Cytogenetic analysis of human lymphocytes exposed to various concentrations of aqueous and methanolic extracts of *Brassica oleracea var. italic* (Broccoli)

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**ABSTRACT**

The chromosomal aberration, Mitotic index (MI) were employed to investigate the *in vitro* effect of broccoli (*Brassica oleracea*) on human chromosomes. *In vitro* incubation for 48 hrs of normal human lymphocytes were studied with various concentrations (6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/ml) of Aqueous and Methanolic extract of broccoli. Results indicated a positive relationship between Mitotic index (MI) values and the methanolic extract at concentrations (0.19±0.012, 0.29±0.021, 0.26±0.003, 0.3±0.006, 0.32±0.011 and 0.34±0.008) respectively, and (0.12±0.006, 0.15±0.002, 0.19±0.011, 0.21±0.015, 0.22±0.021, 0.30±0.003) respectively, for aqueous extract, a significant differences noted (P≥0.01) among effects of different concentrations. MI value of positive control treatment cyclophosphamide (CP) (0.13±0.003) as compared with a negative control group (0.40±0.018). In addition the value of the damaged cells in negative control group was (0.06±0.01), whereas for positive control group was (43.65±0.003). The damaged cells for the treated group with extract revealed increasing in value in a concentration dependent manner from (4.99±0.08) to (26.53±0.004) and (5.75±0.08) to (23.43±0.023) for both aqueous and methanolic crude extract respectively as compared with the negative control (0.06±0.01). This effect maybe attributed to anti oxidative activity induced by *Brassica oleracea*.

**Keywords:**

*Brassica oleracea*

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**1. INTRODUCTION**

In last recent years, researches on plants has been increased all over the world (Nsimba *et al.* 2008). Many secondary metabolites of plant are commercially important and used in a number of pharmaceutical compounds (Joy *et al.* 1998). The use of plants for treating diseases is as old as the human species (Silva and Fernandes, 2010).

Broccoli (*Brassica oleracea L. var. italic*) belongs to *Brassica oleracea* species together with other commonly grown Brassica vegetables. Broccoli has great potential to prevent several diseases, such as cancer (Keck and Finley, 2004; Hartikainen, 2005) and cardiovascular disease (Mukherje *et al.*, 2008), and the intake of this vegetable results in an improvement of the general health status, mainly due to its antioxidant (Borowski *et al.*, 2008) and anticarcinogenic properties (Jefery
and Araya, 2009). The beneficial effects of broccoli is by substantial quantities of bioactive compounds, such as vitamin C, β-carotene, phenolic compounds, and glucosinolates (Heimler et al., 2006; Jagdish et al., 2006), which are good free radical scavengers (Eberhardt et al., 2005).

Polyphenols act as potent antioxidants as they protect the cells against oxidative damage. They exhibit free radical scavenging and metal chelating activities. Therefore, the intake of these compounds may result in reduction of the risk to develop various degenerative diseases triggered by oxidative stress (D’Archivio et al., 2003).

The cellular macromolecules of humans, such as DNA, proteins and lipids, are continuously at risk for endogenous and environmentally induced structural alterations. (Lee and Kohn, 2009). According to rather controversial information, about 5% of environmental chemicals are characterized by potential mutagenic activity. At the same time, data on mutagenic activity of overall pollutants of air, water, and foodstuffs are being accumulated (Bach et al., 1973; Knudsen et al., 1999).

Chromosomal aberrations (CA) are one of the important biological consequences of human exposure to ionizing radiation and other genotoxic agents. In epidemiological studies, it has been shown that people with elevated frequencies of CA in their peripheral blood lymphocytes have a significantly elevated risk of developing cancer (Obe et al., 2002)

The aim of this work was to study the Cytogenetic effect of aqueous and methanolic crude extracts of Brassica oleracea var. Italic on human blood lymphocyte by evaluation MI, damaged cells and chromosomal aberrations.

2. MATERIALS AND METHODS

2.1. Plant sampling:

*Brassica oleracea* was obtained from the wet market in Erbil, identified by herbarium in College of Agriculture, Salahaddin University, Erbil, Iraq. Then dried at room temperature according to Harbon (1984), and ground in to powder by electrical grinder (mesh No. 0.5mm), then extracted and isolated using aqueous and methanol as an extracting solvent in research center laboratories of Erbil Polytechnic University.

2.2. Broccoli extracts preparation:

Repeated extraction with water and methanol recommended by Harborne, was applied as described by (Al-Atby, 2001), fifty grams of the broccoli powder was placed in a flask separately, with 300 ml of deionized distilled water (D.D.W) and absolute methanol then each flask was tightly sealed. The mixture was left to stir on a magnetic stirrer for 3 days at room temperature. Then filtered through gauze and Whatman no.1 filters paper. Filtrate extract was dried in an electric oven at 37°C. Dried extract was scrubbed off the petri dishes, and stored in tightly sealed plastic test tube at -20°C (Harbon, 1984).

2.3. Cytogenetic study on human lymphocyte:

1. Blood collection: The blood samples were taken from apparently normal human adult by venous puncturing, using disposable syringe. Five ml of blood was transferred into heparinized tubes.

2. Procedure

   Blood samples (0.5 ml) was added to 4.5 mL RPMI 1640 medium supplemented with 10% fetal bovine serum, 0.3ml Phytohaemagglutinin (PHA), and then incubated at 37 °C in
CO2-incubator with shaking test tubes gently each 15 minute.

After 24 hrs, cultures were treated with 1ml of each effective concentration of both aqueous and methanolic extracts (6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/ml) (three replicates for each concentration) of *Brassica oleracea var. Italic*, 1ml of PBS as a negative control, and 1 ml of 50 µg/ml of cyclophosphamide (CP), as a positive control. Lymphocytes were harvested after 52 hrs incubation by adding 0.1 g/ml colchicine (to arrest the cells at metaphase in mitosis) and incubation at 37ºC for 1hr, then centrifuging cell suspension to remove culture medium (1000 rpm), addition of hypotonic solution (KCl 0.075 M) at 37 °C for 20 min to swell the cells, and treated twice with Carnoy’s fixative (3:1 ratio of methanol: acetic acid) (Shubber and Juma, 1999).

3. Slide preparation:

The slide was prepared according to the procedure applied by Iraqi center for cancer and medical genetic research (ICCMGR) by which the cell suspension removed from freezer or used immediately. The suspension was mixed very well by Pasteure pipette; 3-4 drops of cells suspension dropped evenly from appropriate distance (30-50 cm) onto wet, chilled, oil-free slides and allowed drying at room temperature.

4. Staining:

The slides were stained using freshly made Giemsa stain (stock solution) and rapidly washed with warmed Sorenson's buffer, after that left to dry at room temperature.

5. Microscopic examination

Microscopic examination was performed to determine the Chromosomal Aberration (CA) analysis, 100 well spread complete metaphase cells in first cell cycle were evaluated per subject under a microscope at 100× magnification to identify numerical and structural CA. Chromosome-type CAs: (break; gap; ring, dicentric) were observed. The MI was determined by scoring at least 1000 cells, and then MI was calculated by the following formula:

Mitotic Index (%) = Number of the dividing cells X 100 / The total number of cells (Shubber and Juma, 1999).

3. RESULTS AND DISCUSSION

The most sensitive tests for the effect of carcinogenic and mutagenic agent are the quantifying of cytogenetic parameters including MI (%MI) (Shubber *et al.*, 1998).

The cytogenetic effects for six concentrations (6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/ml) of *B. oleracea* aqueous and methanolic extract were studied on lymphocytes of peripheral blood in vitro after 48hrs incubation. The cell division induced by mitogen (PHA in comparison with CP 50µg/ml as positive control. (Fig. 1).
The result of the present study revealed that there was significant difference in MI after 48hr treatment from (0.19, 0.12 to 0.34, 0.30) for both aqueous and methanolic extracts respectively. The exposure of lymphocytes to known and potent antimitotic agent (CP) resulted in sharp decrease in MI (0.13±0.003) as compared with a negative control group (0.401±0.018), because CP is an alkylating agent that adds an alkyl group (CnH2n+1) to DNA. It attaches the alkyl group to the guanine base of DNA, phosphoramidate mustard forms DNA crosslinks both between and within DNA strands at guanine N-7 positions (known as interstrand and intrastrand cross linkages, respectively). This is irreversible and leads to cell death (Takimoto and Calvo, 2008). Krishna et al., (1986), observed in their study that CP cause dose related chromosomal aberrations and sister chromatid exchange (SCE).

Figure (2), shows damaged cells (cell containing one or more chromosome aberration) among treatments. The value of the damaged cells in negative control group was (0.06±0.01), whereas for positive control group was (43.65±0.003). The damaged cells of the treated group with extract revealed increasing in value in a concentration dependent manner from (4.99±0.08) to (26.53±0.004) and (5.75±0.08) to (23.43±0.023) for both aqueous and methanolic crude extract respectively, and the percentage of damaged cells that induced after application the plant extract, was less than positive control group which received cyclophosphamide. This observation indicates that B. oleracea extract had an inhibitory effect on cellular proliferation but without increase of chromosomal aberration.

Also the M.I. of the treated groups decreased in a concentration dependent manner. All concentrations (6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/ml) of B. oleracea of both extract have anti mitotic effects as compared with the positive control. (Fig. 3)
The main isothiocyanate in broccoli is sulforaphane, which can up regulate the expression and activity of thioredoxin reductase (TR) in humans as well as thioredoxin thus playing a crucial role in the regulation of the redox state in cells (Bacon et al., 2007).

Yanyan et al., (2010) in their researches about sulforaphane, a dietary component of Broccoli, found that it inhibits Breast Cancer Stem Cells. Munters et al., (2010), was study the effects of broccoli sprouts intake on oxidative stress. Brocoli sprouts have been reported to inhibit skin and urinary bladder carcinogenesis in vivo (Dinkova- Kostova et al., 2007), and also inhibit the proliferation of human bladder and prostate cancer cells in vitro (Tang et al., 2006). The anticancer properties of broccoli sprouts occur through their primary active micronutrient, sulforaphane, by the induction of mitochondria-mediated apoptosis (Tang et al. 2007). Melchini et al., (2013) showed in his study that erucin which is a dietary component of broccoli, is considered to be a major cancer chemopreventive phytochemical, and showed a lower potency in inhibiting the proliferation of prostate adenocarcinoma cells (PC3).

The chromosomal aberrations that include dicentric chromosome, chromosomal gap, chromosomal break and ring chromosome were found in all the positive control and in other different concentrations of extract (Fig. 3) (table 1 & 2).

The number of chromosomal aberrations (damaged cells) of treated groups with extracts decreased in comparison with the positive control. The chromosomal aberrations as chromosomal gap, break and ring chromosome where observed in all concentration but there values were lower in comparison with the positive control, table (1 & 2).

Table (1): Mean±SE for types of chromosomal aberrations among human lymphocyte cells after 48hrs treatment with B. oleracea aqueous extract:
| Treatment                  | Conc. mg/ml | Metaphase examined | Di Centric | Ring     | Gap     | Break    |
|---------------------------|------------|--------------------|------------|----------|---------|----------|
| -ve Control (PBS)         | 0          | 100                | 1.33±0.41  | -        | 1.23±0.33 | 6.66±0.33 |
| +ve control (CP)          | 50         | 100                | 2±1.66     | 4.33±0.33| 21.66±0.33| 15.66±0.66|
|                           | 6.4        | 100                | 2.1±1.33   | 3±0.33   | 8±0.57  | 10.33±0.66|
|                           | 3.2        | 100                | 3.33±0.33  | -        | 8.11±0.66| 10.23±0.33|
| Brassica oleracea var. italica (Aqueos) | 1.6 | 100                | 1.11±0.67  | 2.12±0.66| 6.66±0.33| 4.66±0.66 |
|                           | 0.8        | 100                | 2.33±0.6   | -        | 5.33±0.88| 5.33±1.33 |
|                           | 0.4        | 100                | 1.66±0.23  | 0.33±0.63| 3±0.55  | 3.33±0.33 |
|                           | 0.2        | 100                | 0.66±0.67  | -        | 1.33±0.33| 3.76±0.33 |
| LSD                       | LSD        |                    | 2.57       | 0.79     | 1.8     | 2.93     |

SE=standard error

Table (2): Mean±SE for types of chromosomal aberrations among human lymphocyte cells after 48hrs treatment with *B. oleracea* methanolic extract:

Antioxidant provide protection to living organism from damage caused by uncontrolled production of free radicals, reactive oxygen.
species (ROS) and concomitant lipid peroxidation, protein denaturation and DNA-strand breaking (Yadav et al., 2003). A major advantage of antioxidants is that they are generally effective against a wide range of mutagens, both exogenous and endogenous (Kohlmeier et al., 1995).

4. CONCLUSIONS

Phytochemicals that are present in broccoli have great cytogenetic effect to reduce the risk of chemotherapies on human lymphocytes.

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