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Research Article

STUDY THE CYTOGENETIC EFFECTS OF AQUEOUS (AE) AND ETHANOLIC (EE) EXTRACTS OF *ARTEMISIA HERBA ALBA* ON PERIPHERAL BLOOD LYMPHOCYTES *IN VITRO*

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ABSTRACT

The present study was carried out to evaluate the cytogentic effect of both aqueous (AE) and ethanolic (EE) extracts of Artemisia herba alba on on several cytogenetic parameters such as mitotic index, blast index, sister chromatid exchange/cell, cell cycle progression and replicative index after culturing of peripheral blood lymphocytes in vitro. The results of in vivo study indicate high effectiveness of AE in reducing the tumor volume in a dose- and time-dependant manner. The best effective dose was 0.5 g/kg when administered intraperitoneally or orally. The comparison of relative tumor volumes of different groups revealed high significant differences between all treated groups and those of untreated (control) groups. Coincidently, the histopathological changes in treated and untreated tumor masses showed that necrosis and fibrosis were the predominant features occurring with the advanced time of treatment proportional to the reduction in tumor volume. In advanced time of treatment, there were only few islands of tumor tissue sequestered by massive mature fibrous tissue. The results of cytogenetic study showed good antiproliferative, antimutagenic effects of AE and EE. The results showed significant decrease of mitosis and blast cells formation in AE and EE-treated groups proportional to the concentration. There was a high significant decrease in the average of sister chromatid exchange/cell particularly after treatment of lymphocytes with high concentrations of both extracts. The results of cell cycle progression and replicative index supported the other cytogenetic results, that indicating the lowering effect of both extracts on both parameters. In conclusion, both extract aqueous (AE) and ethanolic (EE) of Artemisia herba alba showed antimitotic and antimutagenic effect on human peripheral blood lymphocytes in vitro.

Keywords: cytogentic effect, *Artemisia herba alba*, antiproliferative, antimutagenic.

INTRODUCTION

Herbal medicines are culturally accepted and widely used in many countries for treatment of disorders and hence are of great importance as a mechanism to increase access to health care services. However, only few countries have some forms of policy/mechanism on traditional/complementary and alternative medicine (TCAM). Other countries need to develop their policy on TCAM to provide a sound basis in defining the role of TCAM in national health care delivery, ensuring that necessary, regulatory and legal mechanisms are created for promoting and maintaining good practices, that access is equitable, affordable and that authenticity, safety and efficacy of therapies in Herbal medicines are culturally accepted and widely used in many countries for treatment of disorders and hence are of great importance as a mechanism to increase access to health care services. However, only few countries have some forms of policy/mechanism on traditional/complementary and alternative medicine (TCAM). Other countries need to develop their policy on TCAM to provide a sound basis in

defining the role of TCAM in national health care delivery, ensuring that necessary, regulatory and legal mechanisms are created for promoting and maintaining good practices, that access is equitable, affordable and that authenticity, safety and efficacy of therapies are ensured (El-Gendy, 2004).

Cytogenetic analysis

Mitotic index

The proliferating cells pass through a regular cycle of events in which the genetic material is duplicated and divided equally between two daughters. The molecular mechanisms occurring through cell cycle are highly conserved in all eukaryotic organisms (Miller, 2001). Before a normal somatic cell enters mitosis, each chromosome replicates its DNA and in fact becomes doubled. During the DNA replication phase the chromosomes are extremely long, diffusely spread through the nucleus, and cannot be recognized with the light microscope. With the onset of mitosis, the chromosomes begin to coil, contract and condense, and these events mark the beginning of prophase. Throughout prophase, the chromosomes will continue to condense and become shorter and thicker, but only at prometaphase will the chromatids become distinguishable. During metaphase, the chromosomes line up the equatorial plane and their doubled structure is clearly visible. Each is attached by microtubule (mitotic spindle) extending from the kinetochore to the centriole. Soon the kinetochore of each chromosome divides, marking the beginning of anaphase followed by migration of the chromatids to opposite poles of the spindle. Finally, during telophase, chromosomes uncoil and lengthen, the nuclear envelope reforms, and division of cytoplasm occurs. Each daughter cell receives one-half of all the doubled chromosome material and thus maintains the same number of chromosomes as the mother cell (Sadler, 1990).

Sister chromatid exchanges (SCEs) test:

SCEs involve breakage of both DNA strands followed by an exchange of DNA segments by mutagens. The formation of SCEs has been correlated with the induction of point mutation, gene amplification and cytotoxicity (Perry and Evans,1975).

The exact mechanisms of SCE production and its relationship with development of chromosomal abnormalities are unclear (Grossen et al., 1981). Increased frequencies of SCE may be induced by ionizing radiation (Perry and Evans, 1975), Ultraviolet light and a variety of mutagens expressing genetic lesions (Kato, 1973). The mutagenic and carcinogenic agents have the ability to increase the frequency of SCEs especially agents that interact with metabolism and DNA repair system or the drugs that cross link with DNA like Cisplatin (Deen et al.,1989), Mitomycin-C (Evans and Vijayalaxmi,1981), ionized radiation (Shubber and Shaikhly,1985), in addition to a number of flavonoids like quercetin and kaempferol (Wolff et al.,1977) and some plants extracts like somac (Al-Ani and Al-Aswad,1997). During the malignant transformation of cultured cells infected by simian virus (SV40) (Wolff et al.,1977), there was an increase in frequency of SCEs. The analysis of the frequency of SCEs in mammalian cells in vivo and in vitro promoted by the knowledge that SCEs are in some way related to the damage-repair mechanisms of DNA (Perry and Evans,1975). Many halogenated pyrimidine analogues have been assessed for the labeling of DNA including bromodeoxyuridine (BrdU), chlorodeoxyuridine, iododeoxyuridine, bromodeoxycytidine, chlorodeoxycytidine and iododeoxycytidine (Dufrain, 1983; Kubbies et al., 1985). However, BrdU is a large supplement of tritiated thymidine for the purpose of differential labeling of sister chromatid (Allen et al., 1977; Zakharov and Bairamjan, 1980). When incorporated into DNA or chromatids, BrdU can quench the fluorescence of DNA binding dyes such as 33258 Hoechst (Craig-holmes and Shaw,1976), acridine orange (Kato,1974), and 4,6-diminido-2-phenylindol (DAPI) (Lin and Alfi,1976). The use of fluorescent stain, such as DAPI, has the advantages of rapidity and simplicity of the technique and the age of the slide is not crucial to the technique. The advantages of fluorescent plus Giemsa technique are the ability to reduce non-fading, permanently stained slides. Moreover, small exchanges near to tips of the chromosomes are clearly visible, however, this technique is time consuming (Shubber et al., 1991). BrdU has a variety of effects on mammalian cells, including toxicity, suppression of differentiation and mutagenesis. However, it was shown that the major factor in determining in frequency of SCE induced by BrdU is the concentration of BrdU in the medium (Morris, 1991). Furthermore, it was found that the amount of BrdU in

DNA has only a small role in determining the frequency of SCE (Davidson et al., 1980).

The cell cycle progression (CCP):

The BrdU-Giemsa technique is a useful method for identifying with accuracy, the percentage of cells which have gone through one, two or three divisions in vitro and in vivo (Bianchi et al.,1990). this phase all appear bright under the fluorescent microscope. The in vitro CCP was found to be affected by the medium, BrdU levels and colchicine (Shubber and Al-Allak,1986). The nomenclature utilized for the evaluation of CCP pattern according to the number of cell cycle was (Becher et al., 1984):

1. First cell division (M1): this group of cells has no incorporated BrdU or is able to incorporate BrdU during a single phase. The chromosomes of
2. Second cell division (M2): this group contains cells which incorporated BrdU during two 'S' phases and display a typical differential staining of sister chromatids (one dull and one bright).
3. Third cell division (M3): these metaphases incorporated BrdU during three 'S' phases and contain chromosomes with double standard BrdU-substituted DNA in both sister chromatids (both dull), for this reason the study aimed to identify the Cytogenetic effect of aqueous and ethanolic extracts o peripheral blood lymphocytes of human.

MATERIALS AND METHODS

Extraction of test plant

Plant collection

Artemisia herba alba was collected from Al-Najaf province, south of Baghdad in December 2003, and was shed and dried at room temperature. A voucher specimen of the plant was deposited to be identified and authenticated at the National Herbarium of Iraq Botany Directorate in Abu-Ghraib (Certificate no. 3522 in 23/12/2003).

Figure 1: Artemisia herba alba

The dried plant then was separated into roots and aerial parts, then the aerial(leaves and barks) parts were ground into powder by coffee electrical grinder (mesh no.50), and the powdered parts were kept in a plastic bags in deep freeze (-20˚C) until the time of use (Harborne et al., 1975).

Preparation of aqueous and ethanolic extracts of A. herba alba

According to Harborne et al. (1975), aqueous extract of plant was prepared as follows:

1. Aliquots of 50 g of the powdered plant were suspended in 200 ml of distilled water (D.W.) in Erlyn Myer flask and stirred on a magnetic stirrer over night at 45˚C.
2. After 24 hours, the sediments were filtered by gauze and then by filter papers.
3. Steps (1) and (2) were repeated 4-5 times.
4. The pooled extract was evaporated to dryness (45˚C) under reduced pressure in a rotary evaporator.
5. The weight of crude extract resulted from that amount of powdered plant was measured.
6. The crude extract then was kept at -20˚C until the time of use.
7. The ethanolic extract of A. herba alba was prepared in the same manner as that of the aqueous extract except using of 70% ethyl alcohol instead of D.W.

For following experiments, 1 g of powdered plant extract was dissolved into 100 ml PBS (as solvent), the suspension then filtered and sterilized by using 0.4 µm sterile millipore filter and kept in deepfreeze(-20˚C) until use.

Blood collection

Blood was taken from normal adult of human by puncturing, using disposable syringe. 5 ml of blood was transferred into heparinized tubes.

Procedure

Blood culture with plant extracts

1) Add 0.5 ml of peripheral blood into all test tubes containing (5 ml) of culture medium (either medium I or II).

2) Added (0.3 ml) of PHA into all test tubes, mixed the components very well and transfer to CO2 incubator at 37˚C.

3) After 24 hrs. of incubation, added to each test tube 1 ml of different concentrations (156.25,312.5,650,1250) µg/ml prepared from aqueous and ethanolic extracts of A. herba

alba (Three replicates for each concentration. Total=24 test tubes). Also added 1 ml PBS for other three test tubes and these considered as negative control (Shubber et al.,1991). For other three test tubes, added 1 ml of 50 µg/ml of Cyclophosphamide (CP) and these considered as a positive control.

4) Put back all the test tubes to the CO2 incubator, and gently shake each 12 hrs. One tries at least.

Harvesting

1) After (45hrs) of incubation, 0.7 µg/ml of colchicine added for 3 hrs.

2) Centrifugation of samples for 10 min. at 1500 rpm.

3) Withdraw the supernatant by Pasteur pipette and the precipitated cells with a little culture medium left in the test tube.

4) Mixing of precipitate very well by the vortex mixer, then 5-10 ml of warmed (37˚C) potassium chloride (0.075M) gradually and gently added with mixing.

5) Incubation of the samples in shaker water bath for 30 min. at 37˚C.

6) Centrifugation of the samples for 10 min. at 1500 rpm, then the supernatant discarded.

7) Few drops of the freshly made fixative (methanol and glacial acetic acid 3:1 V/V) added drop wise with gently mixing till reaching 5 ml. Later on centrifugation performed for 10 min. at 1500 rpm then fixative decanted off and the process repeated for 2-3 times. At the final change, the cells re-suspended in a 3 ml of freshly made fixative and stored at - 20˚C.

Slide preparation:

The procedure which was followed according to ICCMGR. The cell suspension removed from freezer and centrifuged at 1500 rpm for 10 min.

The supernatant decanted off and the cells resuspended in appropriate amount to make thinly cloudy suspension. By using Pasteur pipette, 3-4 drops of cells suspension dropped evenly from appropriate distance (30 cm) onto wet, chilled, grease-free slides and allowed drying at room temperature.

Staining

a)The slides stained using freshly made (Giemsa stain (stock solution) and Sorenson's buffer 1:4 V/V) which was applied for 2 min., then rapidly washed with Sorenson's buffer, after that left to dry at room temperature.

Microscopic examination under low magnification using 10X objective lens was performed to determine mitotic index (MI %) and blast index (BI %).

MI % analysis: The MI % was determined as a ratio of the mitotic cells to the cells in interphase in 1000 calculated cells.

M.I. % = (No. of dividing cells / No. of dividing cells + No. of non-dividing cells) X 100. (Shubber and Al-Allak, 1988).

BI % analysis: The BI % was determined as a ratio of the cells in blast form to the other cells in 1000 calculated cells.

b) In case of G-banding stain, after slide preparation, trypsin added to the slide for 30 seconds, then rapidly washed with Sorenson's buffer, Giemsa stain then applied for 1.45 min., also rapidly washed with Sorenson's buffer. After that left to dry at room temperature then microscopically examined fewer than 100X oil immersion objective lens.

c) The slides from the samples cultured in (Medium I) were, stained for 10 min. using DAPI solution then washed with D.W. and try at room temperature and mounted with few drops of an alkaline phosphate buffer (pH=11.0). The slides then examined fewer than 100X oil immersion objective lens using Olympus BH2 standard microscope equipped with a fluorescent epi illumination system.

This process aimed to score sister chromatid exchange per cell (SCE/cell), cell cycle progression (CCP), replicative index (RI %).

Sister chromatid exchange (SCE) analysis:

The SCE was scored in 50 well spread second metaphases containing 23 pairs of chromosomes presented as mean of SCE/cell±Standard error (S.E).

Cell cycle progression (CCP) analysis:

The CCP analyzed in a 100 consecutive metaphase cells presented as percentage of M1, M2, M3 cells. The replicative index (RI %) measured by application of the following equation:

RI %= M1% X 1 + M2% X 2 + M3% X 3 / 100 (Schneider and Lewis, 1982).

Statistical analysis

Analysis of variance (ANOVA) and the least significant difference (LSD) were used for the statistical analysis of the results and P-values at levels ($P<0.05$-$P<0.0001$) were considered to be statistically significant. These calculations

were carried out according to program SPSS, version 10 (Susan et al., 1997). All experiments were performed in the Iraqi center for cancer and medical genetic researches.

RESULTS

Cytogenetic study on lymphocytes of human circulating blood:

Mitotic index (MI %):

The results showed a similar effect of AE and EE on mitotic incidence (Table1). The mitotic index of control (-ve) group was (5.22±0.26) for AE and (6.05±0.42) for EE. The mitotic index values of treated group revealed high significant decrease in a concentration dependant manner (3.89±0.79, 2.03±0.20, 1.13±0.21 & 0.73±0.15) for AE, and (3.38±0.42, 1.33±0.48, 0.81±0.19 & 0.47±0.03) for EE. The exposure of lymphocytes to known and potent antimitotic agent (CP) in control (+ve) group resulted in sharp decrease in mitotic index (0.60±0.04 & 0.46±0.02). The results revealed that the highest concentrations (1250, 625 µg/ml) of AE and (1250, 625, 312.5 µg/ml) of EE caused antimitotic effect similar to that of control (+ve) group.

Blast index (BI%):

Similar results obtained with blast cells formation (Table2). The control (-ve) groups showed high percentages of blast cells (26.25 and 30.24), while the CP-treated groups revealed high significant decrease in blast formation (3.91 and 2.49). The AE treated groups as well as those treated with EE showed high significant decrease in blast formation in a concentration dependant manner. The comparison with control (-ve) groups showed high significant decreasing effect proportional to AE and EE concentrations (20.06±2.28, 9.39±0.58, 6.17±0.7 & 3.9±0.11) for AE and 19.93±0.95, 6.21±2.19, 3.85±0.75 & 2.33±0.12) for EE. These values except those of 156.25 µg/ml of both extracts revealed antiblastic cells formation effect almost like the control (+ve) groups.

Sister chromatid exchange (SCE):

The incidence and distribution of SCE in control groups mentioned in table (3), which revealed a variation in number of SCE in scored cells. There was increase in number of cells undergoing high number of SCE as well as average of SCEs

Table 1: Mean values of mitotic index (M.I.%) of circulating normal lymphocytes after treatment with aqueous and ethanolic extracts of *Artemisia herba alba.*

Concentration (µg/ml)	Aqueous extract (Mean±SE)	Ethanolic extract (Mean±SE)
Control (-ve)	5.22±0.2696 **	6.05±0.4248 **
156.25	3.89±0.7968* **	3.38±0.5914* **
312.50	2.03±0.2050* **	1.33±0.4875*
625.00	1.13±0.2194*	0.81±0.1922*
1250.0	0.73±0.1528*	0.47±0.0393*
Control (+ve)	0.60±0.0404*	0.46±0.0240*

*The mean difference is significant in comparison with control (-ve) group at levels (P<0.005-P<0.0001)

**The mean difference is significant in comparison with control (+ve) group at levels (P<0.001-p<0.0001)

Table 2: Mean values of blast index (B.I.%) of circulating normal Lymphocytes after treatment with aqueous and ethanolic extracts of *Artemisia herba alba*

Concentration (µg/ml)	Aqueous extract (Mean±SE)	Ethanolic extract (Mean±SE)
Control (-ve)	26.65±1.7622 **	30.24±2.5320 **
156.25	20.06±2.2834* **	19.93±0.9518* **
312.50	09.39±0.5848* **	06.21±2.1915*
625.00	06.17±0.7069*	03.85±0.7578*
1250.0	03.90±0.5703*	02.33±0.1202*
Control (+ve)	03.91±0.1170*	02.49±0.1968*

*The mean difference is significant in comparison with control (-ve) group at levels (P<0.005-P<0.0001)

**The mean difference is significant in comparison with control (+ve) group at levels (P<0.01-P<0.000

Table 3: Distribution of sister chromatid exchange/cell in normal lymphocytes treated with phosphate buffer saline (control –ve) and 50 µg/ml of Cyclophosphamide

	Cell no. With 0-5 SCE	Cell no. With 6-10 SCE	Cell no. With 11-15 SCE	Cell no. With 16-20 SCE	Total cell count	Total SCEs	Average Of SCEs/cell
Control (-ve)	38	12	0	0	50	192	3.84
	40	10	0	0	50	198	3.96
	37	13	0	0	50	203	4.06
Control (+ve)	23	15	11	1	50	616	12.32
	22	14	12	2	50	712	14.24
	21	16	10	3	50	674	12.24

Table 4: Mean values of sister chromatid exchange per cell (SCE/cell) in circulating normal lymphocytes after treatment with aqueous and ethanolic extracts of *Artemisia herba alba*

Concentration (µg/ml)	Aqueous extract (Mean±SE)	Ethanolic extract (Mean±SE)
Control (-ve)	03.95±0.2282 **	03.95±0.2282 **
156.25	05.25±0.3180* **	04.28±0.1906 **
312.50	04.02±0.0755 **	03.82±0.0461 **
625.00	03.61±0.3174 **	03.10±0.2173* **
1250.0	02.99±0.0940* **	02.14±0.2615* **
Control (+ve)	12.93±0.6539*	12.93±0.6539*

*The mean difference is significant in comparison with control (-ve) group at levels (P<0.005-P<0.0001)

**The mean difference is significant in comparison with control (+ve) group at level (P<0.0001)

Table 5: Mean values of (M1, M2, M3 and RI)% in circulating normal lymphocytes after treatment with aqueous extract of *Artemisia herba alba*

Concentration (µg/ml)	**M1** (Mean±SE)	**M2** (Mean±SE)	**M3** (Mean±SE)	**RI** (Mean±SE)
Control(-ve)	** 33.1333±0.6363	** 42.0667±0.6360	** 24.8009±0.7024	** 1.9133±0.0133
156.25	* ** 36.4000±0.3786	** 40.7667±0.2186	* ** 22.8333±0.2772	* ** 1.8600±0.0057
312.5	* ** 43.4000±0.3512	* ** 36.8333±0.3480	* ** 19.7767±0.3055	* ** 1.7700±0.0115
625	* ** 45. 6333±0.712	* ** 37.0667±0.7839	* ** 17.3000±0.2517	* ** 1.7133±0.0088
1250	* ** 50.4333±0.2963	* ** 36.2333±0.7881	* ** 13.3333±1.0667	* ** 1.6267±0.0133
Control(+ve)	* 59.1000±0.2646	* 30.2000±0.0577	* 10.7000±0.0517	* 1.5100±0.0057

*The mean difference is significant in comparison with control (-ve) group at levels (P<0.05-P<0.0001)

**The mean difference is significant in comparison with control (+ve) group at levels (P<0.01-P<0.0001)

Table 6: Mean values of (M1, M2, M3 and RI)% in circulating normal lymphocytes after treatment with ethanolic extract of *Artemisia herba alba*.

Concentration (µg/ml)	M1 (Mean±SE)	M2 (Mean±SE)	M3 (Mean±SE)	RI (Mean±SE)
Control(-ve)	** 33.1333±0.6360	** 42.0667±0.3712	** 24.8000±0.7024	** 1.9133±0.0133
156.25	* ** 40.0333±2.1365	* ** 37.6667±1.4170	* ** 22.3000±1.100	* ** 1.8167±0.0290
312.5	* ** 45.7000±0. 6658	* ** 36.9667±0.7424	* ** 17.3333±0.2728	* ** 1.7100±0.0057
625	* ** 50.4000±0. 6658	* ** 37.1667±0.9333	* 12.5000±1.2423	* ** 1.6167±0.0185
1250	* ** 51.8333±0.3756	* ** 38.000±0.2000	* 10.1667±0.4933	* ** 1.5767±0.0088
Control(+ve)	* 59.1000±0.2646	* 30.2000±0.0577	* 10.7000±0.2517	* 1.5100±0.0057

*The mean difference is significant in comparison with control (-ve) group at levels (P<0.05-P<0.0001)

**The mean difference is significant in comparison with control (+ve) group at levels (P<0.05-P<0.0001)

per cell in control (+ve) group (Mean value=12.93), while the control (-ve) group showed more than 38 cells out of 50 had 0-5 SCE and fewer number with 6-10 SCE but there was no cells with larger number of SCE. The average of SCEs/cell was 3.95.

The highest concentrations of AE (1250 µg/ml) and (1250 and 625 µg/ml) of EE showed significant decrease of SCEs / cell while the lowest concentrations showed no significant effect. All groups treated with AE and EE showed high significant decreasing effect (P< 0.0001) for the incidence of SCEs / cell in comparison to control (+ve) group (12.93 Vs 2.99, 3.1, 4.02 & 5.25 for AE and 2.14, 3.10, 3.82 & 4.28 for EE) (Table4).

Cell Cycle Progression (CCP):

Coincidentally with the above parameters, the results of cell cycle progression were supporting them. Tables (5) and (6) revealed the high significant variation in cell cycle groups. In addition, the results of treated groups pointed to the significant increase of M1 phase (up to 50.45 for AE and 51.83 for EE) and decrease of M3 phase (drop to 13.33 for AE and 10.16 for EE) in comparison with control (-ve) group (M1=33.13, M3=24.80). The comparison of CCP values of the control (+ve) group (M1=59.10, M2=30.20, M3=10.70) with those of treated groups showed significant differences in M1, M2 and M3 values in all concentrations of AE and EE. This was reflected on replicative indices of treated groups which showed significant differences at wide range of probability (P<0.05-P<0.0001) with both control groups.

G-banding preparation:

After slide preparation, the examination revealed no morphological or numerical changes in chromosomes after treatment by all concentrations of aqueous and ethanolic extracts of the plant (figure2).

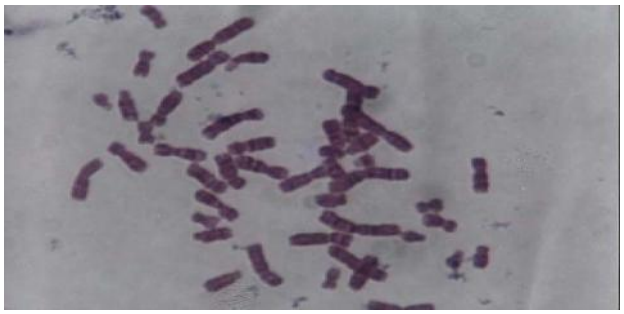

Figure 2: The chromosomes of lymphocytes showed no structural and numerical changes after exposure to 1250 µg/ml of ethanolic extract of *Artemisia herba alba* (1000X, Giemsa stain)

DISCUSSION:

The in vitro experiments have been shown adequate as a routine cytotoxic research of different compounds used in the treatment of human disease. For this reason we analyzed some genotoxic effects of the medicinal plants extracts in human lymphocytes culture. The most sensitive tests for the effect of potentially mutagenic and carcinogenic agent are the quantifying of cytogenetic parameters including mitotic index (MI%), blast index (BI%), sister chromatid exchange (SCE), replicative index (RI%) and cell cycle progression (CCP). (Shubber et al.,1998). The results revealed that AE and EE played significant roles in decreasing the number of cells in mitotic phase (antiproliferative effect), decreasing the average of SCE/cell that indicate the antimutagenic activity as well as decrease the replicative index that indicate the inhibitory action of cell cycle progression. The used concentrations of AE and EE also revealed no morphological or numerical changes in mitotic chromosomes. Our results were consistent with that of AlKhayat (1999), who studied the effect of Artemisia herba alba extract on the same parameters when it administered before, with and after mitomycin C to the normal lymphocytes. Also our results were agreed the results that obtained by Hassan (2002) and Al-Qadoori (2004). They found a decreasing effect of MI%, RI% and CCP in cultured lymphocytes treated with some of the plant extracts and methotrexate. Our findings agreed the results of Krishna et al. (1986), they observed that CP caused dose-related SCEs both in vivo and in vivo/in vitro. In the in vivo group, both bone marrow and spleen cells showed approximately a five-fold increase in SCEs over controls following 40 mg cyclophosphamide / kg. Treatment the same dose, under in vivo/in vitro conditions, caused about three- and six-fold increases in SCEs over controls in bone marrow and spleen primary cells, respectively. Similar results have been reported in Chinese hamster cells. A large increase of SCE was found after in vitro treatment with alkylating agents such as CP, Actinomycin D, Methotrexate. It was possible that agents exerting their cytostatic action by metabolic inhibition of DNA synthesis or after the S phase have little or no capacity for SCE induction, while agents with a maximum effect during G0-G1 phase or G1-S transition are more potent SCE inducers (Littlefield et al., 1979). The capacity of AE and EE to reduce the MI could be interpreted

that the extracts contain β-Sitosterol, compound that replace the cholesterol in cellular membranes leading to mitotic impairment (Hayatsu et al., 1988). For the same reason we can understand the reduction in RI% and CCP. The sister chromatid exchange may represent a form of normal DNA repair and has been used frequently to indirect assess the DNA damaging potential of various physical and chemical treatments (Latt et al.,1984). The chemical mutagens known to cause DNA damage in any way are divided into direct mutagens that exert their effects without any chemical modification, and indirect mutagens that need chemical modification to be active (Wolff et al.,1977). Porter and Singh (1988) were mentioned that rats treated with increasing doses of CP yielded serum which had increasing mutagenic and toxic effects on mouse embryos during 48 hrs in vitro cultures. The SCEs/cell ranged from 17.6±0.31 for serum from control (0 mg/kg CP) rats to 40.4±0.22 for serum from rats given 250 mg/kg CP. In cultured lymphocytes, CP has been shown to produce gene mutations, chromosome aberrations, micronuclei and sister chromatid exchanges in the presence of the metabolic activation (Anderson et al., 1995). This indicates that the cultured lymphocytes possess the metabolic capacity to convert this indirect-acting mutagen (Waalkens et al., 1981). The ability of AE and EE of A. herba alba for significant decrease of SCE frequency results in classification of this plant primarily as bio-antimutagen due its contents of tannins and β-Sitosterol (Gebhart,1992; Wong et al.,1992), and secondarily as desmutagen, probably due to its contents of coumarins and flavonoids (Cai et al.,1993; Edenharder et al.,1997). The glutathione-S-transferase group of enzymes play a major role in the detoxification pathway and help in conversion of reactive chemicals to non reactive polar compounds which can excreted from the body (Cheng et al., 1998). Antioxidants provide protection to living organisms from damage caused by uncontrolled production of free radicals/reactive oxygen species (ROS) and concomitant lipid peroxidation, protein denaturation and DNA-strand breaking. Antioxidants exert their effect by donation of electron to instable oxygen species generated from endogenous processes or formed as a result of radiations or chemical exposure. The oxidative attack on chemical bonds at other points in the cell including DNA helices, may reduce the incidence of mutations arising from oxidative-induced mutations (Yadav et al.,2003). A major advantage of the antioxidants is that they are generally effective against a wide range of mutagens, both exogenous and endogenous (Kohlmeier, 1995).

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