	Control 1 %	Control 2 %
M1	21. 4	3. 9	5. 3
M2	22. 2	4. 1	4. 8
M3	18. 8	5. 3	3. 6
M4	20. 3	4. 8	4. 5
M5	18. 4	4. 9	6. 3
M6	19. 4	4. 1	5. 5
Mean ± SE	a 20. 08 0. 61	b 4. 51 0. 22	b 5. 0 0. 37

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

After 10 days of injection

Mice number	Sample %	Positive control %	Negative control %
M1	14. 1	6. 1	4. 1
M2	13. 5	4. 8	3. 2
M3	15. 3	4. 6	4. 6
M4	13. 9	5. 7	5. 3
M5	16. 4	5. 2	4. 5
M6	18. 7	6. 4	3. 7
Mean ± SE	a 15. 31 0. 8	b 5. 46 0. 29	b 4. 23 0. 3

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (7)

The effect of extract on phagocytic cells (*in vivo*).

After 5 days of injection

Mice number	Sample %	Control 1 %	Control 2 %
M1	90. 9	32. 9	27. 4
M2	90. 0	30. 2	28. 6
M3	85. 0	26. 4	23. 5
M4	83. 1	23. 9	20. 1
M5	86. 5	20. 7	17. 8
M6	87. 6	23. 3	21. 3
Mean ± SE	a 87. 18 1. 2	b 26. 23 1. 86	b 23.11 1. 72

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

After 10 days of injection

Mice number	Sample %	Control 1 %	Control 2 %
M1	84. 9	18. 7	20. 3
M2	87. 3	20. 1	34. 1
M3	90. 4	25. 2	22. 4
M4	89. 6	23. 8	23. 1
M5	84. 7	19. 8	24. 6
M6	86. 8	21. 3	22. 8
Mean ± SE	a 87. 28 0. 96	b 21. 48 1. 02	b 26. 21 1. 97

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (8)

The effect of extract on spleenocyte blastogenic response (*in vitro*).

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	66. 2	44. 5	24. 0	13. 8	29. 7	15. 7
M2	75.7	59. 7	20. 3	12. 4	21. 5	11. 3
M3	66. 9	54. 8	23. 4	15. 4	24. 5	13. 5
M4	70. 8	60. 3	19. 1	10. 9	20. 8	12. 5
M5	68. 7	58. 5	20. 5	11. 6	22. 3	14. 1
Mean ± SE	a 69. 66 1. 7	a 55. 56 2. 92	b 21. 46 0. 94	b 12. 82 1. 24	b 23. 76 0. 72	b 13.42 0. 74

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (9)

The effect of extract on blood lymphocytes blastogenic response (*in vitro)*.

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	80. 9	55. 9	27. 7	15. 9	25. 7	14. 8
M2	86. 0	63. 6	24. 3	14. 1	26. 0	15. 7
M3	72. 8	54. 8	20. 5	11. 7	24. 5	13. 2
M4	77. 1	58. 8	22. 4	12. 3	23. 4	11. 6
M5	80. 3	60. 2	25. 1	13. 5	21. 9	10. 7
Mean ± SE	a 79. 42 2. 18	a 58. 66 1. 54	b 24. 0 0. 81	b 13. 5 0. 71	b 24. 3 0. 75	b 13. 2 0. 93

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (10)

The effect of extract on spleenocyte mitosis (*in vitro*).

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	7. 1	4. 1	2. 1	1. 3	2. 2	0. 9
M2	10. 3	5. 8	1. 8	0. 5	2. 9	1. 2
M3	7. 7	6. 2	2. 4	1. 7	2. 4	1. 5
M4	9. 4	6. 8	3. 1	1. 2	3. 3	1. 9
M5	11. 1	7. 2	2. 4	0. 7	2. 8	0. 8
Mean ± SE	a 9. 12 0. 75	a 6. 02 0. 53	b 2. 36 0. 21	b 1. 08 0. 21	b 2. 72 0. 19	b 1. 26 0. 2

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (11)

The effect of extract on blood lymphocyte mitosis (*in vitro*).

Mice number	Sample %		Control 1 %		Control 2 %	
	With PHA	*Without PHA*	*With PHA*	*Without PHA*	*With PHA*	*Without PHA*
M1	5. 2	4. 8	3. 0	1. 8	3. 4	1. 8
M2	8. 3	4. 7	1. 7	0. 5	2. 5	0. 8
M3	13. 5	7. 6	1. 5	0. 3	1. 7	0. 4
M4	9. 7	5. 8	2. 6	0. 7	3. 1	1. 6
M5	10. 8	6. 5	2. 8	1. 3	2. 2	0. 6
Mean ± SE	a 9. 5 1. 37	a 5. 88 0. 54	b 2. 32 0. 3	b 0. 92 0. 27	b 2. 58 0. 3	b 1. 04 0. 27

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Appendix (12)

The effect of extract on phagocytic cells (*in vitro*).

Mice number	Sample %	Control 1 %	Control 2 %
M1	86.1	18. 7	27. 3
M2	83. 5	24. 2	20. 8
M3	90. 4	20. 1	25. 6
M4	80. 2	23. 6	17. 3
M5	88. 3	20. 5	18. 5
Mean ± SE	a 85. 7 1. 79	b 21. 42 1. 05	b 21. 9 1. 95

Differences (a and b) are significant (b<0.05) to comparison rows between samples, controls 1 and 2.

Conclusions

1- The extract has immune stimulation on mice immune response in both *in vivo* and *in vitro* study.

2- The extract has no effect on visceral organ.

3- In the cytotoxicity assays, the extract showed an inhibitory effect on tumor cell line growth rate. While no effect was observed on normal cells growth rate.

Contents

ألإهداء

إلى من جعلني زهرة تكبر كل يوم من حنان يروى ببلسم وعبير والداي .

إلى الذي سهر الليل يناطرني وضحى براحته من أجلي زوجي الحبيب .

إلى من كانوا لي قناديل وشموع تنير دربي بالـدعم و المحبة أخوتي

روز

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Supervision Certificate

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

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Recommendations

1- Further purification and identification of the active compounds.

2- Study on the effect of extract on different of tumor cell lines.

3- Carry out cytogenetic studies of *artemisia* active compounds.

الخلاصة

صممت هذه الدراسة لتحديد الفعالية البايولوجية للمستخلص النباتي لنبات الشيح على الاستجابة المناعية للفئران و فعالية النطف وتأثيره على بعض الخلايا الطبيعية والسرطانية وعلى الانسجة الحيوانية. تم أختيار 112 من ذكور الفئران نوع BALB/c لأستخدامها في هذه الدراسة.

تضمنت الدراسة ثلاثة تجارب رئيسية . التجربة الاولى شملت دراسة تأثير المستخلص داخل الجسم الحي ، حيث تم أختيار 92 فأرة. بفحص الاستجابة الانقسامية للخلايا اللمفاوية المأخوذة من الطحال والدم المحيطي لدراسة تأثير المستخلص على الاستجابة الانقسامية لخلايا الطحال وخلايا الدم اللمفاوية ، بينت النتائج زيادة في نسبة تحفيز الخلايا اللمفاوية على الانقسام مقارنة بعينات السيطرة ١ و ٢ ($b<0.05$) .

بينما اظهرت نتائج تجربة البلعمة على وجود زيادة في نسبة فعالية الخلايا البلعمية لألتهام الخلايا البكتيرية مقارنة بعينات السيطرة ١ و ٢ ($b<0.05$) .

أما فيما يخص فحص الوراثة الخلوية فقد تم أستخدام الخلايا اللمفاوية للطحال والدم بالأضافة الى خلايا الخصى لدراسة تأثير المستخلص على قابلية الخلايا للانقسام الخيطي، وقد بينت نتائج هذا الفحص على وجود زيادة في معامل انقسام الخلايا مقارنة بعينات السيطرة١ و ٢ ($b<0.05$) .

أظهرت نتائج الفحوصات النسيجية لعينات الطحال و الكلية و الكبد و الخصى عدم وجود أي تغيير على مستوى الانسجة و كذلك عدم وجود ترسبات للمستخلص النباتي فيها.

تمثلت التجربة الثانية دراسة تأثير المستخلص النباتي خارج الجسم الحي. تم أجراء ثلاثة فحوصات رئيسية. الفحص الاول تضمن دراسة تأثير المستخلص على الاستجابة الانقسامية للخلايا اللمفاوية ، و قد وضحت النتائج وجود زيادة في نسبة معامل الانقسام في كل من الخلايا اللمفاوية للدم والطحال مقارنة بعينات السيطرة١ و ٢ ($b<0.05$) .

اما الفحص الثاني فقد تضمن فحص معامل البلعمة لخلايا الدم وقد أظهرت النتائج هناك زيادة في فعالية الخلايا البلعمة لألتهام الخلايا البكتيرية مقارنة بعينات السيطرة١ و ٢ ($b<0.05$) .

في الفحص الثالث تم دراسة تأثير المستخلص النباتي على قابلية الخلايا للانقسامية الخيطي لخلايا الدم والطحال اللمفاوية، وقد أظهرت النتائج وجود زيادة في نسبة معامل الانقسام الخيطي للخلايا مقارنة بعينات السيطرة ١ و ٢ ($b<0.05$) .

تضمت التجربة الثالثة دراسة سمية المستخلص على الخلايا الطبيعية والسرطانية ، فقد بينت النتائج وجود تثبيط في نسبة نمو الخلايا السرطانية مع فترات الحضن مع المستخلص النباتي (أيام 4 و 24,48,72 hr.) مقارنة بعينات السيطرة ١و٢. بينما أظهرت نتائج التأثير السمي للمستخلص على الخلايا الطبيعية وجود تحفييز في نسبة نمو الخلايا في أخر تركيزين، أما في التراكيز الأخرى لم يؤثر المستخلص على نمو الخلايا مقارنة بعينات السيطرة ١و٢ .

Summary

This study was designed to determine the biological effect of diethyl ether extract of *Artemisia herba-alba on* mice immune response, normal and tumor cell line and visceral organs. A total of 112 male BALB/c mice have been used in this study.

In the *in vivo* study, 92 mice have been used. In lymphocyte transformation assays spleen single cell suspensions have been prepared as well as peripheral blood samples have been taken to examine the effect of extract on lymphocyte transformation. The results were showing a significant increasing in the percentage values of blastogenic response in comparision with control 1 and control 2 ($b<0.05$). While the results of phagocytosis assay showed a significant increasing in the percentage values of phagocytic cells in comparision with control 1 and control 2.

The cytogenetic assay was done by using spleen and blood lymphocytes as well as testis to study the effect of extract on cells mitosis. The results of this test were indicating a significant increasing in mitotic index values when compared with control 1 and control 2 ($b<0.05$).

Spleen, kidney, liver and testis samples have been prepared for histological studies and the results cleared that there was no changes in the level of tissues and no extract precipitation have been observed.

In the *in vitro* study. A total of 20 mice have been used. In this study three tests have been done. The first test on studying the effect of extract on lymphocyte transformation. The results showed a significant increasing in the percentage values of blastogenic response in both blood and spleen lymphocytes compared with control 1 and control 2 ($b<0.05$).

The second test was phagocytosis assays. The results also revealed an increase in the percentage values of phagocytic cells in compared with control 1 and control 2 ($b<0.05$). While the third test was cytogenetic study of the effect of extract on spleen and blood lymphocytes mitosis. The results were showing a significant increasing in the percentage of mitosis index values in compared with control 1 and control 2 ($b<0.05$).

The finale experiment was applied to study the cytotoxic effect of extract on both normal and tumor cell line. The results of the cytotoxic effect of extract on tumor cell line showed growth inhibition of cells in different times of incubation with extract (24, 48, 72 hr. and 4 days) in compared with control 1 and control 2. The results of cytotoxic effect of extract on normal cells pointed a growth stimulation of cells in the last two concentrations (60 and 45 mg/well),

while no effect was detected on cells growth by other concentrations compared with control 1 and control 2.

CHAPTER ONE

INTRODUCTION

CHAPTER TWO

LITERATURE REVIEW

CHAPTER THREE

MATERIALS & METHODS

CHAPTER FOUR

RESULTS

CHAPTER FIVE

DISCUSSION

APPENDIXES

CHAPTER SIX

REFERENCES

List of Abbreviations

Ag	Antigen.
D.D.W	Deionised distilled water.
D.W	Distilled water.
DHA	Dihydroartemisinin.
DMSO	Dimethel sulfoxide.
I.P	Intraparitonialy injection .
PBS	Phosphate buffer solutin.
PHA	Phytohemagglutinine.
T_C	T-cytotoxic lymphocyte.
T_H	T-helper lymphocyte.

جمهورية العراق
وزارة التعليم العالي والبحث العلمي
جامعة النهرين
كلية العلوم

دراسة سمية مستخلص نبات الشيح على خطوط خلايا البلازما السرطانية S.U.99 cell line و على بعض جوانب الاستجابة المناعية للفئران

رسالة مقدمة إلى
كلية العلوم – جامعة النهرين
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