International Journal of Technical Research and Applications e-ISSN: 2320-8163,
www.ijtra.com Volume 2, Issue 1 (jan-feb 2014), PP. 13-16

STUDY OF CYTOGENETIC EFFECTS OF CRUDE EXTRACT OF PORTULACA OLERACEA L. ON PERIPHERAL BLOOD LYMPHOCYTE OF HUMAN IN VITRO

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***Abstract*— This study was designed to evaluate cytotoxic of 70% ethanolic crude extract of Portulaca oleracea L on normal human lymphocytes (in vitro) In vitro two, parameters were conducted; mitotic index (MI) and blast index (BI) .The cytotoxic effect of plant extract of Portulaca oleracea at (6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/ml) on normal human peripheral lymphocytes in vitro, for 72 hrs was studied. Results indicated a positive relationship between Mitotic index (MI) values and concentration of ethanolic plant extract (0.467±0.03, 0.467±0.09, 0.300±0.06, 0.267±0.07, 0.300±0.07 and 0.267±0.07) respectively, no significant differences noted (P≥0.01) among effects of different concentrations. Mitotic index value of positive control treatment (PHA) (0.700±0.12) differed significantly (P≤0.01) with all concentrations. Blast index (BI) values following treatment with different concentration of ethanolic extract of Portulaca oleracea (6.4, 3.2, 1.6, 0.8, 0.4 and 0.2 mg/ ml) were (4.03±0.37, 3.70±0.17, 3.30±0.61, 3.13±0.24 and 2.77±0.20), without significant differences (P≥0.01).Following treatment with the highest concentration of plant extract (6.4 mg/ml), the BI value was (5.37±0.30) significantly higher (P≤0.01) than those following treatment with other concentrations. Positive control treatment showed highly significant elevation (P≤0.01) in BI (7.67±0.56) compared with all treatments.**

***key words* — Purslane cytogenetic effect human lympho cytes.**

I.INTRODUCTION

A. Chemical compounds of Portulaca oleracea

Purslane appears to be the only higher plant reported to contain the beneficial omega-3 fatty acids (O3FAs)-eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Purslane also contains carbohydrates, lipids, glycosides, alkaloids, sterols, triterpenes, and flavonoids (1). Phenolic constituents of the plant include scopoletin, bergapten, isopimpinellin, lonchocarpic acid, robustin, genistein, and others (2). Purslane is a rich source of vitamins A, B, C, and E (3). Purslane is high in carotenoid content, including beta-carotene (4) , (5). Purslane contains large amount of norepinephrine (noradrenalin), dopa, dopamine, citric acid, waxes, tannic acid, alkaloid, flavinoid, coumarone, saponines, cardiac glycoside, amino acid, anthraquin, oxilic acid (6, 7) determined levels of endogenous antioxidants (alpha-tocopherol, ascorbic acid, beta-carotene and glutathione) in plant leaves. In addition, it was (8) showed that portulaca oleracea have antioxidant and free radical scavenging activities. Portulaca oleracea L. (PO) It is an edible plant and is usually cut into small pieces and eaten with salt (9). In the United Arab Emirates and Oman, a cultivated variety of PO is used as a vegetable (10). Portulaca oleracea L, has many folkloric uses, It is used in the Arabian peninsula as antiseptic, anti-scorbutic, antispasmodic (10). In China, it is used as an anti-bacterial and anti-viral agent and for the treatment of viral hepatitis and in diabetes management (11). Portulaca oleracea L showed a tumoricidal activity against KATO III (human gastric carcinoma cell (line) and COLO 320 HSR cells (human colon adenoma cell line) in vivo and in vitro (12, 13) Purslane acts as analgesic, antiarthritic, antiartheriosclerotic and anticancer (breast, colon, forestomach, liver, skin) activities. (14) mentioned that Polysaccharide from Portulaca oleracea L. has immune effects on mice with tumor S180.

This study was designed to asses the cytogentic effect of portulaca oleracea crude extract on normal human lymphocytes (in vitro).

II.MATERIALS AND METHODS

A. Cytogenetic study on lymphocyte of human circulating blood

1. Blood collection

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

2. Procedure

a) Blood culture with plant extracts

1. Ethanolic extract of P.O at different concentrations were added to each test tube containing the whole media which prepared in 3.1.4.5 that final concentration of each extract in the tubes being (6.4, 3.2 ,1.6 ,0.8 ,0.4 0.2 mg/ml) (three replicates for each concentration.), to the other three tubes only PHA was added as control.
2. To all test tubes 0.3 ml of PHA was added, mixed the components very well.
3. Peripheral blood 0.5 ml was added into all test tubes containing (5 ml) of culture medium and then transferred to CO2-incubator at 37□C, and the tubes were shacked gently each 24 hours.

b) Harvesting

1. After 71.30hrs of incubation of lymphocyte, 0.1 ml of colcemide was added for 30 minte.
2. Samples were centrifuged for 10 min. at 250 Xg rpm.
3. The supernatant was withdrawn by pasture pipette and the precipitated cells with a little culture medium were left in the test tube.
4. The precipitate was mixed very well by the vortex mixer, and then 5-10 ml of warmed (37□C) Potassium chloride (0.075 M) was gradually and gently added with mixing.
5. The samples were incubated in shaker water bath for 30 min. at 37□C.
6. Samples were centrifuged for 10 min .at 250 Xg, then the supernatant discarded.
7. Few drops of the freshly made fixative (methanol and glacial acetic acid 3: l V/V) were added drop wise gently mixing till reaching 5ml and left at -20□C for at least 30 mints. Later on centrifugation performed for 10 min.at 250 Xg then fixative decanted off and the process repeated for 2-3 times until the supernatant looked clear. At the final change, the cells resuspended in (1-1.5) ml of freshly made fixative.

c) Slide preparation

The slide was prepared according to the procedure applied by ICCMGR by which the cell suspension removed from freezer or used immediately.

The suspension was mixed very well by pasture 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.

d) Staining

1. The slides were stained using freshly made Giemsa stain (stock solution) and warmed Sorenson's buffer (37□C) 1:4 V/V) which was applied for 2 min., then rapidly washed with warmed Sorenson's buffer, after that left to dry at room temperature. Microscopic examination was performed to determine mitotic index (MI %) and blast index (BI %) and chromosomal aberration (C.A.) and slides treated with trypsin before staining.

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

M.I. %=No. of dividing cells in metaphase / {Total No. of dividing cells +No. of non- dividing cells (1000) cells} X 100. (15

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

M.I. = No. of cells in blast form / Total No. of dividing cells +No. of non- dividing cells (1000) cells} X 100. (16).

III.RESULTS AND DISCUSSION

A. Cytogenetic study on peripheral human lymphocytes

1. MI

Figure, (4-45) show that MI value of human lymphocyte was increased at concentrations (6.4, 3.2, 1.6, 0.8 , 0.4 and 0.2 mg/ml) in a concentration dependent manner , but without significant differences (P ⩾ 0.01) between them (0.467±0.03, 0.467±0.09, 0.300±0.06,0.267±0.07, 0.300±0.07 and 0.267±0.07) respectively. Compared with MI value of positive control group was showed a highly significant difference (P ⩽ 0.01), (0.700±0.12) compared with all concentrations.

2. BI

Figure, (4-45) show that BI value was increased at concentrations (3.2, 1.6, 0.8, 0.4 and 0.2 mg/ml) in a concentration dependent manner, but without significant differencesm (P⩾0.01) (4.03±0.37, 3.70±0.17, 3.30±0.61, 3.13±0.24 and 2.77±0.20) respectively .In the highest concentration (6.4 mg/ml) the BI was (5.37±0.30) which significantly different (P⩽0.01), compared with previous concentrations. Positive Control group showed highly significant increase (P⩽0.01) in BI (7.67±0.56) compared with all concentration.

The used concentration of portulaca oleracea plant extract showed no stimulatory effects on human blood lymphocytes as a mitogene without PHA and anti- mitotic effect when using the same concentration of plant extract instead of colcemide.

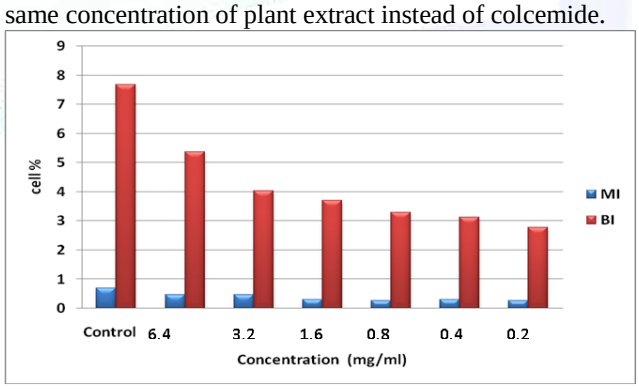

Figure 1: MI and BI values at different concentration of 70% ethanolic extract of Portulaca oleracea.

Cytogenic study is a routine cytotoxic research of different compounds used in treatment of human diseases. For this reason we analyzed some genotoxic effects of the medicinal plants extracts in human lymphocyte culture. The most sensitive tests for the effect of potentially mutagenic and carcinogenic agents are the quantifying of cytogenetic parameters including mitotic index (MI %) and blast index (BI %) (17).

The results showed no karyotyping and phenotyping changes in chromosomes of human lymphocytes when exposed to all concentration of 70% ethanolic extract of Portulaca oleracea. Portulaca oleracea have alkaloid pigments, the reddish β-cyanine (visible in the coloration of the stems), this pigments are potent antioxidant and have been-mutagenic properties (18). 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 (19) .A major advantage of antioxidants is that they are generally effective against a wide range of mutagens , both exogenous and endogenous (20).Tannic acid reduced mutagen–induced chromosomal aberration in mammalian cell (21). Flavonoids are probably the best known of these substances due to their properties (22).

The reason of increased MI and BI values in peripheral blood lymphocyte but without significant difference($P \geqslant 0.01$), compared with positive control group, might be belong to several reasons, ethanolic extract of P.O have different compound alkaloids, flavenoids, saponines, glycosides and tannins. This compound may cause increase MI and BI in concentration depended manner with presence of mitogen (PHA).

Carotenoid (β–carotein) has a role on immunesupresion state by increased lymphoblastiogensis with mitogen that stimulant T cells in vitro (23). Also P.O have other compound may decreased MI and BI proliferation such as poly unsaturated fatty acid (PUFA) which have the capacity to inhibit T cell signal transduction, due to their ability to remove protein tyrosine kinases (24). Protein tyrosine kinases are critically involved in the initiation of T cell receptor (TCR) signal transduction (25).

Other plant compound may decrease lymphocyte proliferation such as dopamine. Dopamine receptors (D1 and D2) are known to be on many cells of human body. Lymphocytes have these receptors (26).Activation D1 receptor in vitro by dopamine causing decrease T cell proliferation by degradation of nuclear factor-kappa beta (NF-KB) (27), because NF-KB appear to be among the transcription factors that have been implicated in the expression of a wide range of proinflammatory genes, dopamine induce immune modulation can be explained via this pathway (28).

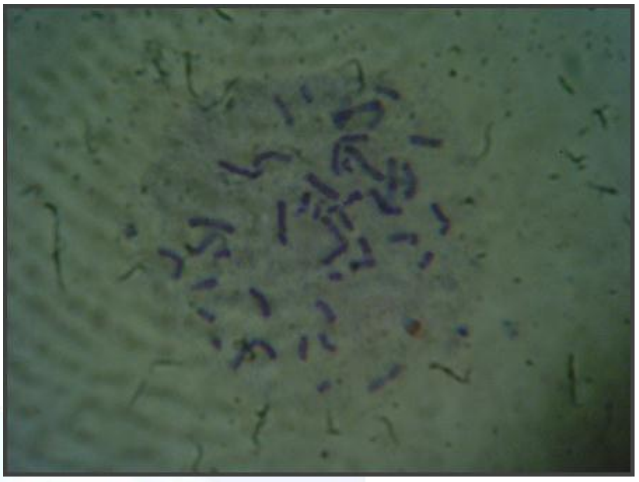

Figure 2: Normal karyotyping of human chromosome at different concentration of 70 % ethanolic extract of Portulaca oleracea, XY, G-band (400X).

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